KINETICS OF THE SULPHITE-INHIBITED MAILLARD REACTION

by

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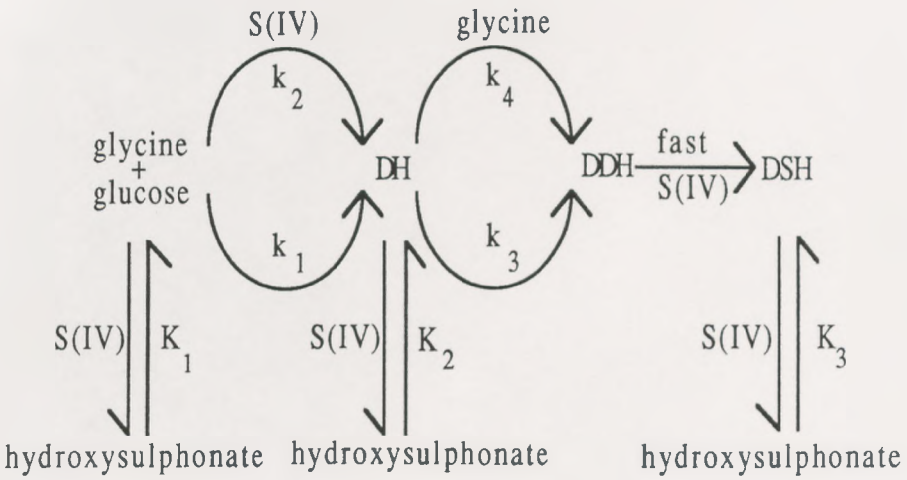
Abstract

The kinetics of the inhibition of Maillard browning of glucose and glycine by sulphur(IV) oxospecies, S(IV), are reported in detail in water, and solutions containing either 40% w/w ethanol, 40% w/w polyethylene glycol 400 or up to 81.5% w/w glycerol. The progress of the reaction was followed by measuring free and reversibly bound S(IV), production of H^+ and formation of sulphate ion. Equilibria between S(IV)-species were investigated by Fourier Transform Infrared (FTIR) Spectroscopy.

Kinetic data show that despite earlier reports demonstrating a lack of an effect of pH on this reaction, maintaining of reaction mixtures at a constant pH causes considerable increases in rate. Overall the kinetics support the following 3-step mechanism,



where DH, DDH and DSH represent 3-deoxyhexosulose, 3,4-dideoxyhexosulos-3-ene and 3,4-dideoxy-4-sulphohexosulose. Some autoxidation of S(IV) also takes in practice. The kinetically measured dissociation constant of the hydroxysulphonate of DH is found to be 0.28 M and this is the first report of kinetic evidence for such an interaction in the S(IV)-inhibited Maillard reaction. Hydrogen ion production during the reaction is the result of a condensation

reaction between glucose and glycine, and autoxidation of S(IV).

FTIR spectroscopy shows that addition of glycerol as a humectant does not cause the expected conversion of HSO_3^- to $S_2O_5^{2-}$ in solution. Instead new species, e.g. ion pairs such as $NaS_2O_5^-$, are formed and $S_2O_5^{2-}$ is probably less important in concentrated foods, than once thought. The effect of humectant is to increase both k_1 and k_2 (a_w 1-0.43) and the effects are explained in terms of equilibria involving water as a reactant and interactions between transition states of the solvent.

CONTENTS.

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Finally, I would like to dedicate this piece of work to my parents, as a measure of thanks for their support and considerable help during the many years that I have been a student.

I. List of abbreviations.

a_w	Water activity
DDH	3,4-dideoxyhexosulos-3-ene
DDP	3,4-dideoxypentosulos-3-ene
DFG	Difructose glycine
DH	3-deoxyhexosulose
DP	3-deoxypentosulose
DSH	3,4-dideoxy-4-sulphohexosulose
DSP	3,4-dideoxy-4-sulphopentosulose
DTNB	5,5-dithiobis (2-nitrobenzoic acid)
FTIR	Fourier Transform Infrared Spectroscopy
Fruc	Fructose
Gluc	Glucose
HMF	Hydroxymethylfurfural
MFG	Monofructoseglycine
PEG-400	Polyethylene glycol 400
S(IV)	Sulphur(IV) oxospecies

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CHAPTER 1: INTRODUCTION.

1.1. Non-enzymic browning.

1.1.1. Introduction.

When browning has taken place in a food it is often difficult to determine whether the reaction has occurred by an enzymic or non-enzymic mechanism, unless the enzymes are deactivated by the conditions under which the food has been treated. If this is the case, then, only non-enzymic browning is said to occur. However, colour production could occur as a result of intermediates formed through oxidation reactions involving enzymes, prior to enzyme deactivation, becoming subsequently involved in non-enzymic browning. However, it is generally understood that the term non-enzymic browning refers to one of the following categories of reactions:

(a) Maillard browning or the reaction of amino acids, peptides or proteins with reducing sugars,

(b) ascorbic acid browning, that is the spontaneous thermal decomposition of ascorbic acid under both aerobic and anaerobic conditions and either in the presence or absence of amino-compound,

(c) caramelisation or the pyrolysis of food carbohydrate, and

(d) lipid browning, which is probably oxidative degradation followed by polymerisation.

However, as much of the research into non-enzymic browning has been on Maillard browning, the two terms are often used interchangeably (Mauron, 1981). The above list of reactions covers a diversity of chemical types with regard to reactants and involves a variety of reaction conditions. However, non-enzymic browning

reactions are characterised by the participation of carbonyl compounds as reactive intermediates and also the ability of S(IV) oxoanions to inhibit, at least partially, the formation of coloured products. These products are frequently referred to as melanoidins and probably consist of a mixture of discrete, high molecular weight components (Motai and Inoue, 1974) rather than having the nature of random polymers of intermediate length. Usually, the yellow colour is due to the trailing of an intense ultraviolet peak into the visible region, and there is no single wavelength at which the products of non-enzymic browning can be quantified.

1.1.2. The Maillard reaction.

The Maillard reaction was probably first discovered accidentally with the advent of the use of heat to cook foods, the application of fire for food preparation being rapidly reinforced by the interesting new tastes and flavours thus produced. As a consequence of heating, some food became safer to eat due to the destruction of bacteria. Heating also meant that the choice of food was improved enormously, since many foods are palatable only after prolonged heat treatment.

Under certain conditions, however, the Maillard reaction does not lead to the increased enjoyment of food, but to food spoilage. This can occur as a result of the production of brown pigments, off-flavours and aromas. A classic example is the production of aged or stale flavour during the storage of dairy products. Such flavours are caused by lipid deterioration as well as by Maillard reactions (Henry *et al.*, 1948; Parks, 1967; Scanlan *et al* ; 1968). However, much more importantly the reaction can produce potentially toxic or mutagenic compounds, thus affecting food safety. Also the reaction can cause a reduction in the nutritional quality of food. In addition to

amino acid destruction, the Maillard reaction can cause a decrease in amino acid availability by at least three mechanisms. These are the blocking of amino acid side-chains, the formation of cross-links between peptide chains leading to absorbable but unavailable peptides and the decreased overall digestibility of the protein due to a combination of the first two processes (Mauron, 1981). The reaction can also cause destruction of vitamins and a reduction of bioavailability of trace elements. In addition, Maillard reaction products could also interfere with physiological processes, e.g. absorption, retention, biochemical transformation and excretion.

The reaction is named after the French chemist Louis Maillard, who first described the formation of brown pigments or melanoidins when heating a solution of glucose and lysine (Maillard, 1912). It takes place readily at all pH values found in food systems, Fig. 1.1.1 showing the pH dependence in the range pH 1-8 when xylose is the aldose and glycine and alanine are the amino-compounds (Wolfrom *et al.*, 1953). The reaction leading to the formation of colour, measured as absorbance at 490 nm, is independent of pH in the range expected for foods (pH 3-7). Although amino acids readily induce the browning reaction, it takes place with most amines, including peptides and proteins, and the reaction involving ammonia is widely used in the manufacture of ammoniated caramels.

1.1.3. Early Maillard reactions.

The early stages of the Maillard reaction are essentially those of amine-assisted dehydration. The sequence of events is initiated by the condensation reaction between the carbonyl group of the aldose and the amine (a result of nucleophilic attack by the amine) to give the corresponding aldosylamine. Such adducts form readily when

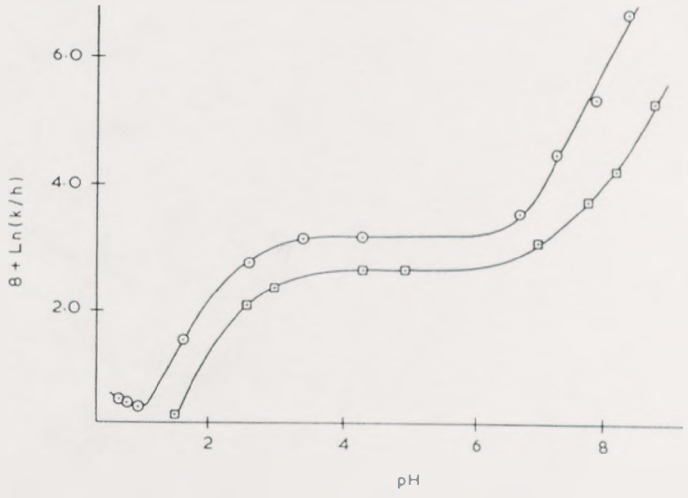


Fig.1.1.1. Effect of pH on the rate constant of the xylose–glycine and xylose–alanine reactions at 65 °C when the concentration of all reactants is 0·25 M: glycine, ⊙; alanine, ⊡ (Wolfrom *et al.*, 1953). Reprinted with permission from *J. Amer. Chem. Soc.*, 1953, **75**, page 3473. © 1953 American Chemical Society

aldoses are heated with primary amines in water and if concentrated solutions are used they separate out on cooling (Weygand, 1939). Primary amines should be able to form dialdosylamines; one such product which has been isolated is di-D-glucosylamine in which the amine is ammonia (Ellis and Honeyman, 1955). The aldosylamine next undergoes a facile irreversible rearrangement, the Amadori rearrangement (Hodge, 1955), to give the 1-amino-1-deoxy-2-ketose or, more simply, ketoseamine. The complete sequence of events from aldose to ketoseamine is as shown in Fig.1.1.2 (Mauron, 1981):

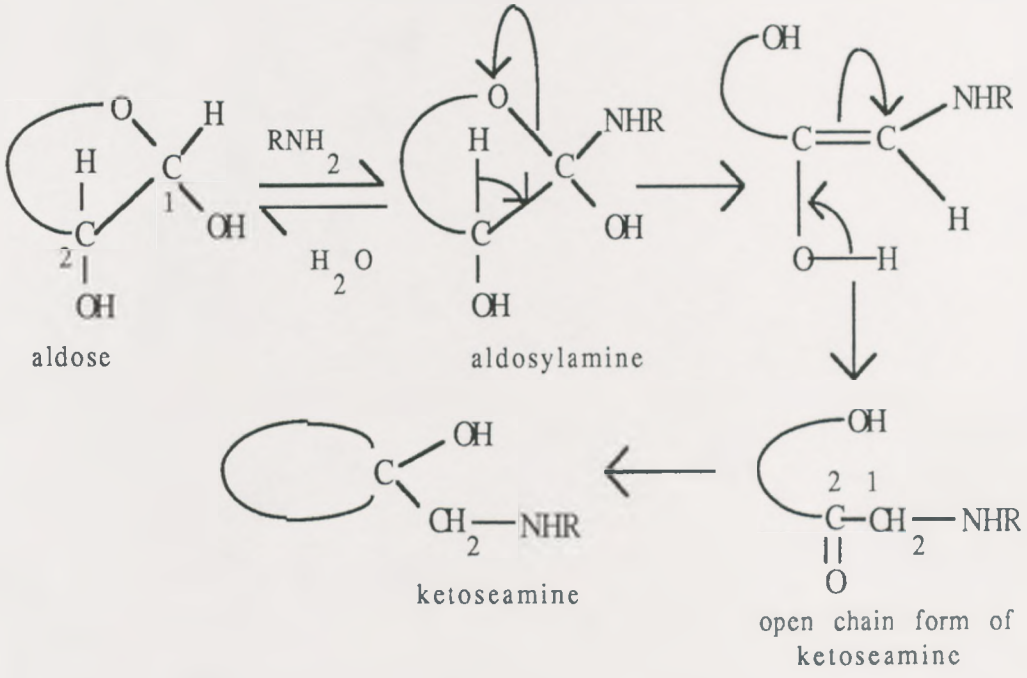


Fig.1.1.2. Reaction of aldose with amine to form a ketoseamine.

If the aldose is D-glucose, then the Amadori product will be the 1-amino-1-deoxy-D-fructose or more simply monofructoseglycine (MFG).

1.1.4.1. Advanced Maillard reactions.

It is evident that the ketoseamine still bears a replaceable hydrogen on its amino group and, since secondary amines react with

aldoses to form aldosylamines with a subsequent Amadori rearrangement (Hodge and Rist, 1952), it is not surprising that the Amadori product shown above will react with a second mole of aldose, undergo a second Amadori rearrangement and, thereby, give rise to a diketoseamine. If the reagents are glucose and glycine, then, the diketoseamine is commonly referred to as difructoseglycine or DFG (Anet, 1959a). The mono- and diketoseamines are regarded as the key intermediates in Maillard browning, coloured compounds resulting from their degradation and polymerisation. Difructoseglycine appears to be much less stable than the monoketoseamine, decomposing spontaneously with a maximum rate in the region of pH 5.5 to a quantitative yield of the monoketoseamine and nitrogen-free carbonyl compounds (Anet, 1959a). The rate of colour development, as judged from absorbance measurement at 440 nm, is also much greater when difructoseglycine is allowed to decompose in the presence of amino acids compared with when monofructoseglycine or glucose are used (Anet, 1959b). The main product of the decomposition of difructoseglycine is 3-deoxy-D-erythro-hexosulose in 80-90% yield (Anet, 1969a, b). This compound will be referred to simply as 3-deoxyhexosulose or 3DH, and is accompanied by two unsaturated compounds, the cis- and trans-isomers formed by the removal of water between positions 3 and 4 of 3DH (Anet, 1962) and known collectively as 3,4-dideoxyhexosulos-3-ene.

The nature of the product arising from the degradation of the monoketoseamine depends on whether the Amadori compound undergoes 1,2- or 2,3-enolisation, the former leading to the formation of osuloses as shown in Fig.1.1.3 (Anet, 1960). In the case when glucose is the aldose, R = $(CHOH)_2CH_2OH$. In contrast, 2,3-enolisation

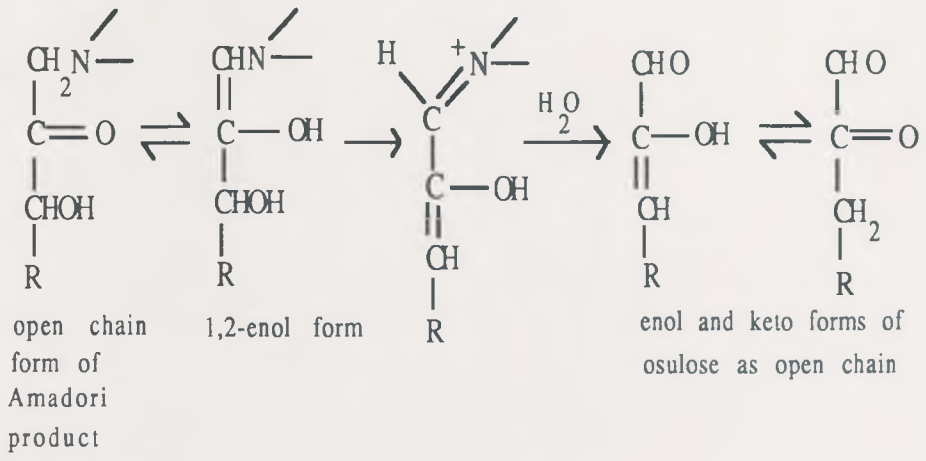


Fig.1.1.3. Conversion of monoketoseamine to a 3-deoxyosulose.

leads to a suspected dehydroreductone intermediate as shown in Fig.1.1.4.

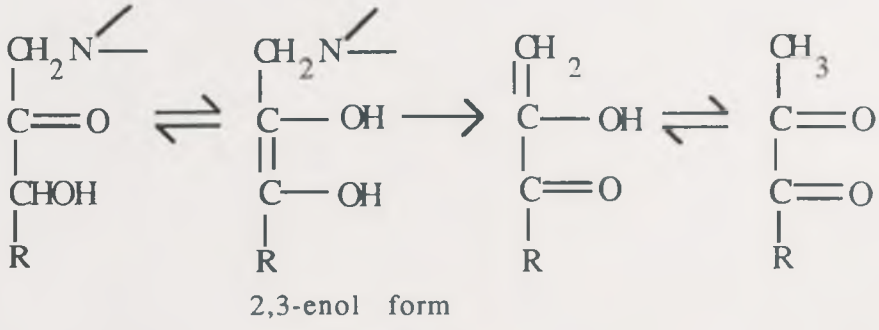


Fig.1.1.4. 2,3-enolisation of monoketoseamine.

Whereas the osuloses have been isolated and identified in non-enzymic browning systems, the existence of the 1-deoxy-2,3-dicarbonyl intermediate is proposed on the basis of its possible cyclisation to maltol and iso-maltol whose structures are shown in Fig.1.1.5 (Hodge *et al.*, 1963, 1972). 1,2-Enolisation is favoured when the amino group of the Amadori compound is almost completely protonated, that is in acid solution, whereas in weakly acidic and neutral media, 2,3-enolisation is favoured (Feather, 1981).

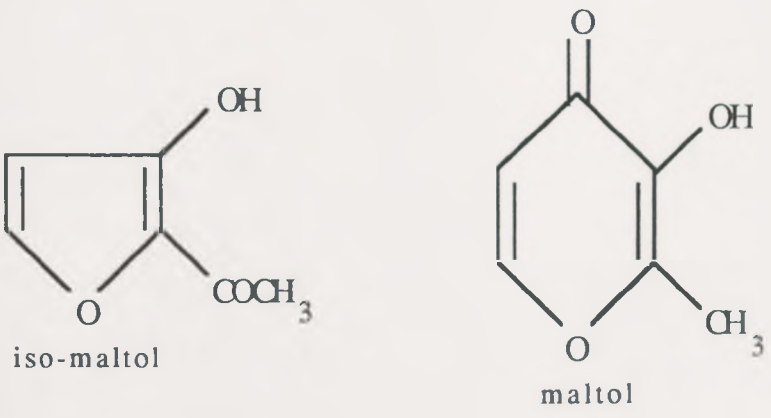


Fig.1.1.5. Structures of iso-maltol and maltol.

A simplified scheme, based on one devised by McWeeny and Burton (1963), showing the relationships between products in the early stages of the glucose-glycine reaction is shown in Fig.1.1.6.

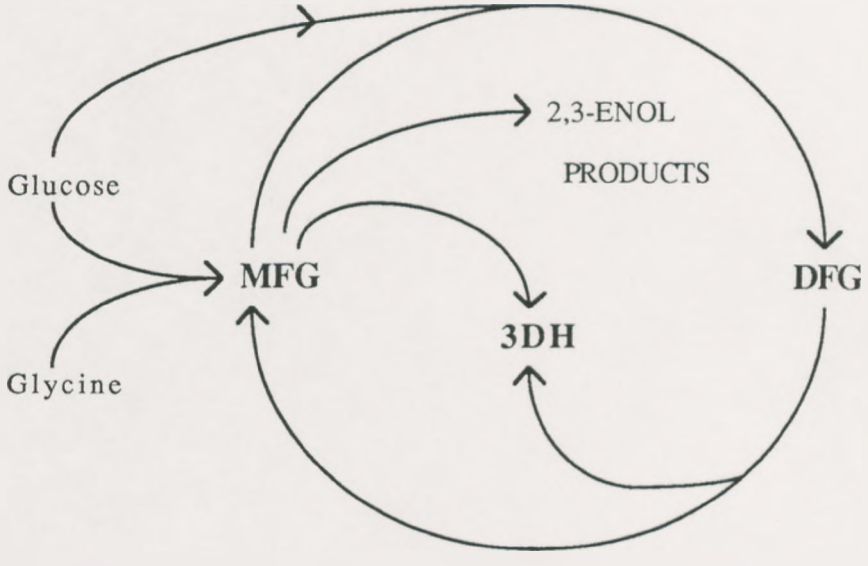


Fig. 1.1.6. Simplified scheme for the early stages of the reaction between glucose and glycine.

Almost all the known mechanisms whereby S(IV) inhibits the consequences of Maillard reactions involve pathways connected with 3-deoxyhexosulose. 2,3-Enolisation reactions, however, ultimately lead to the formation of low molecular weight sugar fragments such as

acetaldehyde, pyruvaldehyde, diacetyl and acetic acid, as well as being able to cyclise to oxygen heterocycles, and are, therefore, capable of producing distinctive flavours, even if the extent to which they occur is relatively small.

Both pathways for the breakdown of Amadori product (MFG) lead to the formation of α-dicarbonyl compounds (e.g. diketo or glyoxal groupings) and, therefore, a reaction common to both sets of intermediates is the Strecker degradation reaction (Schönberg and Moubacher, 1952). The mechanism involves formation of a Schiff's base adduct between the dicarbonyl compound and an α-amino acid, enabling facile decarboxylation of the amino acid as shown in Fig.1.1.7:

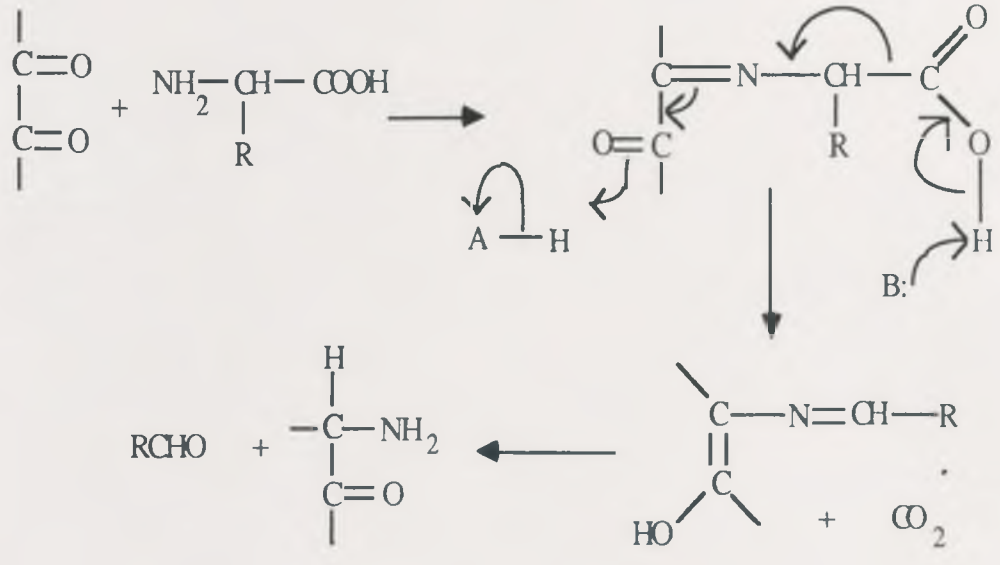


Fig.1.1.7. Scheme for Strecker degradation.

In his original report, Maillard (1912) described the formation of carbon dioxide is 36% yield when glucose (1.67 mol) and glycine (1 mol) were heated at 100 °C for 7 h. The reaction is generally regarded as being favoured at higher temperatures, the aldehyde produced contributing directly to the flavour and odour of the

mixture. The amine product may well undergo further reaction, such as conversion to pyrazines, again resulting in products with characterstic organoleptic properties (Dawes and Edwards, 1966; Kochler and Odell, 1970).

Høltermand (1966) proposed a pathway to Strecker degradation products, which bypasses completely the Amadori rearrangement. It starts with the Schiff base and involves the migration of the CN double bond and subsequent hydrolysis with H_2O. It represents a transamination reaction in which the amino acid is converted into the corresponding oxo-acid and the sugar into a non-reducing amino sugar. The oxo-acid reacts further with an intact amino acid resulting in decarboxylation and liberation of an aldehyde by a Strecker degradation.

1.1.4.2. Formation of heterocyclic compounds.

A great number of heterocyclic compounds are formed during the later stages of the advanced Maillard reactions. They include N-, S- and O-heterocyclic structures. They play an important role as food flavours and some of them might also have physiological significance.

Among the N-heterocyclic structures the pyrazines have received most attention and, during the past decade, much evidence has accumulated indicating that pyrazines contribute directly to the roasted or cooked flavour of various foodstuffs. They are typical products of the advanced Maillard reaction and have been reported in many heated food systems including beef products, toasted barley, cocoa products, coffee, peanuts, popcorn, potato products, rye crisp bread, soy products, roasted pecans and chicken broth. In their review, Maga and Sizer (1973) list 70 different pyrazines that have been isolated from food systems.

Pyrroles, pyrrolidines and pyridines are another class of N-heterocycles that have been observed in a wide variety of heated foodstuffs.

Thiazoles are compounds which contain both nitrogen and sulphur in their ring structure and are formed in the Maillard reactions involving the sulphur amino acids. A number of thiazoles have been isolated from foods which have undergone heat processing or Maillard-type reactions. These include coffee (Stoll *et al.*, 1967), roasted peanuts (Waldradt *et al.*, 1971), cooked beef (Wilson *et al.*, 1973), stale dried milk (Ferretti and Flanagan, 1972) and potato chips (Buttery and Ling, 1974). Several alkyl thiazoles were described as having nutty or roasted flavours.

Oxygen-heterocycles are responsible for the fragrant caramel aroma and can be formed by sugar pyrolysis in the absence of amino compounds, but they can be similarly formed much more rapidly or at lower temperatures as a result of a Maillard reaction. They have the structure of cyclic enolones, the best known structures being maltol and isomaltol (Fig.1.1.5).

1.1.5. Final Maillard reactions.

In contrast to the relatively good understanding of the early stages of Maillard browning, information regarding the structure of the pigments is fragmentary. The products clearly contain a number of different functional groups but the situation is further complicated by the effects of reaction conditions on the composition of the melanoidins. The pigments isolated from the reaction of aldoses and amines contain nitrogen, some are readily soluble in water, some are slightly soluble and others insoluble. In all cases, however, the solubility of the products decreases with increasing reaction time

(Reynolds, 1965). Pigment formation is the result of polymerisation of the many highly reactive compounds formed during the advanced Maillard reactions, especially the unsaturated carbonyl compounds and furfural, the latter yielding water-insoluble brown melanoidins (Reynolds, 1965). In addition to the brown colour formation, these polymerisation reactions lead to toughening of stored food. This was demonstrated by Labuza *et al* (1977), who showed that an increase in browning of an intermediate moisture food was directly correlated with an increase in toughness as measured by an Instron. The browning that occurred was not the result of lipid oxidation, as the same changes occurred even if the food was protected with antioxidants.

1.2. Chemistry of S(IV) oxospecies.

1.2.1. Nomenclature and structure of S(IV) oxospecies.

This work will be concerned primarily with reactions of the anions hydrogen sulphite, HSO_3^-, sulphite, SO_3^{2-}, and disulphite, $S_2O_5^{2-}$. The disulphite ion is more commonly known as metabisulphite but IUPAC recommends the former name.

The structural parameters of some oxosulphur species in oxidation state +4 and of sulphate ion are shown in Fig.1.2.1.

Originally, two isomeric forms (**I**, **II**) were suggested for hydrogen sulphite ion as shown in Fig1.2.2 (Schaeffer and Köhler, 1918). Golding (1960) proposed the presence of **I** in dilute solutions. However, Meyer *et al.* (1979) suggested structure **II** on account of the ion having C_{3v} symmetry necessary for this species. These findings are in agreement with those of Simon (1947) and Simon and Waldman (1955), who showed by observing the H-S Raman band that this

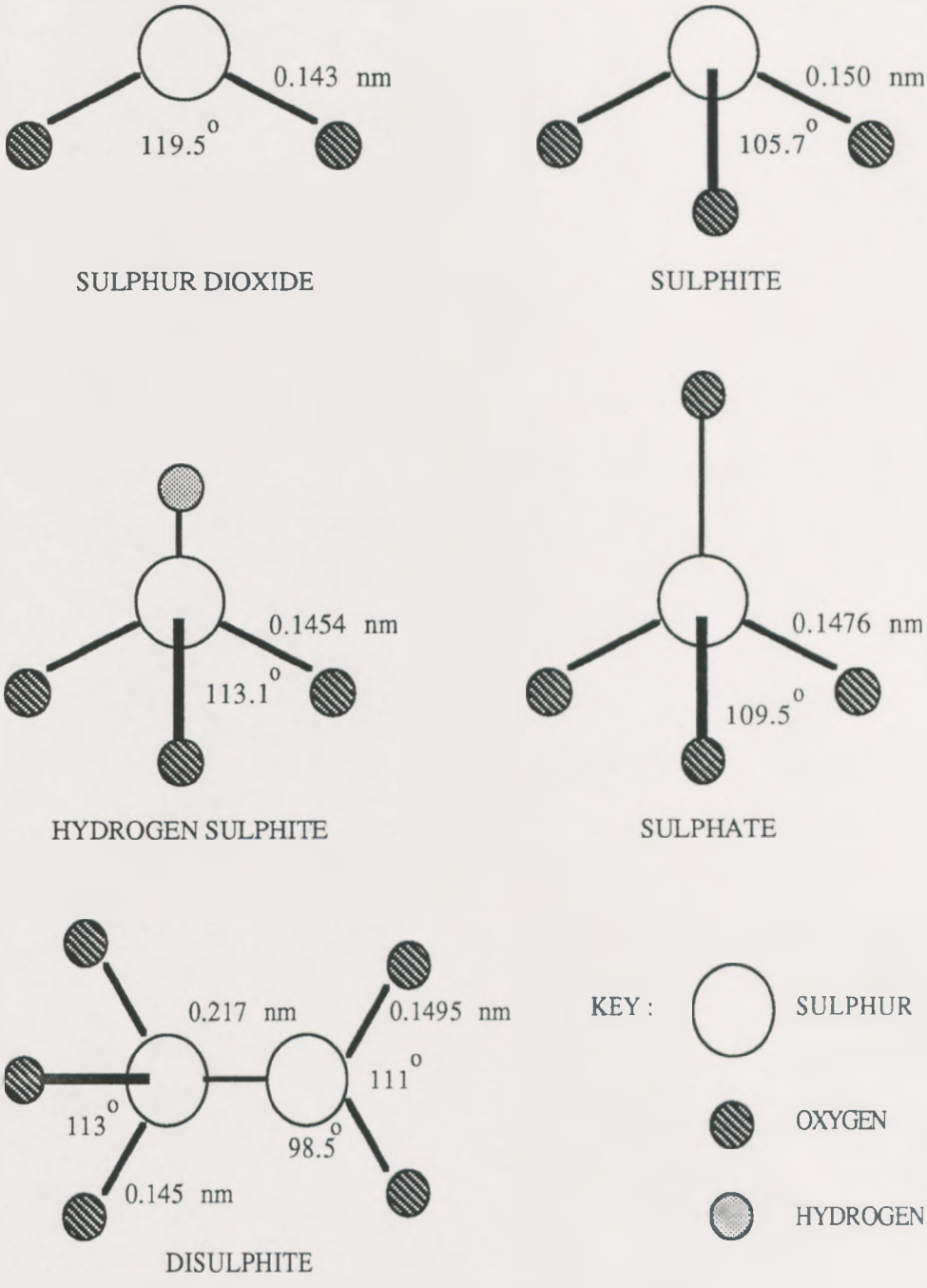
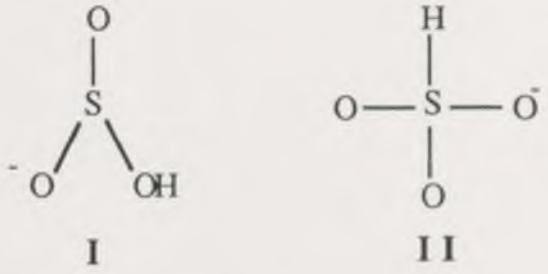


Fig.1.2.1. Molecular structures and dimensions of S(IV) oxoanions and of sulphate ion. Sources: SO_2 and $S_2O_5^{2-}$, Meyer and coworkers (1979); SO_3^{2-}, Larsson and Kirkegaard (1969); HSO_3^-, Johansson and coworkers (1980); SO_4^{2-}, Nord (1973). Based on Meyer and coworkers (1979) © 1979 Pengamon Press LTD. Reproduced from Wedzicha (1984b) page 7 © 1984 Elsevier Applied Science Publishers LTD.

structure exists in aqueous solution as well as in $RbHSO_3$ and $CsHSO_3$[18]. The first reported crystallographic work on the hydrogen sulphite ion



I II

Fig.1.2.2. Isomeric structure of hydrogen sulphite ion.

was that of Johansson and coworkers (1980), whose results are given in Fig.1.2.1. The S-O bond length in hydrogen sulphite ion is similar to 0.1456 nm in CH_3-SO_3^- (Charbonnier *et al.*, 1977), in which a similar bonding situation is expected (Johansson *et al.*, 1980). Meyer and coworkers (1977) considered the electron distributions around the sulphur atom of the sulphite ion with a view to establishing whether there existed any possibility of protonation on oxygen. The results, which suggest structure **II**, are surprising on account of the normal expectation that SH bonds are weaker than OH bonds. Analysis using an extended Huckel model based on spectral atomic parameters shows that the vacant position on the sulphur atom is occupied by an S-O antibonding orbital which, when protonated, reduces the antibonding electron density and, thereby, shortens the S-O bonds in HSO_3^-.

On the basis of Raman data Simon and coworkers (1956) suggested that both isomers (**I** and **II**) are present in solution. Simon did not consider the evidence entirely conclusive and that the question of the occurrence of $HOSO_2^-$ was still open to question. Appreciable concentrations of $HOSO_2^-$ are not unexpected on the basis of its acid ionisation constant. Kossiakoff and Harker's (1938) treatment of ionisation constants of oxygenated acids with the proton on the oxygen leads to a predicted K_a of this species of 10^{-7}. Since the observed

ionisation constant of hydrogen sulphite ion is of the order of 10^{-7}, there should be an appreciable concentration of the species $HOSO_2^-$ in solutions of hydrogen sulphite. Indeed, it was later concluded by Connick *et al* (1982) that both isomeric forms are present in solution in appreciable amounts. Evidence for this was based on the fact that in aqueous solution, in addition to a band due to the H-S bond of HSO_3^- at 1128 cm^{-1} (Meyer *et al*, 1979), there are three detectable Raman bands belonging to hydrogen sulphite ion in the S-O stretching region: 1052, 1021, and 730 cm^{-1}. None of these appears to be combinations or overtones. Since HSO_3^- has C_{3v} symmetry, it should only have two S-O stretching frequencies. It was concluded, therefore, that another hydrogen sulphite ion species must be present, presumably $HOSO_2^-$. The definite identification of three S-O stretching frequencies for hydrogen sulphite ion provides direct evidence for the presence of $HOSO_2^-$ in aqueous solution. Additional evidence is provided by the presence of a band at 709 cm^{-1}, which did not fit with the frequencies predicted for HSO_3^- by comparison with the Raman spectrum of $CH_3SO_3^-$ and $C_2H_5SO_3^-$. Whereas this band is consistent with a frequency predicted for $HOSO_3^-$ by extrapolation of the spectrum of $CH_3OSO_2^-$ (Simon and Kriegsmann, 1956). From *ab initio* calculations on the hydrogen sulphite ion, Strömberg *et al* (1983) found the isomers HSO_3^- and SO_2OH^- to be of comparable energy. This agrees with the proposal that appreciable concentrations of both ions exist in equilibrium. However, the most convincing evidence for the existence of the two isomers of hydrogen sulphite ion is derived from oxygen-17 NMR spectra of sodium hydrogen sulphite, where separate peaks corresponding to each of the isomers were obtained (Horner and Connick, 1986). From these data the lowest value of the equilibrium constant, K, for the reaction,

$$HSO_3^- \rightleftharpoons SO_2(OH)^-$$

given by,

$$K = [SO_2(OH)^-] / [HSO_3^-]$$

was 4.89, clearly indicating that under the experimental conditions used by these workers $SO_2(OH)^-$ is the predominant species. This was in agreement with *ab initio* calculations performed by Strömberg *et al* (1983) who stated that the SO_2OH^- ion is of lower energy than HSO_3^-. However, these findings were in contrast to those of Baird and Taylor (1981) deduced from similar calculations and the earlier work of Guthrie (1979) who estimated ΔG^o for the reaction above to be $+9 \pm 8$ kJ mol^{-1}, indicating that the species HSO_3^- predominates and that $SO_2(OH)^-$ accounts for only some 2.5% of the hydrogen sulphite ion species. Unless it is necessary to make the distinction, the hydrogen sulphite ion will be referred to as HSO_3^- in this thesis.

1.2.2. Aqueous solutions of S(IV) oxospecies and equilibria.

Despite solutions of SO_2 in water being conventionally regarded as H_2SO_3, there is no evidence to support the occurrence of significant amounts of the dibasic acid (Wedzicha, 1984b). Raman and infrared spectra of solutions of sulphur dioxide indicate solutions of molecular SO_2 (Falk and Giguere, 1958; Jones and McLaren, 1958; Davis and Chatterjee, 1975). Also, as the Raman spectrum of dissolved SO_2 is identical to that of liquid SO_2 (Meyer, *et al*, 1977; Simon and Pischfschan, 1961); this indicates that the interactions with water are weak. Hence, the species formed as a result of dissolution of SO_2 in water is normally given the formula $SO_2.H_2O$, the limit of the amount of H_2SO_3 present being set at 3% by Falk and Giguere (1958). The results are, however, still inconsistent with the relatively strong acid

behaviour of $SO_2 \cdot H_2O$ and also the high solubility of the gas in comparison with that of carbon dioxide (Bell, 1973). Guthrie (1979) has considered the possibility of tautomeric equilibria between 'sulphurous' acid species,

$$SO_2 \cdot H_2O \rightleftharpoons SO(OH)_2 \rightleftharpoons HSO_2(OH)$$

by means of a thermodynamic analysis based on the observation that the free energies of esters of inorganic oxoacids, for which resonance effects are not important, can be calculated with useful precision from the pK_a of the corresponding oxoacid; free energy changes for each of the equilibria have been estimated. The free energy of covalent hydration of SO_2 was found to be 6.7 ± 4.2 kJ mol^{-1}, in satisfactory agreement with the suggested limit on the amount of the acid present at equilibrium (Falk and Giguere, 1958). The calculated pK_a value for $SO(OH)_2$ was 2.3 and that for $HSO_2(OH)$ was -2.6. The value of ΔG^o for the reaction,

$$SO(OH)_2 \rightleftharpoons HSO_2(OH)$$

was found to be 19 ± 5 kJ mol^{-1} and it is interesting that the greater stability of the species $SO(OH)_2$ than that of $HSO_2(OH)$, predicted by Guthrie (1979), is the reverse of the order of stability predicted by Meyer and coworkers (1977) from electron density studies. Baird and Taylor (1981) used *ab initio* calculations to obtain the energies of the hexavalent sulphur isomer, $HSO_2(OH)$, and the tetravalent species, $SO(OH)_2$. They concluded that $SO(OH)_2$ was more stable, thus confirming the findings of Guthrie (1979).

The first dissociation of the dibasic acid form is,

$$SO_2 \cdot H_2O \rightleftharpoons HSO_3^- + H^+$$

and the equilibrium constant is given by,

$$K_1 = [H^+][HSO_3^-] / [SO_2 \cdot H_2O]$$

From ultraviolet absorption measurements this has a value of

0.0139 ± 0.0002 M at 25°C, corrected to zero ionic strength (Huss and Eckert, 1977). The result is in excellent agreement with conductivity data and confirmed previously reported values of 0.0139 M (Britton and Robinson, 1932; Ellis and Anderson, 1961) and 0.0142 M (Deveze and Rumpf, 1964), but was considerably lower than the often used value of 0.0172 M (Tartar and Garretson, 1941; Beilke and Lamb, 1975). The pK_a for the first dissociation of 'sulphurous acid' is, therefore, 1.86. This apparent value is close to the value of 2.3 predicted by Guthrie (1979) assuming that the major species in solution was $SO(OH)_2$. However, the amount of this acid actually present is too small to be detectable and it does not account for the acidity of solutions of sulphur dioxide. The pK values reported above for $HSO_2(OH)$ and $SO(OH)_2$, indicate that the former is much the stronger acid. Baird and Taylor (1981) calculated deprotonation energies of these two isomers and also came to this conclusion. This is not surprising on account of the lower stability of $HSO_2(OH)$, deduced by the same workers from *ab initio* calculations. The occurrence of $HSO_2(OH)$ may, therefore, account for the acidity of sulphur dioxide solutions, even if the proportion is very small. Unless differentiation between sulphurous acid and dissolved sulphur dioxide is required, the substance will, in future, be expressed in formulae as $SO_2 \cdot H_2O$.

In aqueous solutions of hydrogen sulphite ion the following equilibrium is rapidly established,

$$2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O$$

$$K = [S_2O_5^{2-}] / [HSO_3^-]^2$$

Reported values of the equilibrium constant, K, for this dimerisation reaction are shown in table 1.2.1. It can be seen that there is little agreement between the values. These results were all obtained from measurements of the UV absorption of $S_2O_5^{2-}$ in a region where HSO_3^-

T/°C	Ionic strength/M	K/M	Source
20.0	0.07-0.32	0.07	Golding (1960)
22.0	0.18	2.0	Arkhipova and Chistyakova (1971)
25.0	2.0	0.34	Bourne, Higuchi and Pitman (1974)
	0.0	0.076	

Table 1.2.1. Literature values of the equilibrium constant, K, for the reaction: $2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O$

absorbs negligibly and are based on the assumption that both K and the molar extinction coefficient of $S_2O_5^{2-}$ remain constant during the experiments. However, Connick *et al* (1982) showed that this was not the case and hence the values of K are considered unreliable. To avoid these assumptions Connick *et al* (1982) used the intensity of the Raman line of the H-S stretch at 2532 cm^{-1} and the UV absorption spectrum of the equilibrated solutions measured at 320 nm, a wavelength at which $S_2O_5^{2-}$ absorbs strongly and the absorption of hydrogen sulphite ion is negligible, to calculate reliable values of K. The variation of K with ionic strength is described approximately by,

$$K = 10^{(-1.398 + 0.35\sqrt{I})}$$

for 0.15 M < I < 4 M (Wedzicha *et al*, 1991). At ionic strengths of 0.18 and 2 M the corresponding values of K are 0.056 and 0.125 M respectively, these differing significantly from the values shown in table 1.2.1. Connick *et al* (1982) states that if one recalculates the absorbance data of Bourne *et al* (1974) using an improved value of extinction coefficient, the K values obtained are in good agreement.

In dilute solutions of HSO_3^- (10 mM) the amount of disulphite ion is negligible, but the proportion increases rapidly with increasing HSO_3^- concentration. The ionic strength dependence of K is presumably dominated by the rapidly decreasing activity coefficient of the doubly charged $S_2O_5^{2-}$. As a result, dimerisation becomes more important at high ionic strength. The variation of apparent equilibrium constant with ionic strength is shown in Fig.1.2.3.

The second dissociation of sulphurous acid,

$$HSO_3^- \rightleftharpoons H^+ + SO_3^{2-}$$

$$K_2 = [H^+][SO_3^{2-}] / [HSO_3^-]$$

has a pK_a of 7.18 ± 0.03 at 25 °C and zero ionic strength, which represents an equilibrium constant five orders of magnitude smaller than that for the corresponding ionisation of HSO_4^- ($pK_a = 1.99 \pm 0.01$) (Smith and Martell, 1976). Using this pK_a value and also the result, $pK_a = 1.86$ for the ionisation of $SO_2 \cdot H_2O$, the amounts of the species $SO_2 \cdot H_2O$, HSO_3^- and SO_3^{2-}, present in equilibrium in aqueous solution may be calculated as a function of pH; the result is shown in Fig.1.2.4. Such calculations are for solutions at high dilution, hence no disulphite ion is formed. Contributions from possible tautomeric species are also neglected.

Insufficient activity data are available to predict the behaviour at high concentration. The effect of increasing ionic strength on the pK_a of HSO_3^- is to reduce its value, Shapiro (1977) giving a value as low as 6.25 in concentrated salt solution. It is evident that in the normal pH range of food, that is pH 3-7, the predominant species will be hydrogen sulphite ion, with significant amounts of disulphite ion being formed as the concentration is increased, although sulphite ion is likely to become important at the higher end of this pH range and particularly at high ionic strength.

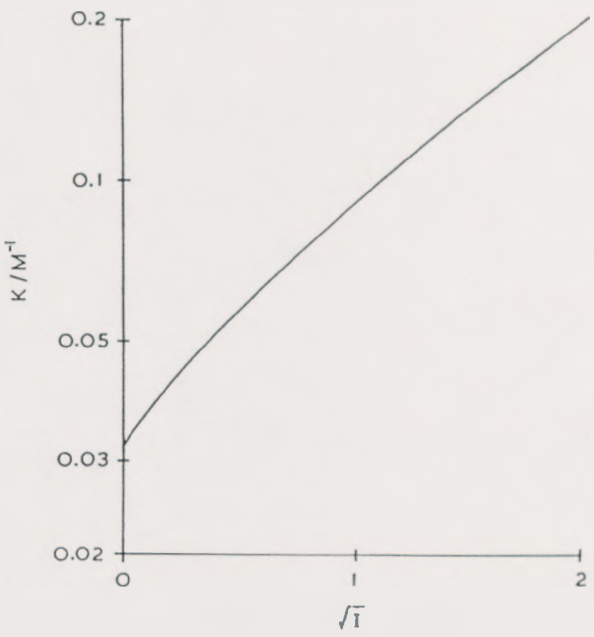


Fig.1.2.3. Effect of ionic strength, I, on the equilibrium constant, K, at 25 °C for the formation of $S_2O_5^{2-}$ from HSO_3^- plotted as K versus $\sqrt{I}$. Solutions contained $[HSO_3^-] = 10$–100 mM and ionic strength was controlled by the addition of NaCl and $NaClO_4$. Adapted from the paper by Connick *et al.* (1982).

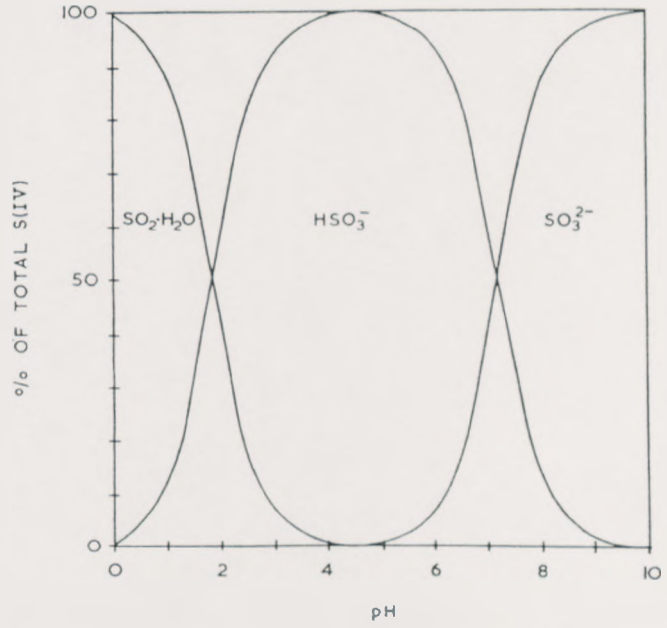


Fig.1.2.4. Distribution of the species $SO_2 \cdot H_2O$, HSO_3^- and SO_3^{2-} as a function of pH in dilute solution.

1.2.3. Effect of ion pair formation and nonelectrolytes on the dissociation of S(IV) oxospecies.

The activities of inorganic ions in solution are reduced through interactions with counter ions, ion pairs being produced. Whereas such species in 1:1 electrolytes (e.g. $Na^+ NO_3^-$ and $K^+ NO_3^-$) are highly dissociated (pK, -0.6 and -0.2 respectively), those of 1:2 electrolytes (e.g. $Na^+ SO_4^{2-}$ and $K^+ SO_4^{2-}$, pK, 0.68 and 0.92 respectively) show significant stability (Sillen, 1964; Reardon, 1975) and need to be taken into account when, for example, kinetic salt effects on reactions involving these oxoanions are being studied. There is very little information regarding the dissociation constants of ion pairs involving SO_3^{2-} and $S_2O_5^{2-}$ species. Reported findings include those of Young and Jencks (1977) who determined the formation constant for KSO_3^- at an ionic strength of 1.0 M. The value of the formation constant was found to be 1.42 M^{-1} at 25°C, which compares well with the value for the formation constant of KSO_4^- under these conditions. Also Wedzicha and Goddard (1991) considered whether or not any interaction between SO_3^{2-} and Na^+ could contribute to the effects of salts on the pK of HSO_3^-. It was found that an increase in the concentration of Na_2SO_3 or the addition of NaCl to a solution of Na_2SO_3 caused a decrease in the UV absorbance of SO_3^{2-} at 255 nm, a wavelength at which the absorbance due to HSO_3^- is negligible. This change in absorbance was attributed to ion association occurring between Na^+ and SO_3^{2-}, the formation constant for the complex being 6 M^{-1} at zero ionic strength. It was concluded that significant concentrations of ion pairs are to be expected in concentrated systems.

Wedzicha and Goddard (1991) considered the effect of ethanol, glycerol, polyethylene glycol PEG-400 and sucrose on the dissociation constants of $SO_2.H_2O$ and HSO_3^-. The corrected effect of ethanol, as

described by Wedzicha and Goddard (1991), and the uncorrected effects of glycerol, PEG-400 and sucrose on the pK of a 45 mM solution of $SO_2.H_2O$ are shown in Fig.1.2.5; clearly, sucrose and glycerol show little or no effect. The effect of the nonelectrolytes on the pK of a 50 mM solution of $NaHSO_3^-$ is shown in Fig.1.2.6. Wedzicha and Goddard (1991) showed that in the case of sucrose, the change observed in Fig.1.2.6 was no greater than the effect of sucrose on the pH of a solution of HCl. The increase in pK in the presence of ethanol, glycerol and PEG-400 could be due to the lower water activity environment favouring formation of HSO_3^- as a result of less efficient solvation of the more highly charged SO_3^{2-}. However, a contribution from the formation of $S_2O_5^{2-}$ is likely because further addition of, say, ethanol leads to the precipitation of NaS_2O_5 and it is reasonable to imagine the extent of association of HSO_3^- ions gradually increasing with addition of solvent until the solubility of NaS_2O_5 is exceeded.

1.2.4. Chemical reactivity of S(IV) oxospecies.

The observed chemical reactivity of S(IV) oxospecies stems from their ability to act as reducing agents or to take part in nucleophilic attack.

Sulphite ion behaves as a Lewis base and is classified on the 'hard' and 'soft' convention as borderline. Lewis acids which are small, have a high positive charge and no unpaired electrons, are termed hard. Conversely, acids which are easily polarisible and have a low positive charge are referred to as soft. A similar definition exists for hard and soft bases (Pearson, 1968). A typical example of a hard acid is the proton. A very simple statement of fact regarding the stability of acid-base complexes is that 'hard acids prefer to bind to hard bases and soft acids to soft bases'. In view of its borderline classification,

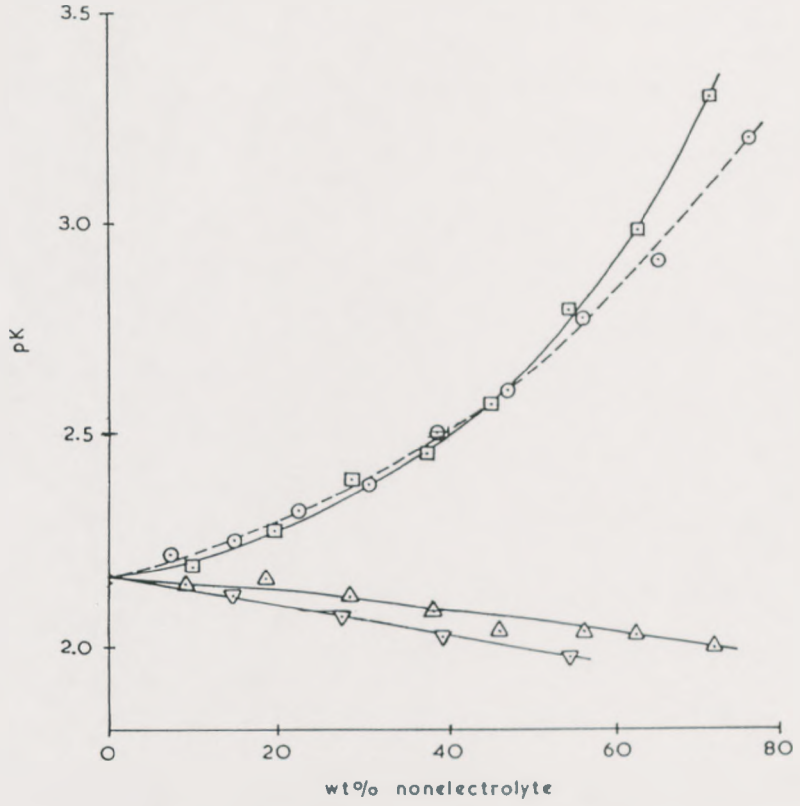


Fig.1.2.5. Effect of concentration of nonelectrolyte on the pK of a 45 mM solution of $SO_2 . H_2O$ at 30°C. ⊙ Ethanol; △ Glycerol; ⊡ PEG-400; ▽ Sucrose.

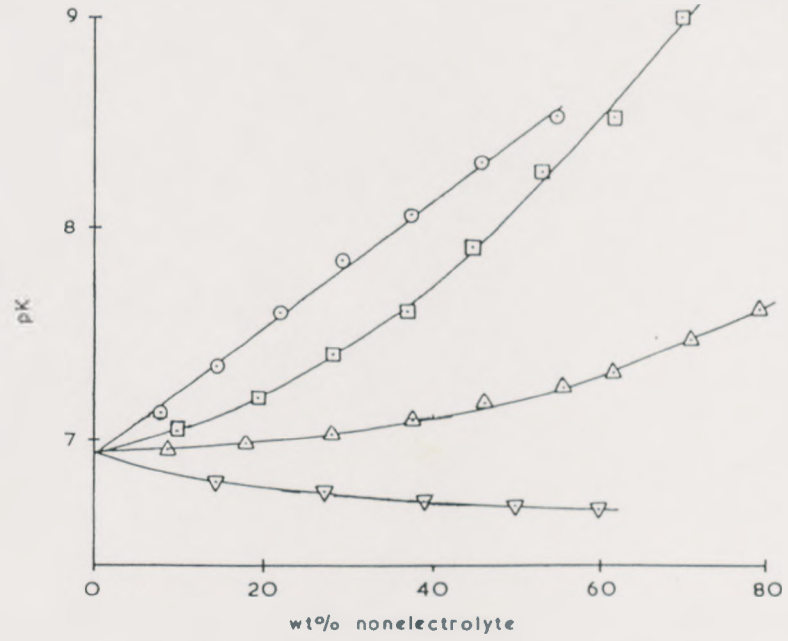


Fig.1.2.6. Effect of concentration of nonelectrolyte on the pK of a 50 mM solution of $NaHSO_3$ at 30°C. ⊙ Ethanol; △ Glycerol; ⊡ PEG-400; ▽ Sucrose.

sulphite ion is expected to interact widely with Lewis acids of all classifications. However, owing to its ability to accommodate 3d-electrons, the sulphite ion may also act as an electron acceptor, and, therefore, is capable of acting as a Lewis acid when appropriate bases are available.

Edwards and Pearson (1962) describe factors determining nucleophilic reactivities, the series of reactivity being $SO_3^{2-} > S_2O_3^{2-} > SC(NH_2)_2 > I^- > CN^- > SCN^- > NO_2^- > OH^- > N_3^- > Br^- > NH_3 > Cl^- > C_5H_5N > H_2O$. Recently, Wedzicha and Goddard (1991) determined the nucleophilic reactivity of S(IV) oxospecies in equilibrium with HSO_3^- by measuring reaction rates with malachite green. The data indicate that $S_2O_5^{2-}$ ion does not behave as a nucleophile in comparison to SO_3^{2-}. The predominant S(IV) species in aqueous solution a low concentration, 10^{-2} M, and pH 5.5 is HSO_3^-. However, it has been suggested that this ion exists in equilibrium with the isomer SO_2OH^-, appreciable concentrations of both species being present. It is not unreasonable to expect that the electron distributions around these ions will not be the same and hence the nucleophilicity of these two species may differ.

1.2.5. Sulphite ion oxidation by oxygen in aqueous solution and its inhibition.

The stoichiometric equation for the oxidation of sulphite ion by oxygen,

$$SO_3^{2-} + {}^1/_2\,O_2 \longrightarrow SO_4^{2-}$$

suggests none of the complexities inherent in its mechanism. The reaction can occur alone or it can be catalysed by transition metal ions. Fuller and Crist (1941) give the rate equation for the uncatalysed

reaction as,

$$d[SO_4^{2-}] / dt = (0.013 + 6.6[H^+]^{1/2}) [SO_3^{2-}]$$

from experiments carried out in the range pH 5.1-7.8 and the rate expression for the reaction catalysed by copper at pH 8.7 as,

$$d[SO_4^{2-}] / dt = (0.013 + 2.5 \times 10^6[Cu^{2+}]) [SO_3^{2-}]$$

The two expressions are very similar except that the hydrogen ion term has been replaced by one containing Cu^{2+}. The S(IV) term in both equations involves SO_3^{2-} rather than HSO_3^-, as the latter is less susceptible to oxidation. As there are no changes in rate constant of the uncatalysed reaction between pH 7 and 9, the rates may be compared at, say, pH 9 where, for the uncatalysed reaction, the rate of formation of sulphate is very nearly $0.013[SO_3^{2-}]$. The catalysed reaction will proceed at approximately three times this rate when the cupric ion concentration is 10^{-8} M, and significant effects of Cu^{2+} are likely at 10^{-9} M. With such a small metal ion concentration capable of catalysing the reaction, it is not surprising that many rate equations have been reported for the oxidation of sulphite species by oxygen and that these expressions and their rate constants seldom show a high degree of correlation with each other. Despite this, the reaction is consistently shown by the majority of data to be of first order with respect to the concentration of sulphite ion, independent of the concentration of oxygen in the range pH 3-7, and of first order with respect to oxygen above pH 9. The variations in the data are in the absolute magnitude of rate constants and pH effects.

The literature concerning the mechanism of the oxidation reaction is divided according to whether or not the superoxide ion, $^{\cdot}O_2^-$, is thought to be involved. The mechanism originally suggested by Bäckström (1934) is one of the most widely accepted. In this scheme the involvement of the free radicals $SO_3^{\cdot-}$ and $SO_5^{\cdot-}$ was

proposed, the presence of these species being confirmed later by Hayon and coworkers (1972). The mechanism also allows for gaseous O_2 to be the main source of oxygen, as suggested by ^{18}O isotope exchange experiments (Winter and Briscoe, 1951; Halperin and Taube, 1952), and predicts the formation of dithionite (Bassett and Parker, 1951) which is formed in small quantities. The mechanism does have a number of shortcomings and required the addition of extra steps to account for the effect of pH on the autoxidation and the occurrence of an efficient chain reaction on light-induced oxidation of sulphite at high pH. However, Hayon and coworkers (1972) comment that the main difficulty encountered in the Bäckstrom mechanism is concerned with the effects of alcohols on the progress of the reaction. They showed that neither $SO_3^{\cdot-}$ nor $SO_5^{\cdot-}$ were scavenged effectively. The concentrations of alcohols required to reduce the depletion of sulphite in light-induced oxidation to approximately 30% of its value in alcohol-free solutions were:

propan-2-ol	4×10^{-5} M
ethanol	1×10^{-4} M
methanol	2×10^{-4} M
tert-butanol	2×10^{-2} M

when the sulphite concentration was 5×10^{-4} M at pH 9.7. Therefore, these compounds should react with chain carriers at a rate sufficiently high for a significant reduction in their concentrations.

Superoxide ion involvement in sulphite oxidation was suggested in a series of papers by Fridovich and Handler (1958, 1959, 1961); its importance may be demonstrated by the fact that erythrocuprein, an excellent catalyst for the disproportionation of the superoxide ion, is a very effective inhibitor of the spontaneous oxidation in the presence of EDTA (McCord and Fridovich, 1960a, b). Similarly, tiron,

a potent superoxide scavenger (Greenstock and Miller, 1975), inhibits the sulphite mediated destruction of β-carotene in the presence of oxygen (Peiser and Yang, 1979). Yang (1970, 1973) also states that addition of superoxide dismutase inhibits sulphite oxidation. These results considered together provide strong evidence for the involvement of $\cdot O_2^-$ in the chain propagating stages.

The situation regarding the metal ion catalysed reaction appears to be even less well defined and the change from 'metal initiated' to 'metal catalysed' is also not clear. In summarising a large number of experimental rate laws, Hegg and Hobbs (1978) suggested that the rate equation for the catalysed oxidation of sulphite is,

$$d[SO_4^{2-}] / dt = k[H^+][SO_3^{2-}][M^+]$$

Although free radical mechanisms similar to those of the uncatalysed oxidation have been proposed, the formation of sulphito complexes with transition metal ions may be involved in the initiating steps (Schmidkunz, 1963; Barrie and Georgii, 1976).

The chain propagation and termination in sulphite autoxidation are still very much 'possible' schemes rarther than proven pathways; it is clear, however, that they must involve reactive free radical species. Hence, any substance which can effectively scavenge these intermediates should act as an inhibitor to autoxidation. Bigelow (1898) showed that alcohols have a strong inhibiting effect on the oxidation of sulphite to sulphate. Addition of 6.25×10^{-6} M of mannitol to 5×10^{-3} M of sulphite solution halved the rate of oxidation by air. Later, Kashtanov and Ryzhov (1936) demonstrated that the presence of small amounts of phenol in solution retards autoxidation of SO_2. Darykina (1954) showed that the inhibiting effect of phenols increases with the number of hydroxy groups and molecular weight. Chertkov (1960) stated that the addition of 2×10^{-3} to 4×10^{-3}% of

p-aminophenol to calcium sulphite solution decreased the amount of calcium sulphate formed by a factor of three to five. Sapotnitskii and Glushchenko (1962) showed that glucose can inhibit autoxidation of sulphite ion. Oxidation of calcium sulphite was completely arrested even at 0.5:1 mole ratio of glucose to sulphite ion. For ammonium, sodium and magnesium sulphites, a small amount of glucose (0.1 mole per mole of sulphite) decreased the degree of oxidation by about one half. Further increases in the amount of glucose had little effect on the course of oxidation at the molar concentration ratios studied, i.e. 0:1 to 2:1 glucose : sulphite. In reviewing the early work on the inhibition of sulphite oxidation, Schroeter (1966) stated that the reaction was also sensitive to the presence of inorganic ions, organic acids, glycols, polysaccharides, amines, amides, aldehydes and ketones. In the case of inhibition by mannitol (Fuller and Crist, 1941) and by N, N-dimethylformamide and N, N-dimethylacetamide (Schroeter, 1963), the rate law for the inhibited reaction is given by,

$$-d[S(IV)] / dt = k[S(IV)](A / A + [I])$$

where A is a constant describing the inhibition, I represents the inhibitor and k is the rate constant for the uninhibited reaction. This rate law is in agreement with the experimental data over a change in inhibitor concentration of two orders of magnitude, with a value of A of 10^{-5} M in all cases.

As expected, solutions of sulphur(IV) oxoanions containing a large proportion of ethanol are stable to oxidation. Wedzicha and Lamikanra (1983) have studied the progress of manganese catalysed sulphite oxidation in such solutions by observing the consumption of oxygen by means of a Clark-type oxygen electrode. An interesting feature of this reaction is that the addition of glycine, which acts as an inhibitor in aqueous media, considerably enhances the rate of loss of

oxygen in the presence of ethanol.

More recently, Steelhammer *et al* (1982a, b) showed that the oxidation of SO_3^{2-} to SO_4^{2-} was inhibited by water-soluble polyethyleneamines having one or more secondary amine and two primary amines and also by adding a P-containing water soluble topping agent (e.g. a polyphosphonate, organophosphonic acid compounds, their oligomers and mixtures of these compounds), to enhance the antioxidant activity of linear polyethyleneamines and substituted secondary and tertiary aromatic amines. Rochelle and Owens (1985) state that thiosulphate can inhibit SO_3^{2-} oxidation. The Mitsubishi Chemical Industries Co. Ltd. (1980a) showed that mixtures containing tris (nonylphenyl) phosphite (I), hydrolysis products of I, nonyl phenol (II), and/or H_3PO_3 could inhibit SO_3^{2-} oxidation. The same company (1980b) demonstrated that nonyl phenol (II), II-H_3PO_3 mixture, or II-dinonyl phenol mixture could also have the same affect.

1.2.6. Reaction of S(IV) oxospecies with carbonyl groups.

Sulphur(IV) oxospecies are often used in organic chemistry for the isolation of carbonyl compounds as crystalline derivatives, this being by hydroxysulphonate adduct formation. The original carbonyl species can be easily recovered as such adducts are decomposed above pH 7.

The carbonyl group is polarised as a result of oxygen being more electronegative than carbon. A partial positive charge resides on the carbon, hence this site is susceptible to attack by nucleophiles, aldehydes being more reactive in this respect than ketones.

Hydroxysulphonate adduct formation occurs spontaneously in solutions containing carbonyl compounds and sulphur(IV) oxospecies

in the following manner:

The equilibrium constant for the reaction defined by the following equation,

$$K_{obs} = [\text{adduct}] / [\textit{iso}\text{-PrCHO}][\text{free S(IV)}]$$

is practically unaffected by pH in the range pH 2-6, as illustrated, in Fig.1.2.7, by the formation of 1-hydroxy-2-methylpropanesulphonate from *iso*-butyraldehyde (Green and Hine, 1974). In the equation above, the concentrations are analytical and include all states of protonation or ionisation of the reactants and products in question. This result is typical, except in the absolute value of the equilibrium constant, for adducts involving a wide range of carbonyl compounds such as benzaldehyde (Kokesh and Hall, 1975), 1,3-dimethoxyacetone (Hine *et al.*, 1976) and glucose (Vas, 1949), for which extensive pH dependent equilibrium constant data are available. The adducts become less stable as the pH is reduced below pH 2 (Stewart and Donnally, 1932a). Of equal importance to the equilibrium data are the rates of formation and dissociation of hydroxysulphonate adducts. First order rate constants for the decomposition of *iso*-butyraldehyde (Green and Hine, 1974) and 1,3-dimethoxyacetone adducts (Hine *et al.*, 1976) show inverse dependence of hydrogen ion concentration in the range pH 4-8. Stewart and Donally (1932b) showed that the apparent first order rate constant for the decomposition of the benzaldehyde adduct decreased to approximately pH 2, below which it begins to rise. Young and Jencks (1977) provided more detailed data, shown in Fig.1.2.8, illustrating this effect for the dissociation of

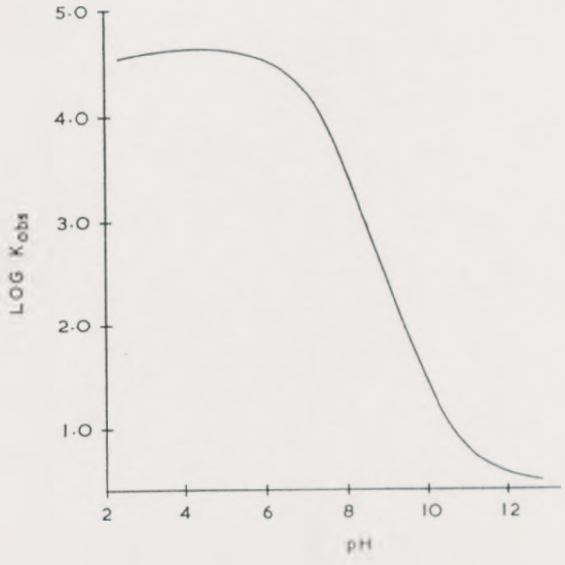


Fig.1.2.7. The variation of equilibrium constant for the formation of the hydroxysulphonate adduct of isobutyraldehyde with pH, in water at 25 °C (Green and Hine, 1974). Reproduced with permission from *J. Org. Chem.*, 1974, **39**, page 3898. © 1974 American Chemical Society.

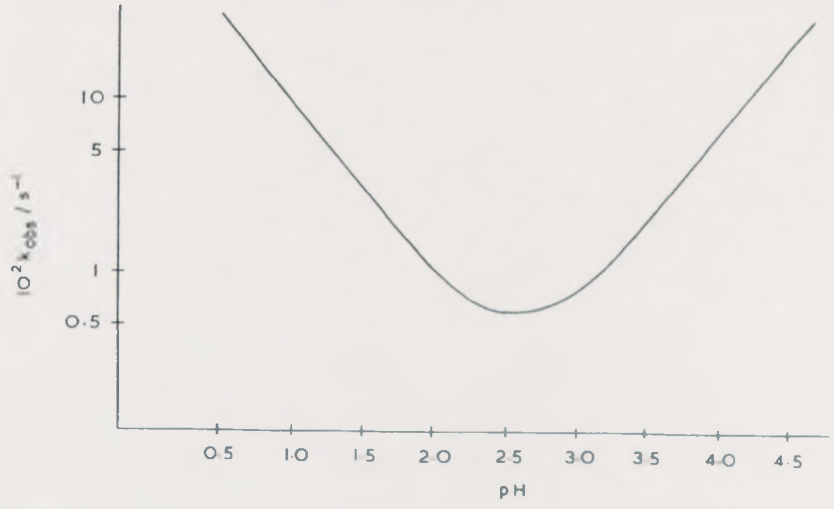


Fig.1.2.8. The variation of rate constant for the dissociation of the hydroxysulphonate adduct of *p*-methoxyacetophenone with pH at 25°C (Young and Jencks, 1977). Reproduced with permission from *J. Amer. Chem. Soc.*, 1977, **99**, page 1208. © 1977 American Chemical Society.

the adduct with p-methoxyacetophenone. Thus, it is evident that the reaction of sulphur(IV) oxospecies with carbonyl compounds shows three types of behaviour according to pH. Below pH 2 the adducts become increasingly labile with decreasing pH, both in terms of equilibrium constant and kinetics. In the approximate range pH 3-6 the complexes have pH-independent stability constants but become increasingly labile with rising pH. Above pH 6 adduct dissociation is favoured on the grounds of both equilibrium constant and rate of dissociation.

The initial step of hydroxysulphonate formation involves attack by the nucleophile at the partially charged carbon atom:

Protonation at either or both negatively charged positions can then occur (Young and Jencks, 1977). Thus, in the pH range 2-7, such protonation leads exclusively to the product, $R_2C(OH).SO_3^-$. Kokesh and Hall (1975) calculated, as follows, the constants for the reactions of HSO_3^- and SO_3^{2-} with benzaldehyde in order to gain some insight into the pH dependence of the overall equilibrium constant:

$$K_{obs} = K_1((1 + K_{a2} / [H^+]) / (1 + K_{a1} / [H^+]))$$

for the series of equations shown in Fig.1.2.9. K_{obs} adequately describes the data between pH 2-12, when $K_1 = 2.4 \times 10^{-7}$ M, $K_2 = 0.912$ M and $K_{a2} = 2.11 \times 10^{-11}$ M, for an ionic strength of 1.0 M at 21 ^oC.

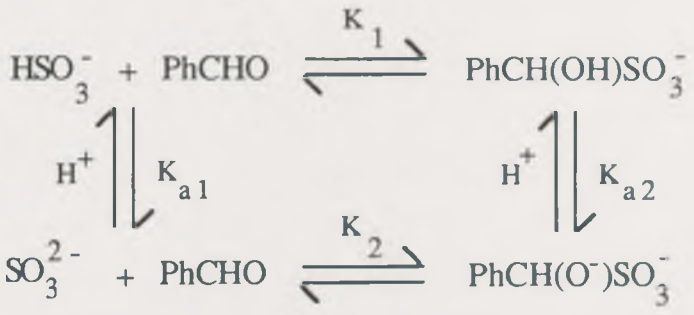


Fig.1.2.9. The reactions of HSO_3^- and SO_3^{2-} with benzaldehyde.

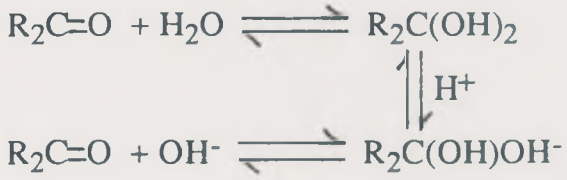
Thus, when $[H^+] > K_{a1}$ (i.e. pH < 6),

$$K_{obs} = K_1(1 + K_{a2} / [H^+]) \approx K_1$$

When the pH < 10 and the denominator of the expression can no longer be ignored, the variation in observed equilibrium constant becomes,

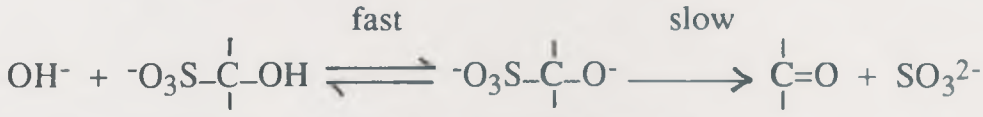
$$K_{obs} = K_1 / (1 + K_{a1} / [H^+])$$

The complete equation is applicable above pH 10. Below pH 2, $SO_2.H_2O$ formation will occur; as SO_2 does not behave as a nucleophile its formation may result in an apparent weakening of the adduct. It has been shown (Green and Hine, 1974; Hine *et al.*, 1976) that the only other reaction which may compete with hydroxysulphonate formation is the addition of water or hydroxide ion to the carbonyl group:



The equilibrium constant of hydration of 1,3-dimethoxyacetone is 0.4 at 25 °C and the pK_a for the adduct is 13.2.

In order to understand the kinetic behaviour of the decomposition of hydroxysulphonates, it is necessary to identify the rate-determining step as the expulsion of the sulphite ion, that is;



It was shown by Young and Jencks (1977) that in the case of

p-methoxyacetophenone, the rate constant for the slow step is at least an order of magnitude less than that for the loss of H^+; the ratio of the two constants is of the order of 100 for p-methoxy-, p-chloro- and p-nitrobenzaldehydes. Since the overall rate of reaction depends on the concentration of intermediate, the observed inverse dependence on $[H^+]$ may readily be explained. One important feature of this reaction is that of general base catalysis by both nitrogen and oxygen-containing bases, which give a Bronstead β value of 0.94 ± 0.05 for both p-methoxyacetophenone and p-methoxybenzaldehyde. The implication of such a high β value is that proton transfer to the base is almost complete in the transition state and, therefore, it is possible to write the following equation:

$$B + R_1HC(OH)SO_3^- \rightleftharpoons BH^+ .[R_1HC(O)SO_3]^{2-} \rightleftharpoons \text{products}$$

1.2.7. Reactions of S(IV) oxospecies with carbon-carbon double bonds.

The reaction of sulphur(IV) oxospecies with carbon-carbon double bonds may proceed by both heterolytic and homolytic routes, depending on the nature of the unsaturated compound. In the case of addition to α,β-unsaturated compounds in which the double bond is attached to an electron-withdrawing group, a simple heterolytic mechanism is observed. The electron-withdrawing groups most commonly found in conjugation with carbon-carbon double bonds in food components are aldehyde, keto, carboxylic acid and ester. In reactions with a nucleophile, shown in Fig.1.2.10, the following sequence of events is required (Sykes, 1965):

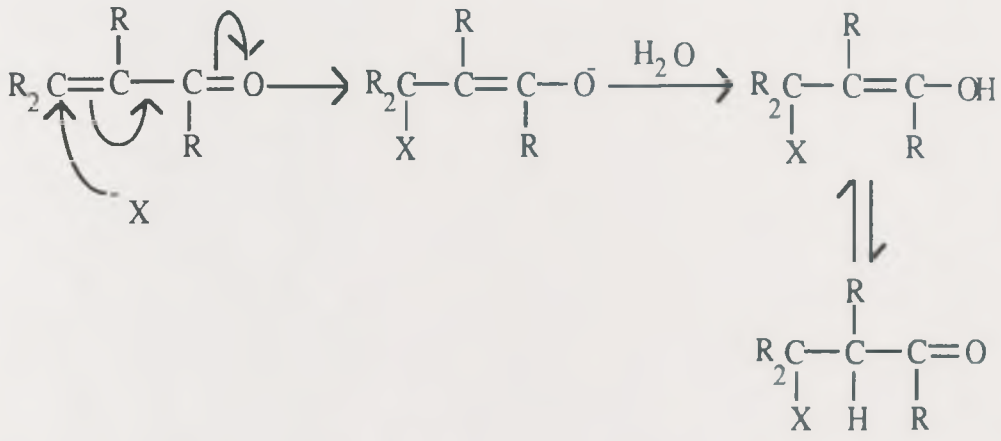
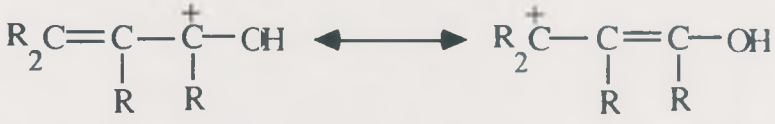


Fig.1.2.10. Nucleophilic attack on an α,β-unsaturated compound.

In the case of a poor nucleophile, prior activation by protonation of the oxygen is required to give a carbonium ion of the type,



In each case the product is the result of overall 1,4-addition. The reaction occurs readily with ketones, acids and esters, but the carbonyl carbon atom of aldehydes is sufficiently positive to allow direct attack leading to 1,2-addition.

When the nucleophile is a sulphur(IV) oxoanion, the reaction is found to proceed over a wide range of pH. As the concentration of S(IV) species is pH dependent, changes in concentration are expected to contribute to the observed pH dependence of the rate of reaction. The most powerful nucleophile is expected to be the sulphite ion on account of its high charge on the exposed sulphur atom. From studies on the rate of addition of sulphur(IV) oxoanions to acrylonitrile it is evident that the reaction is favoured as pH is increased above 7, a maximum rate occurring about pH 9. Since there are no changes to acrylonitrile in this pH range, the results suggest that the effective sulphonating agent is indeed, SO_3^{2-} (Morozov *et al.*, 1972). A

reduction in rate at a pH > 9 was attributed to the alkaline hydrolysis of the nitrile.

1.3. Analytical.

1.3.1. Methods for following the Maillard reaction.

Maillard browning has been followed by several analytical methods, the first and most obvious of these being simple measurement of the absorbance due to coloured products. This has been performed in model systems by McWeeny and Burton (1963) and in a real food situation by Choi *et al* (1949), who used enzyme digested filtrates. However, during the Maillard reaction many coloured products are formed. Thus, at a single wavelength the absorbance may be due to many spectra overlapping as shown in Fig.1.3.1:

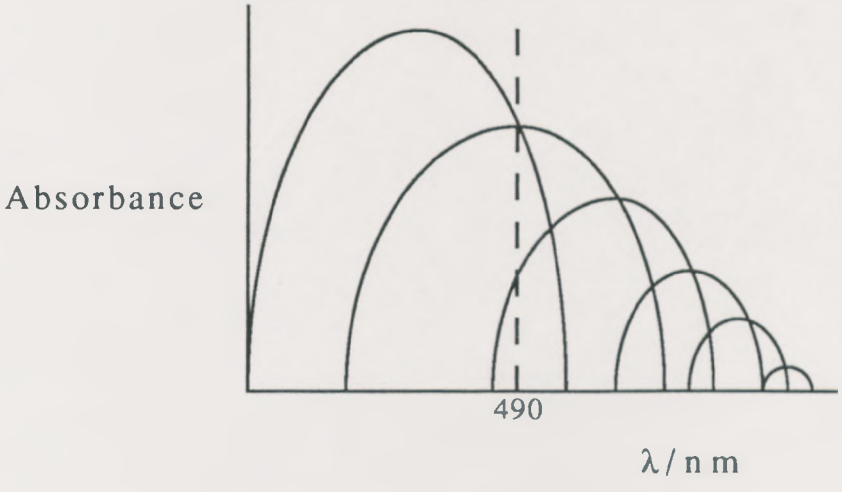


Fig.1.3.1. A diagram showing the the overlap of absorbance spectra of coloured products formed during the Maillard reaction.

Therefore, contributions from many products rather than the accumulation of one will be measured. Also, the solubility of the products, melanoidins, decreases with increasing reaction time

(Reynolds, 1965). Hence, any precipitated products would be unlikely to contribute to a measurement of absorbance. McWeeny (1981) showed that during Maillard browning the colour of the products changes. Therefore, use of a single wavelength absorbance measurement may not accurately measure the rate throughout the entire reaction or be representative of the reaction as a whole. In some systems such as milk, if the heating conditions are not too drastic or the reaction time very prolonged the reaction stops practically at the ketoseamine; such uncoloured products will not be measured at all by such a method. Finally, colour production and measurement occurs after many consecutive reactions and little information as to the early stages of the reaction may be gained from such a measurement. Any deductions made on data produced at the end of the reaction may not accurately extrapolate back to the early stages. Petriella *et al* (1985) used tristimulus colorimetry to get a better measure of "colour". This involves varying the intensity of three different lights of fixed wavelength, untill the combination of these colours matches the unknown colour. The intensities of the lights of fixed wavelength required to give a match being a measure of the unknown colour. However, the method is rather insensitive and it is difficult to get agreement between matches produced by different observers (Judd and Wyszecki, 1963). In addition, the points raised for single wavelength measurements are still applicable. Lea and Hannan (1949), Tarr (1954) and Arnold (1973) used reflectance readings as an indicator of the degree of browning. As this is equivalent to measuring absorbance, the arguments used above also apply to these works.

The accumulation of hydroxymethyl furfural (HMF) or furfural has been used by some investigators as an indicator of the extent of

non-enzymic browning (Reynolds, 1963). However, McWeeny and Burton (1963) concluded that, except in strongly acidic conditions, HMF is a reaction by-product which accumulates in detectable amounts only because of its relatively low reactivity in browning reactions.

Cole (1967) monitored CO_2 evolution as an indicator of the extent of Maillard browning. He concluded that although the Strecker degradation may be the main source of CO_2 produced during the Maillard reaction, it is not the only pathway by which CO_2 may be evolved. If the following of a single pathway is to be used as a measure of progress of the overall complex of reactions, then the relative extent to which each pathway in the reaction occurs must stay constant or the way in which it changes must be known.

Eichner (1975) as well as many others have used carbonyl accumulation as an index. The problem here is that a maximum concentration is reached since the reducing compounds, primarily Amadori rearrangement products, participate in further reactions.

A wide variety of flavour compounds have been used as an index of the Maillard reaction. The Strecker degradation reaction is a source of characteristic browning flavours (Reynolds, 1965). Schonberg and Moubacher (1952) have shown that the type of organic group reacting with the amino acids during the Strecker reaction, determines what type of end-product will be produced. Reynolds (1965) investigated some of the flavour compounds associated with cooked potatoes, soy sauce, bread, cooked milk and meat. Also Markova *et al* (1972) have studied some of the flavour changes associated with cereal products that were subjected to non-enzymic browning. However, the response to flavours differs from person to person and also such an approach is non-quantitative.

Another very important means of monitoring the non-enzymic browning reaction is by observing the protein nutritional changes that occur in the food product after processing and storage. As the free amino-N groups react, they can become bound to the brown pigments and cause the nutritional availability of the protein to decrease. In particular, the N-terminal group of peptides and the ε-amino groups of lysine react and become nutritionally unavailable.

The method of studying the Maillard reaction employed in this work involves the use of S(IV) as a marker for the reaction. Almost all the known mechanisms whereby S(IV) inhibit the consequences of Maillard reactions involve pathways connected with 3-deoxyhexosulose (DH). Kinetic evidence suggests that DH is the main precursor in pigment formation and that the colour-forming reaction departs from the sequence:

3-deoxyosulose $\longrightarrow$ 3,4-dideoxyosulos-3-ene $\longrightarrow$ 2-furaldehyde

before reaching the furan compound (Reynolds, 1965). The latter conclusion is based upon the relative low reactivity of hydroxymethyl furfural (HMF) towards browning when compared with difructoseglycine or DH (Anet, 1958, 1959a, b; McWeeny and Burton, 1963) and is supported by the observation of Yoshihiro *et al* (1961) that the rate of the glucose-glycine reaction is not markedly accelerated by the addition of HMF. In the reaction of glucose and glycine, hydrogen sulphite ion binds irreversibly with 3,4-dideoxyhexosulos-3-ene (DDH). While S(IV) is present the reaction will continue in this way and browning will be prevented. Hence, by measuring the rate of loss of the preservative, it is possible to measure the rate of production of the intermediates that would go on to produce browning and flavour if S(IV) were not present. Measurement of the Maillard reaction by this method has a number of

advantages. The early stages of the reaction can be studied, enabling the rate of production of uncoloured intermediates to be measured. As DH is the main pigment precursor, the obtained rate of reaction, when S(IV) is present, is more representative of the Maillard reaction than when, for example, absorbance measurements at a single wavelength are used. This is because Wedzicha and Garner (1991) showed that in glucose-glycine-S(IV) reaction mixtures, DH and DSH were the main intermediates formed in the presence of S(IV). However, once all the S(IV) has been lost, other more reactive intermediates may be formed. Measurement of S(IV) concentration is a direct and specific method of analysis, whereas absorbance measurements do not measure a single product. Typically, the concentration of S(IV) in a glucose-glycine-S(IV) reaction is much smaller than that of the other reactants. Thus, the concentration of S(IV) falls from 100% to zero, affording good sensitivity to the method. Finally, as S(IV) binding is an indication of the occurrence of two processes in the Maillard reaction, the production of colour precursors such as DH and DDH and the presence of carbonyl groups; a more detailed description of the reaction is produced, enabling a superior model of the reaction to be obtained. Therefore, the number of combinations of the rate and equilibrium constants in a model for the reaction which give a good fit for both of these processes, is likely to be less than the number of such combinations that could be obtained if a single parameter, say HMF concentration, were used as a measure of fit.

1.3.2. Iodimetric determination of S(IV) oxospecies in aqueous solution.

Sulphite ion reacts with iodine according to the following equation,

$$I_2 + H_2O + SO_3^{2-} \longrightarrow 2I^- + SO_4^{2-} + 2H^+$$

this reaction forming the basis on which aqueous solutions of S(IV) oxospecies are standardised. The product of this oxidation reaction, which proceeds rapidly in aqueous solution, is almost exclusively sulphate ion, a small amount of dithionite ion (of the order of 0.5%) also being formed (Basset and Henry, 1935). From electrochemical measurements, the pH dependence of second order rate constants for the reaction show that sulphite ion reacts with iodine an order of magnitude faster than does HSO_3^-, and that the species $SO_2 \cdot H_2O$ is relatively unreactive (Bünau and Eigen, 1962). Increasing iodide concentration reduces the reaction rate, the rate constant for the reaction with I_3^- (2.1×10^5 M^{-1} S^{-1}) being three orders of magnitude less than that for the reaction with molecular iodine (1.7×10^8 M^{-1} S^{-1}).

The reaction is normally carried out in acid solution, the main sources of error apparently lying in the oxidation of S(IV) by air, and in the loss of sulphur dioxide gas from the solution during titration when iodine is added from a burette (Mason and Walsh, 1982a, b). These difficulties may be overcome if the unknown solution of S(IV) oxoanions is added to an excess of iodine, thereby leading to rapid oxidation of the whole of the sample, the excess iodine being determined by titration with standard thiosulphate solution. This procedure constitutes the normally accepted technique for the standardisation of solutions of S(IV) oxoanions (Vogel, 1961), with the additional recommendation that dilute solutions are used. The

incorporation of antioxidants is desirable if the standardisation is to be meaningful and if the solutions are to be stable for a significant period of time. Possible additives include mannitol, sucrose and alcohol (Kolthoff *et al.*, 1957), glycerol (Urone and Boggs, 1951) or a small quantity of EDTA (Humphrey *et al.*, 1970). The use of EDTA to complex metal ions capable of catalysing oxidation appears to be particularly effective. This is because there was no detectable loss of sulphite from a solution containing 1 mM sodium sulphite and an equal amount of EDTA after one week at room temperature. After 2 weeks the loss from this solution was less than 5%, after 3 weeks 10% and approximately15% loss after 4 weeks. The sulphite solutions in EDTA are also quite stable to aeration. No loss was detected after passing air at a rate of 250 ml per minute through a 1 mM sulphite solution for 24 h.

If other reducing agents are present in solution with S(IV) oxoanions, it is still possible to determine the amount of S(IV) in the mixture, providing the reducing power of these interfering substances is known. A simple method, which allows for the presence of other reducing agents, is the conversion of the S(IV) oxoanions to the hydroxysulphonate of formaldehyde or acetone, both of which, at acid pH, are relatively stable and do not react with iodine. Thus, the S(IV) content may be found by comparison of the reducing power towards iodine before and after treatment with the carbonyl compound (Kolthoff *et al.*, 1957).

The apparent lack of reaction between the hydroxysulphonate adduct and iodine has led to the use of iodimetric methods for the study of carbonyl-S(IV) equilibria and the rates of formation or dissociation of hydroxysulphonates. For equilibrium studies, reaction mixtures are 'quenched' by the rapid addition of acid. At low pH the

rate of decomposition of the adducts is sufficiently slow, on the timescale of the titration, to permit reliable determination of the composition of the equilibrium mixtures and the results are consistent with those obtained by spectrophotometric determination of the aldehyde compound in reaction mixtures (Green and Hine, 1974). If necessary, the S(IV) combined in the form of hydroxysulphonate may be estimated by subsequently raising the pH of the reaction mixture to approximately 10, allowing the adducts to decompose, and titrating the mixture after 'quenching' in acid.

Contrary to the normally accepted recommendation that S(IV) oxoanion solutions be added to an excess of iodine for standardisation, the titration of S(IV) in foods involves the addition of iodine from a burette, the only suggestion to combat losses being that of rapid addition. The procedures used for liquid samples generally give the free and combined sulphur dioxide levels separately, and may be divided into two groups according to whether both determinations are carried out on a single sample or whether two samples are used. The accuracy of the determination of any reversibly bound S(IV) depends upon the quantitative decomposition of any hydroxysulphonate-type adducts, these tending to reform to some extent when the pH is lowered. The yield of reversibly bound S(IV) may be improved by use of a two-step decomposition procedure, in which alkaline decomposition and determination of liberated S(IV) is repeated after the normal determination of reversibly bound S(IV). The improvement in analysis of total S(IV) by the introduction of a second decomposition step is illustrated by Jaulmes and Hamelle (1961), for the analysis of wine samples, as follows:

Simple decomposition	Two-step decomposition
437	470
381	422
355	455
367	516
390	430

where amounts of S(IV) are given in units of parts per million in the original wine sample and each pair of results refers to a separate sample. Prolonged alkaline treatment may, however, lead to changes in the components of wines which change their S(IV) oxoanion binding capacity (Lay, 1970, 1971).

1.4. Inhibition of Maillard browning by S(IV) oxospecies.

1.4.1. Initial construction of an experimental model system for the S(IV)-inhibited Maillard reaction.

1.4.1.1. Introduction.

The mechanism of Maillard browning is reviewed in section 1.1. The most common method of inhibiting such browning involves use of sulphur(IV) oxospecies, the presence of the additive thus completing the model system.

Table 1.4.1 gives the composition of some model Maillard browning systems used by previous workers. It is seen that a model system commonly used in the study of Maillard browning is that of glucose and glycine.

1.4.1.2. Selection of a reducing sugar.

Glucose and fructose are the most common and abundant

System	[aldose] (M) [amine] (M)	pH or buffer	T (^{o}C)	Purpose	Reference
Glucose -glycine	0.1-1 0.1-0.5	2-8	50-90	kinetics of browning	Song *et al.*, 1966 Nam & Kim, 1984
	equimolar 0.4, 1.0 2.0	6.8	95	Characterisation of melanoidins	Kim & Park, 1986 Kato *et al.*, 1986/7 Kim *et al.*, 1988 Hayase *et al.*, 1984
	1.67 1.0*		100- 125	products and composition	Maillard, 1912/16
	2.0	6.8	reflux	physiological effects of melanoidins	Fujimaki *et al.*, 1979
	variable		50- 110	Rate of formation of melanoidins	Obretenov *et al.*, 1986
	0.5-2.0 0.25-1.0	5.5	55	Kinetics of inhibition of browning	McWeeny *et al.*, 1969 Wedzicha & Vacalis, 1988
Glucose -glycine or alanine	1.5 1.5		90	kinetics of browning and characterisation of melanoidins	Taguchi & Sampei, 1986
Glucose -glycine or methionine	0.2, 1.0 0.2, 1.0 equimolar	3.5 phthlate	reflux	characterisation of melanoidins	Feather & Nelson, 1984 Feather & Huang, 1985

Table 1.4.1. The composition of some model Maillard browning systems used by previous workers.

Glucose -lysine	0.33 0.054	4-8 methanol	90-110	Kinetics of browning	Lee *et al.*, 1984
	0.6	7.9	100	Physiological effects of melanoidins	Takeuchi *et al.*, 1987
Glucose-arginine or histidine	1.0 2.0	3-11	80-110	Antimicrobial compounds	Einarsson, 1987a, b
Glucose or fructose -glycine	0.5 0.5	5.5	100	Kinetics and mechanism of browning	Kato *et al.*, 1969
Glucose or fructose -glycine	ratio 2.5:1.0	5.0	50	Inhibition of browning	Burton *et al.*, 1962
Glucose, fructose, or HMF -glycine	0.2 0.2	3.5 phthalate	reflux	Characterisation of melanoidins	Feather & Nelson, 1984
Glucose or xylose -butylamine or ammonia		5.2-6.5 water or methanol	100	Characterisation of melanoidins	Kato & Tsuchida, 1981
Glucose or xylose -butylamine, glycine or ammonia	2.0 2.0	5.2-6.5 water or methanol	100	Reductone-content of melanoidins	Kato *et al.*, 1968

Table 1.4.1. Continued.

Xylose -glycine	0.2 0.2	5.0 acetate	100	Separation and characterisation melanoidins	Motai, 1974 Motai & Inoue, 1974
	2.0 2.0	6.5	90	Reductone-content of melanoidins	Gomyo *et al.*, 1972
	0.8 0.8	acetate	90-100	Antioxidant activity of products	Kirigaya *et al.*,
	1.0 1.0	8.2 phosphate	reflux	Isolation of coloured product	Nursten & O'Reilly, 1986
Xylose or Arabinose -glycine, alanine or urea	1.0 1.0		22, 68 100	Characterisation of melanoidins	Benzing Purdie *et al.*, 1983 Benzing Purdie & Ratcliffe, 1986
Various	1.0-1.25 0.69	3-9	50	Rate of browning	Spark, 1969
Various	variable	6-7.2	65, 100	Rate of browning	Wolfrom *et al.*, 1974
Various	variable	alkaline	hot	Characterisation of melanoidins	Rubinsztain *et al.*, 1984

Table 1.4.1. Continued.

reducing sugars in cabbage, carrot, potato, brussels sprout and swede (Paul and Southgate, 1978), these being vegetables to which sulphite is added to inhibit non-enzymic browning during dehydration. Although the presence of other sugars may be relevant, their relative concentrations are too small (Souci *et al.*, 1981) to make a significant impact on the overall rate of reaction between S(IV) and extracts of the vegetables above (Adamu, 1986). Table 1.4.2 gives the glucose and fructose contents of vegetable extracts. From table 1.4.2 it can be seen that the sugars are present in relatively similar amounts, but that glucose usually predominates. Fructose acts as a reducing sugar, as it is readily isomerised to an aldose by a series of keto-enol tautomeric shifts. Therefore, selection of glucose as the sugar for the model system is advantageous, because it does not

Vegetable	**----Concentration g/100 g of vegetable----**					
	----A----		**-----B----**		**---------C----------**	
	gluc	**fruc**	**gluc**	**fruc**	**gluc**	**fruc**
Cabbage	17.8	27.2	22.9	19.3	22.4 ± 0.17	17.5 ± 0.21
Carrot	17.9	16.1	9.4	9.4	13.0 ± 0.18	11.3 ± 0.12
Potato	2.0	1.5	1.7	1.0	0.8 ± 0.03	0.6 ± 0.42
Brussel sprouts	9.7	8.8	7.3	8.3	6.0 ± 0.26	5.1 ± 0.09
Swede	16.7	13.0	16.7	13.1	29.9 ± 0.39	19.2 ± 0.11

Table 1.4.2. The glucose and fructose contents of various vegetables. A: Winton and Winton (1935). B: Souci *et al.*, (1981). C: Adamu (1986), where 4 extracts were analysed and the results corrected to the amount present in the original dried vegetable at 9% moisture content. gluc = glucose, fruc = fructose.

undergo such isomerisation and study of its kinetics may be easier.

1.4.1.3. Selection of an amino acid.

Glycine is the simplest amino acid as its side group is only a hydrogen atom. Hence, the Maillard reaction of glucose with glycine will involve a carbonyl and an α-amino group. Unless glycine is present in the free form or at the N-terminus of a peptide or protein it is unlikely to participate in Maillard browning in a food; otherwise its amino group will be bound as part of the peptide bond. In this respect an amino acid such as lysine is a more realistic choice as far as Maillard browning in a food is concerned. Lysine contains two amino groups, which may differ in their reactivity, the reaction may be far more complicated; so much so that the kinetics may be unclear. By studying the glucose-glycine system a description of this relatively simple reaction may be obtained and this may be subsequently developed to describe more complex Maillard reactions.

1.4.1.4. Selection of reagent concentrations.

To prevent Maillard browning during the dehydration of cabbage, a residual S(IV) concentration of 2500 ppm is allowed in the final product. If it is assumed that the solids content of fresh and dried cabbage are approximately 10 and 95% w/w respectively, then the mass of solids in 1 kg of fresh cabbage is given by:

$$\text{Mass of solids} = 0.10 \times 1000 = 100 \text{ g}$$

The mass of solids in 1 kg of dehydrated cabbage is given by:

$$\text{Mass of solids} = 0.95 \times 1000 = 950 \text{ g}$$

Therefore, as 1 kg of fresh cabbage contains 100 g of solids, we would require 9.5 kg of fresh cabbage to give 950 g of solids in the dehydrated cabbage. The mass of water contained in 9.5 kg of fresh cabbage is given by:

Mass of water = 0.90 x 9500 = 8550 g

The mass of water contained in 1 kg of dehydrated cabbage is given by:

Mass of water = 0.05 x 1000 = 50 g

Assuming that 1 kg of dehydrated cabbage contains 2500 ppm of SO_2 and that all the preservative is present in the aqueous component. When 9.5 kg of fresh cabbage is dehydrated to give us 1 kg of dehydrated cabbage, the concentration of S(IV) increases from 0.005 M to 0.8 M or even greater. If some of the S(IV) crystallises, then much greater local concentrations are likely. This assumes that all water is capable of acting as a solvent and that all the S(IV) is associated with the water. Dehydrated cabbage contains 15-25% glucose (Wedzicha and McWeeny, 1974), in terms of solids content. Thus, 1 kg of dehydrated cabbage would contain in the region of 0.8-1.4 mol of glucose, and the molar ratio of glucose:S(IV) in the dehydrated product would be in the range 1:0.05 to 1:0.03. The accurate modelling of the S(IV)-inhibited Maillard reaction occurring in dehydrated cabbage requires the concentrations to be within the stated ratio, use of glucose and S(IV) concentrations of 1.0 and 0.04 M respectively satisfies this requirement. Hayon *et al* (1972) stated that chain oxidation of sulphite ions in solution can occur, but, Sapotnikskii and Glushchenko (1962) showed that glucose can inhibit this process. Therefore, a relatively high glucose concentration will serve to guard against possible S(IV) autoxidation. On the other hand, the ratio of concentration of the reagents could become unrealistic if the concentrations are to high; other variables start to become important. For example, the limit of solubility for glucose may be exceeded causing the system to become nonhomogeneous, the viscosity will increase, which may effect the mobility of the reactants

and increase sample handling problems. The water activity may be significantly reduced, leading to an acceleration in reaction rate. When initially studying the reaction it is desirable that the model system is relatively simple. A compromise is reached and a range of concentrations is chosen that does not introduce too many variables into the model at an early stage.

1.4.1.5. pH selection.

Consider the pH of cabbage at a number of stages during its conversion to the dehydrated product, i.e.

Food (Heterogeneous) ⟶ Blanched material (Less heterogeneous) ⟶ Drainage ⟶ Drying

The initial food sample

Fig. 1.4.1 shows the cross-sectional structure of a foliage leaf.

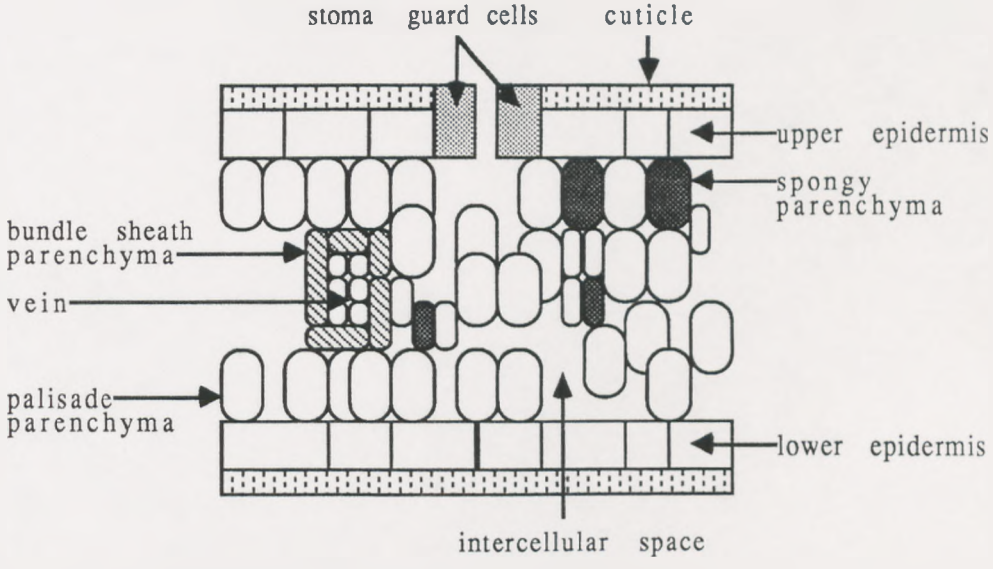


Fig. 1.4.1. Cross-sectional diagram of a foliage leaf.

In Fig. 1.4.1 we see three principal tissues: (a) epidermis, (b)

mesophyl, (c) veins or vascular bundles. The epidermis usually consists of a single layer of cells that covers the entire leaf surface, protecting the tissues within the leaf from drying out and mechanical injury. The mesophyll is composed of parenchyma cells, most of which contain chloroplasts and thus are able to carry out photosynthesis. Veins transmit water, inorganic salts, and "nutrients" (Weir *et al*, 1982). It may be that each of these types of tissue has a different pH. If the food was homogenised at this stage a measurement of pH could be made.

The blanched food.

If two samples of fresh cabbage were blanched, chopped and then boiled, separately using the same liquor, it is unlikely that all the microenvironments of the food will be equally disrupted and hence that the pH-influencing components will not diffuse to exactly the same extent. Measurement of the pH of the blanching liquors could yield differing values. If liquors of different ages were used, then this would have an affect on the value of pH obtained. Because, a 'new' liquor would leach out a different proportion of the soluble matter in the food compared to an 'old' liquor, as an 'old' liquor already has an appreciable solids content. Furthermore, browning reactions taking place in the liquor would alter its pH. Values of pH obtained after blanching would probably differ from those obtained after simple cold homogenisation of fresh cabbage, because of the differing extents to which leaching of water extractable components occurs in these two processes and also because of browning reactions occurring in blanch liquor.

Drainage and drying

If the cabbage is processed further after blanching, it is likely that any subsequent measurement of pH will yield a value different from those obtained above. For example, instead of measuring the blanch liquor pH, this liquor could be discarded and the pH of the drained cabbage obtained after homogenisation. However, the amount of water extractable components remaining in the food, will be dependent on the extent of leaching of these components during blanching. If the food is drained these components will be lost and will have no influence on any pH measurement subsequently made.

Acid is produced during the early stages of the Maillard reaction, prior to colour formation. In addition, the products of Maillard browning, melanoidins, are also acidic. If the drained sample is dried, the a_w of the food will fall as water is lost from the sample. As the rate constants for the Maillard reaction have been shown to reach a maxima in the a_w range 0.3-0.7 (Eichner, 1975), the actual pH of the cabbage is likely to change as the a_w falls. Hydrogen ion activity, a_{H^+}, is related to hydrogen ion concentration in the following manner:

$$a_{H^+} = \gamma [H^+]$$

γ is the activity coefficient of H^+. As the a_w falls the food will become more concentrated, reducing the value of the activity coefficients. The activity of hydrogen ions and, therefore, the actual pH of the food will change.

It appears that the measured pH of cabbage is dependent on both the actual pH of the food and preparation of the sample for pH measurement. The measured pH is probably related to the actual pH in the microenvironments of the food in a complex way and is at best an overall measure of the contribution of each of these

microenvironments. It was shown by Wolfrom *et al* (1953), that the rate of the xylose-glycine reaction is independent of pH in the range expected for foods (pH 3-7). It is convenient, therefore, to study the glucose-glycine-S(IV) reaction at pH 5.5, because if the initial pH of reaction mixtures varies within the stated range, this should have no effect on the observed reaction rate.

1.4.1.6. Selection of a temperature.

Study of the Maillard reaction at 55 °C is advantageous, as at this temperature the Strecker degradation and caramelisation are said to be slow. In addition, the combination of this temperature and reagent concentrations of [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.05 M enables the S(IV)-inhibited Maillard reaction to proceed at a slow but conveniently measurable rate.

1.4.2. General trends of behaviour.

When present in food, the additive may be found in three chemical forms which are distinguishable by the analytical methods available for the determination of S(IV):

1. Free S(IV). Gaseous or aqueous SO_2 or the ionic forms HSO_3^-, SO_3^{2-}, $S_2O_5^{2-}$.
2. Reversibly bound S(IV). Sulphur(IV) in reversible combination as hydroxysulphonate adducts with carbonylic constituents of food. These may be released during Monier-Williams distillation analysis (Monier-Williams, 1927) or by raising the pH.
3. Irreversibly bound S(IV). Sulphur(IV) that is not recoverable by Monier-Williams analysis, for example as SO_4^{2-}, C-sulphonates and S-sulphonates. S-Sulphonate production by cleavage of disulphide bonds is reversible, but during the distillation analysis procedure

the products are hydrolysed by the acid to thiol and HSO_4^-.

When compared with a system containing no S(IV), the time required for browning to become apparent in a mixture of aldose and amino acid is extended. During the period of inhibition of browning, a proportion of the additive becomes irreversibly bound. The relationship between the amounts of free, reversibly bound and total (that is, free + reversibly bound) S(IV) oxoanion and the development of colour is illustrated in Fig.1.4.2 (McWeeny *et al.*, 1969). These concentration data were obtained by iodine titration and the development of colour determined as absorbance at 490 nm for reaction mixtures containing D-glucose (1 M), glycine (0.5 M) and hydrogen sulphite ion (0.039 M) at pH 5.5 and 55 ^oC. In the early stages of the reaction the rate of irreversible loss of S(IV) follows the amount of free additive in solution, the amount of reversibly bound additive remaining relatively constant. If it is assumed that the reversible binding is a result of the formation of the hydroxysulphonate type adducts between carbonyl groups and S(IV), then the stability of these adducts increases with time. This could be interpreted as the formation of carbonyl compounds which bind the additive more strongly than do the reactants. Consequently, the amount of free S(IV) present in the mixture in the later stages of the reaction is small, and it is apparent also that the rates of loss of reversibly bound additive and of total recoverable additive become similar. It is reasonable to assume that irreversible combination of S(IV) is a result of reactions between the free additive and intermediates in the browning reaction. The commencement of colour development coincides with that of the decomposition of labile products at around 800 h. This dissociation of the adducts is presumably a simple consequence of the depletion of free S(IV). The

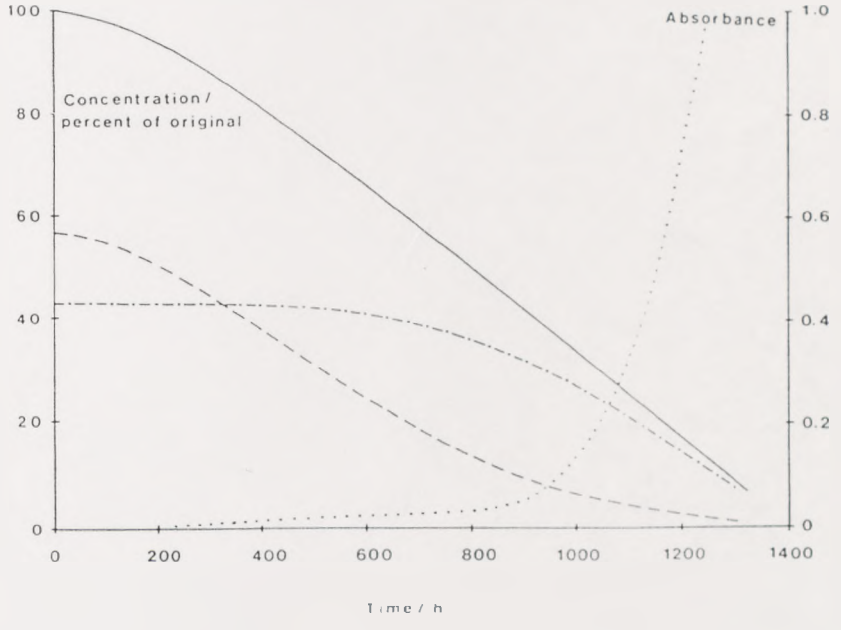


Fig.1.4.2. Concentration time behaviour of total, reversibly bound and irreversibly bound sulphur(IV) oxoanion during the inhibited reaction of glucose and glycine. Absorbance data superimposed. Reaction conditions: [glucose] = 1 M; [glycine] = 0·5 M; [S(IV)] = 0·039 M; pH 5·5; 55 °C. Graphs plotted from the data published by McWeeny and coworkers (1969) to reproduce figure shown by these authors. Total S(IV), ——; free S(IV), - - - -; reversibly bound S(IV), —·—·; absorbance at 490 nm, ·····.

consequent release of reactive carbonyl compounds allows browning to proceed. It is evident also from the results illustrated in Fig.1.4.2 (McWeeny *et al.*, 1969), that the effect of S(IV) is only to delay colour formation. When mixtures of glucose and glycine are allowed to undergo browning and are then treated subsequently with S(IV) oxoanion, an almost instantaneous reduction in colour is noted, although this reduction is only a fraction of the difference in absorbances between the non-sulphited mixture and that which contained the additive from the begining of the experiment. The colour of melanoidins is, therefore, partially bleached by S(IV). However, subsequent development of colour, in the partially bleached sample, occurs at the same rate as in the sample to which S(IV) had been added from time zero. The additive reacts irreversibly with non-enzymic browning model melanoidins (M_r > 12,000, prepared by combination of 1.25 mol glucose with 1 mol glycine (pH 5.5, 90 °C, 22 h)). When the reaction, with initial S(IV) and melanoidin concentrations of 0.0371 M and 5.71 g l^{-1} respectively, is carried out for > 39 days at 40 °C and pH 5.5, up to one sulphur atom is incorporated for every 2 molecules of glucose used to form the polymer.

1.4.3. Sulphur(IV) oxoanion inhibited Maillard browning

The mechanisms of the inhibition of non-enzymic browning reactions by S(IV) have been reviewed by McWeeny and coworkers (1974), McWeeny (1981) and Wedzicha (1987). The interactions of S(IV) with carbonyl components have been summarised in Fig.1.4.3. These possible reactions can be used to account for the observed general trends described above in the following manner. The rate of loss of additive to form products in which it is reversibly or

irreversibly bound is zero at zero time. In the early stages the

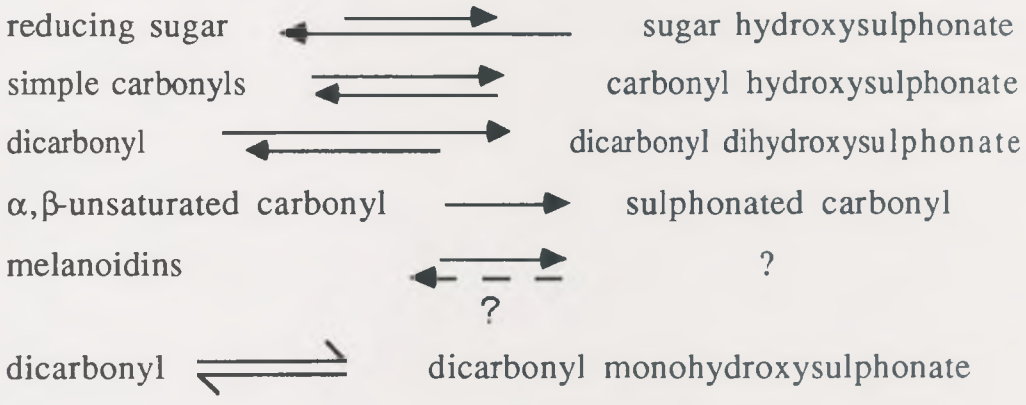


Fig.1.4.3. A summary of the interactions of S(IV) with carbonyl components.

intermediates and products which are potentially capable of reversibly binding S(IV) more strongly than glucose are 3-deoxyhexosulose (DH), 3,4-dideoxyhexosulos-3-ene (DDH) and 3,4-dideoy-4-sulphohexosulose (DSH). The most reactive intermediates towards colour formation are the unsulphonated osuloses, and the inhibition of browning takes place by the effective removal of these compounds. If this inactivation of intermediate is through hydroxysulphonate formation, then the same intermediate will be released once the hydroxysulphonates start to decompose in the later stages of inhibition (Fig.1.4.2). Therefore, as the intermediate in question will be formed during the normal course of reaction and additional intermediate will be present as a result of this release, its concentration at the end of the period of inhibition of browning by S(IV) will be greater than that present during the early stages of the glucose/glycine reaction to which no S(IV) had been added. It would be expected, therefore, that the rate of browning observed once S(IV) has been exhausted would be greater than that in the reaction of glucose and glycine alone. This is found not to be the case. Burton and coworkers (1962) found that the rate of colour

development after the end of inhibition is equal to or slower than that in the uninhibited process and the implication of this result is that the compound or compounds released towards the end of the period of inhibition of browning have a lower reactivity towards colour formation than do the normal intermediates in the Maillard reaction. This observation was used by Wedzicha (1984a) to propose a simple kinetic model to account for the data of McWeeny and coworkers (1969) plotted in Fig.1.4.2. The essential features of this model are the two-step formation of product, in which S(IV) is irreversibly bound, the reaction passing through two rate controlling intermediates and reversible binding of the additive results from combination with both glucose and the final stable sulphonated product. The latter is DSH.

The kinetic model is of the form shown in Fig.1.4.4:

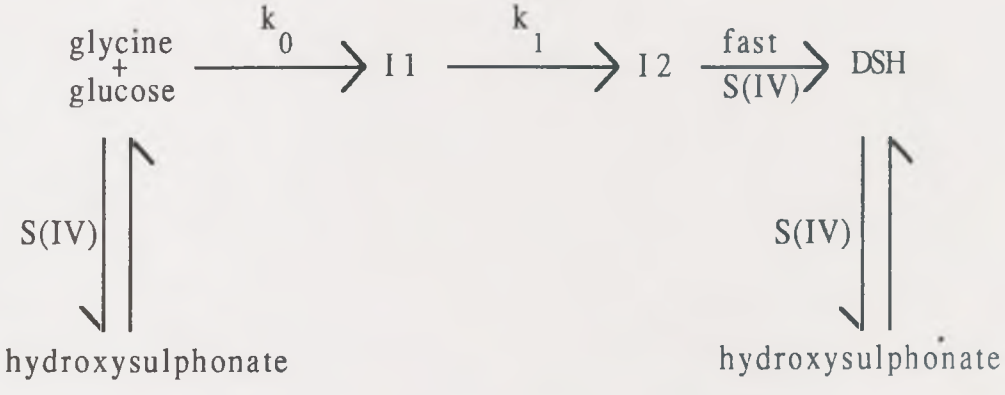


Fig.1.4.4. A kinetic model for the S(IV)-inhibited Maillard reaction (Wedzicha, 1984a).

where I1 is formed at a constant rate during the whole of the period of observation, and formation of I2 is of first order with respect to intermediate 1. Although DSH contains a dicarbonyl function, formation of a dihydroxysulphonate is not favoured (Wedzicha and Smith, 1987). Once all the S(IV) has been lost from the system the intermediates in the normal Maillard reaction, which are more

reactive towards browning than the sulphonated product, will presumably form melanoidins, by-passing the sulphonate. Kinetic studies of the reaction between DH and S(IV) showed that its rate is very similar to that of the step in which I1 is converted to DSH (Wedzicha and Kaban, 1986). It is likely that I1 is DH or some rate determining species derived from it and that the reaction then proceeds by way of DDH to give DSH. The study of the reaction between DH and S(IV) in isolation suggested some additional features which had not been incorporated into the kinetic model of the S(IV)-inhibited Maillard reaction. The most important of these is that DH appeared to form a relatively stable monohydroxysulphonate with a dissociation constant of 0.004 M. This was identified from the observation that the initial reaction of DH with S(IV) was inhibited by S(IV); no such effect could be identified in available data on the glucose-glycine-S(IV) reaction. Another important observation was that glycine appeared to catalyse the reaction between DH and S(IV). It is possible, therefore, that a more complex model applies to the glucose-glycine-S(IV) reaction, perhaps as suggested in Fig.1.4.5.

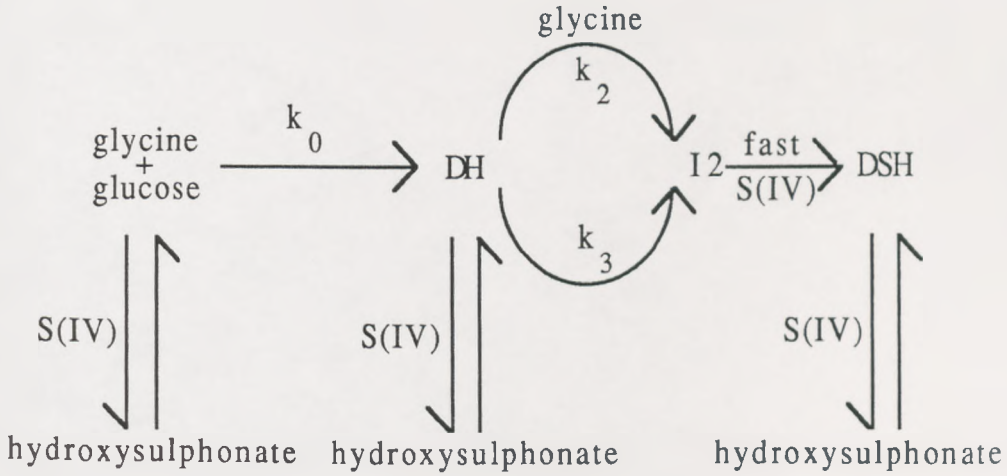


Fig.1.4.5. A kinetic model for the S(IV)-inhibited Maillard reaction (Wedzicha, 1984a).

Wedzicha and Vacalis (1988) found that S(IV) appeared to catalyse the rate of DH formation. A proposed model for the glucose-glycine-S(IV) reaction, that accounts for the first order behaviour of the S(IV)-catalysed Maillard reaction with respect to S(IV) could be as shown in Fig.1.4.6.

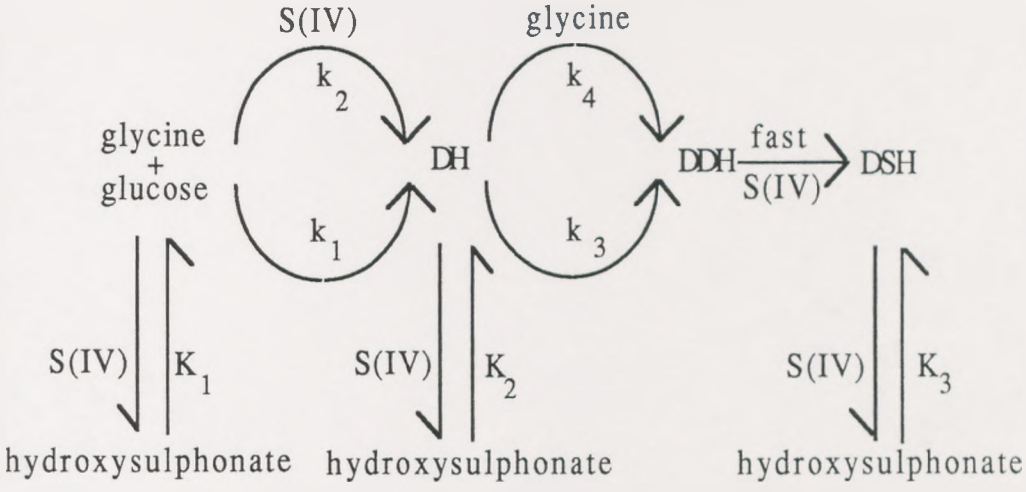


Fig.1.4.6. A kinetic model for the S(IV)-inhibited Maillard reaction (Wedzicha and Vacalis, 1988).

However, it was found that in order to obtain a satisfactory agreement between theoretical data produced using the model and experimental results, DH must not be involved in hydroxysulphonate formation.

1.5. Uses in food.

Sulphur(IV) oxospecies are added to food to inhibit non-enzymic browning, to inhibit enzyme catalysed reactions, to inhibit and control micro-organisms and to act as an antioxidant and a reducing agent (Roberts and McWeeny, 1972). The reasons for which S(IV) oxospecies may be added to foods are summarised in Table 1.5.1, but the appearance of an item in this list does not necessarily imply that the use of the additive is permitted in that application in

Table 1.5.1.

Preservative effect of sulphur(IV) oxoanion in a wide range of foods

Purpose	Inhibition of non-enzymic browning	Inhibition of enzymes	Inhibition of micro-organisms	Antioxidant	Reducing agent	Other
Meat, fish and protein products						
(a) canned, heat processed	√				√	
(b) dehydrated	√			√		
(c) raw and pasteurised			√	√	√	
Vegetable products						
(a) canned, heat processed	√					
(b) dehydrated	√	√[a]				
(c) frozen		√[a]				
(d) raw		√				
(e) pickled	√		√			
Fruit products						
(a) canned, heat processed	√					
(b) dehydrated	√					
(c) frozen		√				
(d) pasteurised	√	√	√			
(e) raw, for manufacturing purposes		√	√			
Beverages						
(a) wine and fruit juices	√	√	√			
(b) beers			√	√		
Starch production		√	√			
Baked products						√
Sugar production	√		√			√

Based on information published by Roberts and McWeeny (1972) with minor changes.

[a] Inhibition of heat stable enzymes if product is blanched.

any given country. Use may also be restricted to certain foods within a certain category. The additive may be applied to solid foods as a solution of one of the salts of 'sulphurous acid' or by fumigation with sulphur dioxide gas, usually obtained directly from burning sulphur. Liquid foods are treated with solid salts of 'sulphurous acid', their solutions or the gaseous additive. The most frequently used sulphiting agents are the disulphites of sodium and potassium on account of there good stability towards autoxidation in the solid phase. Gaseous sulphur dioxide is employed where leaching of solids is likely to be a problem, for simplicity, or where the gas may serve as an acid for the control of pH.

Not all the S(IV) oxospecies found in food is the result of addition, endogenous formation of the species being the consequence of microbial reduction of sulphate ion. Such endogenous S(IV) oxospecies contribute significantly to the total amount found in fermented products. For example, reduction of sulphate may contribute up to 130 ppm sulphur dioxide in large scale fermentation of grape musts (Würdig and Schlotter, 1971).

Thermal processing operations are often effective in reducing the sulphur dioxide content of foods. For example, the boiling of wort in beer manufacture (Thalacker, 1975) and the boiling of sulphited strawberries in the making of jam (McWeeny *et al*, 1980) lead to respective reductions of 94 and 95% of the measurable additive. At lower temperatures desorption of sulphur dioxide may be assisted with a carrier gas, for example in the desulphiting of fruit juices. The relatively simple removal of the additive renders it useful as a possible processing aid with small residues, perhaps the most widely used application in this respect being the manufacture of sugar, during which process the S(IV) oxospecies are removed by

precipitation and boiling in vacuum pans. The wide uses of sulphur dioxide as a processing aid and in ingredients for non-scheduled foods has lead some legislating authorities to specify upper limits of sulphur dioxide in such foods.

1.6. Toxicology of S(IV) and S(IV)-derived reaction products.

The toxicology of S(IV) has been reviewed by Gunnison (1981), Shapiro (1977), the IFT's expert panel on Food Safety and Nutrition (1976) and the Scientific Committee for Food (1981).

Sulphur(IV) oxospecies react with many physiological components of man for example, glucose and other physiological aldehydes and ketones, nicotinamide derivatives, flavin nucleotides, uracil and cytosine and with disulphides of proteins. The toxicological significance of the reversible reactions of S(IV) in the human body is probably slight or nil under conditions of intermittent exposure of body tissue to sulphite.

The irreversible reactions of S(IV) could pose a potentially greater toxicological problem. Metabolic studies (Walker *et al.*, 1983b) showed that DSH is incompletely absorbed from the gastro-intestinal tract of mice and rats after oral administration. 50 to 60% of an intragastrically administered dose was excreted in the faeces of the animal. There is no evidence that DSH is acting as a gastrointestinal irritant unlike sodium disulphite (Til *et al*, 1972a, b). DSH showed no mutagenic or cytotoxic activity in the Ames test, although S(IV) did. Therefore, DSH presents less of a hazard than S(IV) itself (Walker *et al.*, 1983b). It has also been reported that DSH does not liberate sulphur into the body sulphur pool and that the carbon chain in DSH is not degraded to products that can enter pathways of intermediary metabolism (Walker *et al.*, 1983a).

Because of its rapid metabolic clearance by sulphite oxidase, chronically ingested sulphite does not accumulate in the tissues but is rapidly eliminated (Dulak *et al.*, 1984). The only reported case of sulphite-oxidase deficiency disease in a human showed that in the absence of the enzyme extreme brain damage, mental retardation and dislocatation of the occular lenses can occur (Irreverre *et al.*, 1967). However, it is unclear if this was due to S(IV) produced as an intermediate by metabolic pathways, S(IV) inhaled, or that ingested. In addition, anopthalmia, congenital absence of one or both eyes, in rats (Beemer and Delleman, 1980) has been observed. No statistically significant effects on female rats, rabbit and mouse reproductive performances has been detected after exposure to S(IV) and S(IV)-treated diets (Dulak *et al.*, 1984; Murray *et al.*, 1979). Neither teratogenicity nor embryotoxicity attributable to S(IV) exposure was observed.

It has been adequately demonstrated that S(IV), when pre-mixed with food and stored is capable of significant destruction of the dietary thiamine content. Thiamine can also be destroyed in the gut by high concentrations of S(IV), but, there is no evidence for systemic destruction of thiamine in rats, even under conditions of extreme systemic sulphite exposure (Gunnison *et al.*, 1981).

A proportion of the population who suffer from asthma are also unusually sensitive to SO_2 in the headspace above foods. It is alleged that deaths have occurred as a result of such exposure, a possible safe limit being 1 mg m^{-3} air (Wedzicha, private communication). The adverse reaction is the result of the exposure of lung tissue to SO_2. It is of course possible that such exposure could also occur as a result of SO_2 being liberated from sulphited food and subsequently transferred to the respiratory system.

The acceptable daily intake (ADI) of S(IV) is 0.011 mmol S(IV) $kg^{-1}day^{-1}$ (Til *et al*, 1972a), which is recommended by the United Nations FAO/WHO. The Scientific Committee for Food (1981) give the estimates per capita for daily consumption for Europe as 0.25 mmol from food and non-alcoholic beverages and 0.63 mmol and 1.47 mmol if 300 ml or 700 ml additionally of wine are taken, respectively

1.7. Object

Maillard browning is one of the causes of food spoilage, leading to the production of brown pigments, off-flavours and aromas. More importantly the reaction can result in the production of potentially toxic or mutagenic compounds affecting food safety and causes a reduction in the nutritional quality of food, e.g. destruction of vitamins, loss of lysine and other essential amino acids and reduction of bioavailability of nutrients and trace elements. In addition, Maillard reaction products could also interfere with physiological processes, e.g. absorption, retention, biochemical transformation and excretion. The most common method of inhibiting Maillard browning is by use of sulphur(IV) oxospecies.

The experimental model system studied contained glucose, glycine and S(IV), the reaction conditions being pH 5.5, 55 ^{o}C and an a_w in the range 0.43-1.0. The reaction in the model system, and foods, proceeds through reactive intermediates. In the model system glucose-glycine the precursors of coloured products are DH and DDH, the latter being formed as a result of dehydration of DH. The only known products which account for the irreversible binding of the additive in the S(IV)-inhibited Maillard reaction, are the organic sulphonates. Knowles (1971) reported the existence of DSH in a heated reaction mixture containing glucose, glycine and S(IV), this being confirmed by Wedzicha and Garner (1991) during subsequent radiolabelling experiments. Wedzicha and McWeeny (1974) demonstrated the existence of 3-deoxypentosulose, DSP, in a mixture of ascorbic acid, glycine and S(IV) that was heated at 55 ^{o}C. Hydroxysulphonate formation can occur in the model Maillard system as a result of reversible binding of S(IV) with carbonyl containing compounds. Wedzicha (1984a) proposed a kinetic model to account

for both irreversible and reversible binding of S(IV) during the glucose-glycine-S(IV) reaction. This model was subsequently improved by Wedzicha and Vacalis (1988). The later model suggests that rate determining formation of DH from glucose and glycine occurs by two parallel pathways, one of which is catalysed by S(IV). DDH is formed from DH by two parallel pathways, one of which is catalysed by glycine. DSH is formed in a fast step as a result of irreversible binding of S(IV) to DDH. Hydroxysulphonate formation occurs due to reversible binding of the additive to glucose and DSH. No reversible binding of S(IV) to DH has been identified despite the fact that Wedzicha and Kaban (1986) showed that DH hydroxysulphonate could be formed from pure DH in the presence of glycine.

The objectives of this investigation are:

(I) to critically review the kinetic model proposed by Wedzicha and Vacalis (1988) for the glucose-glycine-S(IV) reaction. In constructing this model the following assumptions have been made: (a) Wedzicha and Vacalis (1988) looked at the effect of reaction mixture pH on the kinetics of the reaction. However, after the initial adjustment of pH no further measurements of pH were made. The conclusion that: "the rate is essentially independent of pH between pH 4.5 and 5.5 but there is an increase in rate of loss of S(IV) above pH 5.5" is open to question, because it is unclear whether or not the pH of reaction mixtures has remained constant during these experiments. Also, if a change in pH occurs it is not known if buffering of reaction mixtures would alter the rate. (b) S(IV) is lost by one route only, this being by irreversible binding to DDH to produce DSH. In the proposed research, the assumptions on which the model is based are to be reconsidered.

(II) to obtain rate constants for the uncatalysed and

S(IV)-catalysed Maillard reactions as a function of a_w. This will show how the rate-determining step is affected by a_w and may, therefore, help to improve the description of this step. In addition, knowledge of the extent to which each step of the Maillard reaction occurs with changing a_w may enable the degree to which browning occurs during, say, dehydration of foods to be limited.

(III) to construct a computer programme to integrate the rate equations describing the kinetic model for the glucose-glycine-S(IV) reaction and to examine the extent of agreement between calculated values of S(IV) concentration and experimental results. This will give an indication of the accuracy of the kinetic model used.

(IV) to suggest ways in which the kinetic model of the glucose-glycine-S(IV) reaction proposed by Wedzicha and Vacalis (1988) may be improved. In order for Wedzicha and Vacalis (1988) to obtain a satisfactory agreement between S(IV) concentrations calculated theoretically and experimental results, some of the values of experimentally obtained rate and equilibrium constants used in the model had to be significantly adjusted. If the model accurately described all the processes occurring in the reaction and the experimental results were reliable, then one would expect that little or no adjustment of the constants in the model would be required in order to obtain a satisfactory fit. The fact that this is not the case demonstrates that the model requires improvement. Such improvements could include: (a) A more detailed description of the rate determining step. (b) Identification of the catalytic S(IV) species. (c) determination of whether or not DH forms a hydroxysulphonate adduct.

(V) to illustrate the use of the rate expression for the constant rate period of S(IV) concentration/time plots (in reaction mixtures

containing glucose, glycine and S(IV)), as a novel method of following the progress of the Maillard and S(IV)-catalysed Maillard reactions. This is because methods used by previous workers to study the Maillard reaction, particularly absorbance measurements, have been shown to suffer from a number of disadvantages.

CHAPTER 2: MATERIALS AND METHODS.

2.1. Materials.

All chemicals were of AnalaR grade and were supplied by BDH chemicals Ltd. or M&B Ltd.

Measurements of pH were made using a Jenway PHM6 pH meter. The meter was calibrated with potassium hydrogen phthalate and anhydrous disodium hydrogen orthophosphate /sodium dihydrogen orthophosphate buffers.

2.2. Methods.

2.2.1. Preparation of reaction mixtures containing glucose, glycine and S(IV) oxospecies.

D-Glucose, glycine and sodium metabisulphite were weighed out and dissolved in the appropriate solvent. Reaction mixture pH was adjusted to 5.50 with sodium hydroxide (1 M) and hydrochloric acid (1 M) at 25 °C, the mixture transferred to a volumetric flask (250 or 500 ml) and made up to volume. The reaction was followed in stoppered reagent bottles (250 or 500 ml) in a waterbath set at 55.0 ± 0.1 °C. The time at which the bottles were placed in the waterbath was taken as the starting time for the reaction.

2.2.2. Measurement of Free and Reversibly bound S(IV).

The method of iodimetric titration is widely used to quantitatively determine S(IV) concentration and forms the standard technique against which all other methods of analysis are compared

(Wedzicha, 1984b). In addition, it enables determination of free and reversibly bound S(IV), present in mixtures containing carbonylic compounds, to be performed. Measurement of both forms of the additive is required for development of a kinetic model to describe the sulphite-inhibited Maillard reaction.

An aliquot of the reaction mixture (5-15 ml) was withdrawn at timed intervals and added to water (*c.* 50 ml) containing sulphuric acid (2.5 ml, 2.5 M). Soluble starch indicator (1% w/v) was added and the solution titrated to the first permanent blue colour using standard iodine solution (0.01 M). Sodium hydroxide (40 ml, 1 M) was added, the solution mixed and left to stand for 10 min. The mixture was reacidified by the addition of H_2SO_4 (15 ml, 2.5 M) and the reversibly bound S(IV) released titrated as above.

2.2.3. pH control and measurement.

The possibility that the browning reaction is catalysed by acids and bases, made it important to avoid adding the components of buffers to control pH. A simple method of pH control is by setting up a pH-stat. Typically reactions were followed for one month and it is not practicable to dedicate a commercial pH-stat instrument.

In order to control pH the reaction vessel was transferred to a water bath (55 °C) associated with the pH meter, the solution stirred and brought to pH 5.5 ± 0.03 with NaOH (0.5-3 M). This was carried out on each occasion the mixture was analysed for S(IV). The volume of NaOH added was recorded.

2.2.4. Measurement of oxygen loss from reaction mixtures containing S(IV).

Oxygen can be quantitatively determined by a number of

methods, including combustion, fluorimetry, titrimetry using sodium thiosulphate and by use of the Clark cell (Vogel, 1989a). Combustion analysis involves placing the sample in a reactor heated at 1060 °C; this determination is impractical. Fluorimetry involves the quenching of fluorescence by O_2. As the reaction mixtures used in the kinetic experiments contain glucose, glycine, SO_3^{2-} and reaction intermediates and products with electrons capable of n-π^* or π-π^* transitions, absorption of ultraviolet radiation could take place. Some of these transitions may also give rise to fluoresence. The principle of quenching methods is that the emission by a fluorescent species is quenched by the analyte, so that the intensity of fluorescence decreases as the analyte concentration increases. However, a major limitation of such procedures is that since quenching is somewhat non-specific, applications are restricted to analyses in which only the analyte is able to quench the fluorescence. Reaction mixtures used in the kinetic experiments may contain other quenching species and consequently it was decided not to use this method. Titrimetric determination of O_2 involves oxidation of iodide ion to iodine, which is titrated with sodium thiosulphate. However, this method cannot be used here because S(IV) is oxidised rapidly by I_2. It was decided, therefore, to use an oxygen electrode to measure O_2 levels because it is quick, simple and a direct readout is obtained.

The Clark-type oxygen electrode operates on the principle of polarography. The apparatus consists of two electrodes connected by a saturated KCl salt bridge. The platinum cathode is maintained at -0.6 v relative to the annular Ag-AgCl reference electrode. The reactions on which the electrode assembly relies for its operation are:

Cathode

$$O_2 + 2H_2O + 2e^- \longrightarrow H_2O_2 + 2OH^-$$

$$H_2O_2 + 2e^- \longrightarrow 2OH^-$$

Anode

$$Ag + Cl^- \longrightarrow AgCl + e^-$$

Hence overall;

$$4Ag + 4Cl^- + O_2 + 2H_2O \longrightarrow 4AgCl + 4OH^-$$

The output current, detected by the recorder, is then a direct indication of the oxygen level in the sample liquid (LeFevre *et al.*, 1970). This sample liquid is kept separate from the electrode by an oxygen permeable membrane (PTFE) to ensure no contamination of the electrode occurs. Homogeneity of the cell contents is achieved using a magnetic stirrer.

An exploded diagram of the oxygen electrode appears in Fig.2.2.1 The platinum (central) and silver-silver chloride electrodes were cleaned with toothpaste and ammonia (15%) solution respectively; thoroughly rinsed with water and dried with tissue. A piece of lens tissue (1 cm^2) was perforated with a single hole (1 mm^2) and positioned so that the hole lay directly above the cathode. The tissue was then wetted using saturated potassium chloride solution (*c.* 0.1 ml) and covered with PTFE film (1 cm^2). An 'O' ring was placed over the film, exerting pressure to exclude any trapped air from the electrolyte, and the cell reassembled.

At 55 ^oC a solution of sodium dithionite (10% w/v) was used to calibrate the meter at the zero oxygen response, whilst water saturated with air was used as the 100% standard. Several rinsings of the cell were performed, prior to sample analysis, to ensure complete removal of dithionite. An aliquot of reaction mixture was introduced into the thermostatically controlled holder (55 ^oC); the cell plug replaced, expelling any head space air, and the oxygen level recorded as a function of time.

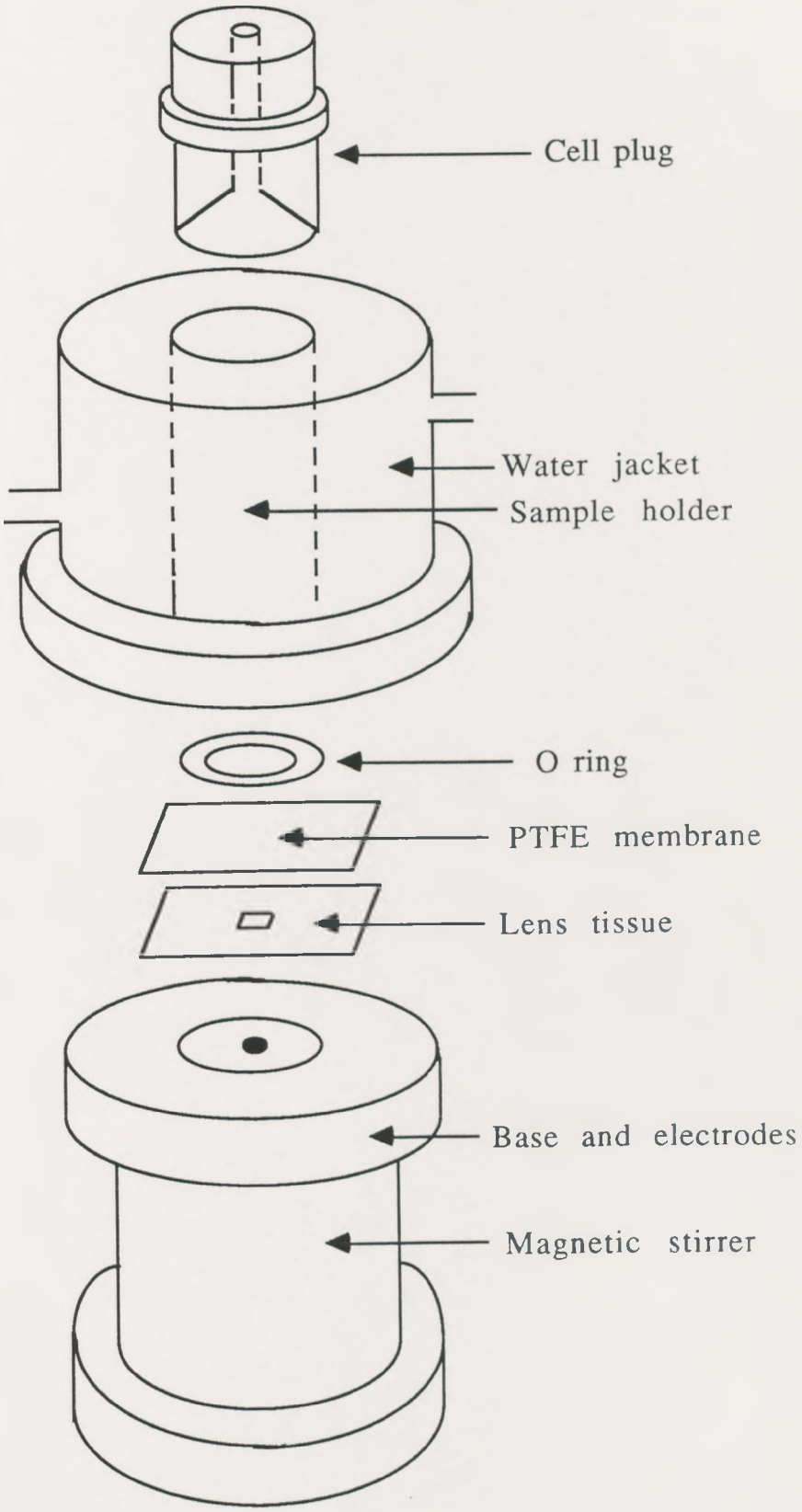


Fig.2.2.1. Exploded view of an oxygen electrode.

2.2.5. Measurement of Sulphate ion.

Reaction mixtures, containing S(IV) (*c.* 0.05 M), were examined, for sulphate ion (S(VI)), to determine whether or not significant S(IV) oxidation occured. The method of analysis chosen, therefore, must be capable of determining all theoretically possible S(VI) concentrations. Hence, of the methods available only the following could be used.

1. Potentiometric titration of sulphate with standard lead nitrate in the presence of a ferrocyanide-ferricyanide redox system.
2. Amperometric titration of sulphate with standard lead nitrate.
3. Precipitation of sulphate with barium chloride. Dissolution of the precipitate in excess EDTA and titration with magnesium chloride.

Iron(III) and S(IV) react to produce sulphate (Wedzicha 1984b). The addition of ferricyanide to the reaction mixture, could cause S(IV) oxidation in addition to that which may have already occurred.

Oxidation of S(IV) could cause reduction of Fe(III), altering the ratio of ferro- to ferri-cyanide, thus changing the redox potential of the system. In addition, increased Fe(II) requires more lead nitrate to ensure complete precipitation as lead ferro cyanide, hence the 'true' endpoint would be exceeded.

The method of amperometric titration requires the precipitation of the soluble lead sulphate produced to minimise the magnitude of the rounded portion of the titration curve. This is achieved by addition of ethanol, the amount depending on the level of sulphate present, to the mixture. In a mixture containing an unknown amount of sulphate excess or insufficient ethanol may be added, leading to uncertainty in the true endpoint.

The method of barium chloride addition enables the

determination of any level of sulphate produced.

An aliquot of reaction mixture (10.0 or 25.0 ml) was transferred to a conical flask (250 ml), diluted to 50 ml, adjusted to pH 1 with conc HCl, and heated until near to boiling. An excess of nearly boiling $BaCl_2$ (15 or 30 ml, 0.05 M) was added rapidly with vigorous stirring, the mixture heated for 1.5 h, allowed to cool and filtered (Whatman No. 42). Concentrated ammonia solution (5 ml) and excess EDTA (25.0 ml, 0.05 M) were added. The mixture was heated for 20 min. and a further addition of conc ammonia solution (2 ml) was made after 10 min to facilitate dissolution of the precipitate. Ammonia buffer (pH= 10, 10ml, $[NH_3]$= 10.31 M + $[NH_4Cl]$= 1.31 M) and Eriochrome Black T indicator were added and excess EDTA was titrated with magnesium chloride (0.05 M) to a clear red colour.

2.2.6. Measurement of water activity of reaction mixtures containing mixed solvent.

Methods for measurement of a_w in foods are based on the colligative properties of solutions. Water activity can be determined by a number of methods.

1. Measurement of the freezing point depression of a liquid and conversion to a_w.
2. Measurement of the equilibrium relative humidity of a solid or liquid. This may be determined by two means:
   (a) A sample of the substance to be measured is enclosed with a small quantity of gas, usually air, and the relative humidity or vapour pressure of this gas measured once equilibrium is reached.
   (b) A sample of the substance is placed in a gas at known temperature and relative humidity. The moisture absorbed

or lost is determined (Labuza, 1984).

The method of freezing point depression is most suitable for high a_w values (> 0.8). The model systems studied covered the a_w range 0.4-1.0, therefore the method is unsuitable.

Transport of water from the sample into the headspace gas, will cause the mixture to lose water to provide the vapour pressure above the sample. If significant quantities of water are released, this would cause a reduction in water content of the mixture and possibly errors in the measured a_w, if a detector were then used to measure the equilibrium relative humidity.

The method of Labuza (1984) can be used over the a_w range 0-0.989, this being ideal for the model systems studied. The method involves suspending a sample over a standard solution of known a_w. If the a_w of the standard is higher than that of the sample, then, the sample will adsorb water until, within the limits of experimental error, the a_w of the standard and the sample are equal. Conversley, if the a_w of the standard is lower than that of the sample, then, the sample will desorb water. The change in mass of the sample is plotted against the a_w of the standard solution over which the sample was suspended and the a_w of the sample is given by the point of zero mass change, the intercept on the a_w-axis. The main advantage of this method is that it effectively measures the a_w under conditions where there is no net transport of water vapour from the sample to the headspace. The a_w of the sample could also be obtained by plotting the initial rate of mass change *vs* the a_w of the standard, as this rate will be dependent on the difference between the a_w of the standard and that of the sample, the larger the difference the faster the rate. However, the rate of mass change will also be affected by the surface

area of the vessel in which the sample is contained and the amount of sample used in the analysis. Therefore, despite the method being much quicker it was decided that it would not be used. It is assumed that there are only two methods by which the amount of water in the sample will change during measurement of a_w. Firstly, mass change due to the process of a_w equilibration. Secondly, loss of water from the mixture due to the Maillard reaction which will continue during equilibration. To reduce water losses from the Maillard reaction to a minimum, it was decided that the sample would be regarded as having the same a_w as the standard once the difference between successive weighings of the sample had fallen below 2% of its original mass.

A diagram showing the apparatus used to measure the a_w of a reaction mixture is shown in Fig.2.2.2. An accurately known weight of reaction mixture (1.0-2.5 g) was allowed to equilibrate over a series of saturated salt (Greenspan, 1977) and sulphuric acid (Murrel and Scott, 1966) solutions at 55 °C. The a_w of the mixture was calculated by graphic interpolation of mass change verses a_w.

2.2.7. Determination of the Fourier Transform Infrared spectra of S(IV) oxospecies.

Hydrogen sulphite ion is in equilibrium with sulphite and disulphite ions i.e.,

$$HSO_3^- \rightleftharpoons SO_3^{2-} + H^+ \qquad (2.2.1)$$

$$2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O \qquad (2.2.2)$$

and the chemical reactivity of the preservative in foods is related to the proportions of each species present. As a_w is reduced it is expected that these proportions will change (Wedzicha and Goddard, 1991). It is required that these changes be measured, as this may

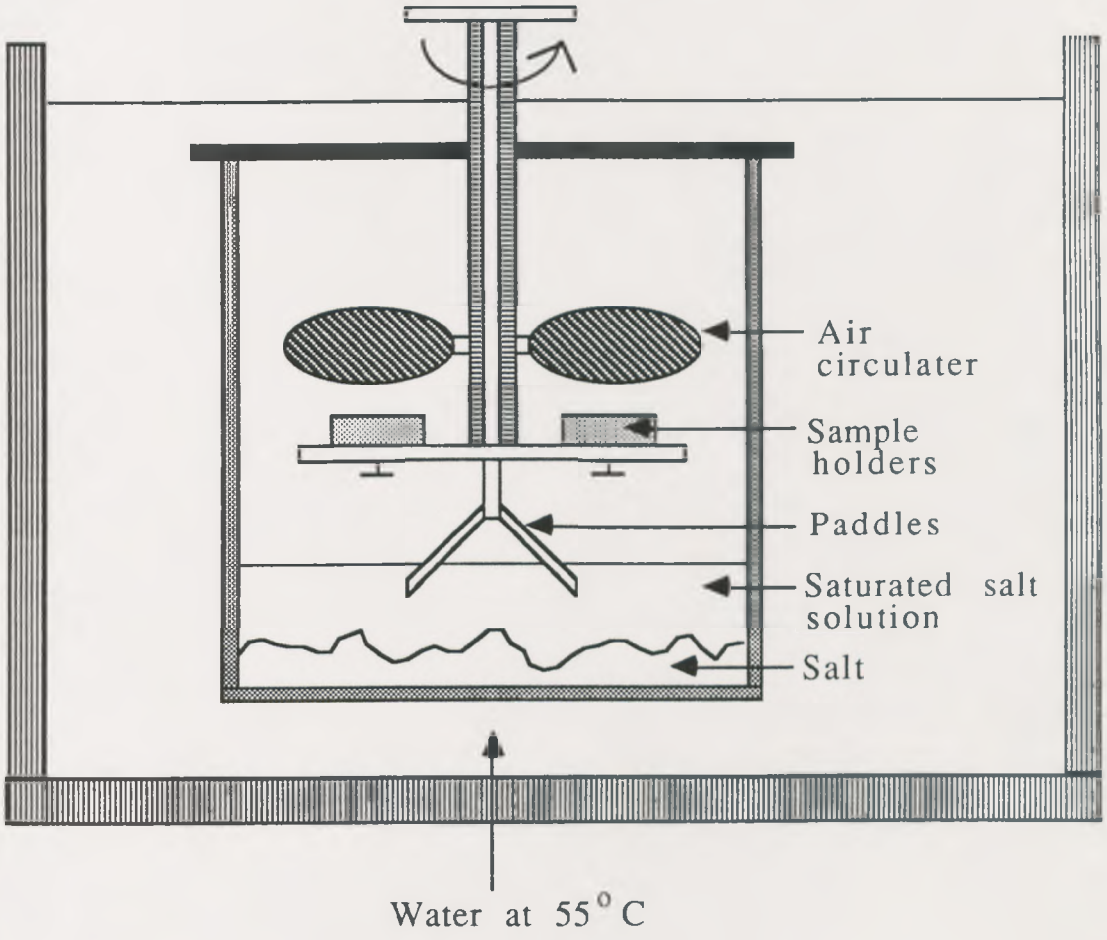


Fig.2.2.2. Apparatus used to measure water activity

explain why the rate constants for the sulphite-catalysed stage of the Maillard reaction are found to vary with a_w.

The use of molecular spectroscopy, electronic and infrared, for the identification and quantitative analysis of S(IV) oxospecies in solution is, perhaps, the simplest available technique for their determination, particularly when no sample work up is necessary. Colorimetric and Fluorimetric techniques measure total S(IV). As the concentrations of the individual anions at reduced a_w are required, these methods are therefore unsuitable.

The most reliable spectrophotometric method for the determination of S(IV) oxospecies in solution and one which gives the greatest resolution is that of Raman spectroscopy, as illustrated by the work of Meyer and his group (Meyer *et al.*, 1980). An appropriate spectrophotometer was not available.

Measurement of the absorbance due to $S_2O_5^{2-}$ at 255 nm (E_{max} = 5790 M^{-1} cm^{-1}; Connick *et al.*, 1982), at which wavelength HSO_3^- has negligible absorbance, offers a convenient approach to measuring the extent of formation of $S_2O_5^{2-}$ in solutions of $NaHSO_3$. Experiments carried out by S. J. Goddard in this laboratory demonstrated that increasing glycerol content brought about a reduction in absorbance at 255 nm and that no shift in wavelength and no new absorption peak was obtained (Fig.2.2.3). This effect was also seen for ethanol and polyethylene glycol 400 (PEG 400), but in these cases the absorbance did not fall to zero. These observations could be the result of less $S_2O_5^{2-}$ being formed at reduced a_w. However this is contrary to expectation as a reduction in water concentration should displace equation 2.2.2 to the right, thus increasing $S_2O_5^{2-}$ concentration. Any precipitated solid obtained when an excessive amount of non-electrolyte is added has an I.R. spectrum

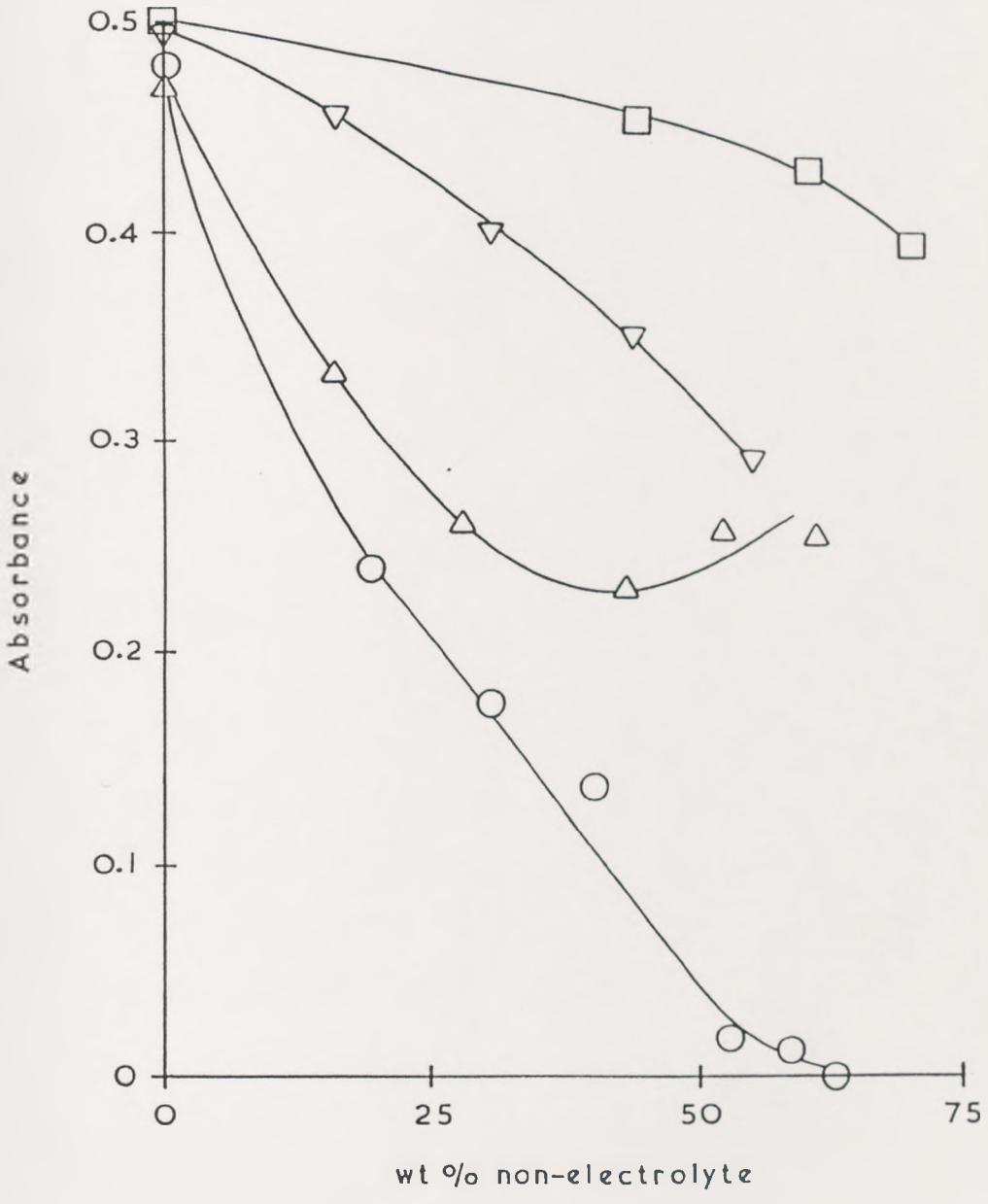


Fig.2.2.3. Effect of non-electrolyte concentration on the absorbance of a 50 mM solution of $NaHSO_3$ at 255 nm. Polyethylene glycol (PEG 400) □ ; Sucrose ▽ ; Ethanol △ ; Glycerol ○ . The data for PEG 400 are displaced upwards by 0.05 absorbance units for clarity.

indistinguishable from that of solid $Na_2S_2O_5$. The main disadvantage of U.V. spectroscopic data is that the extinction coefficient of the solute could depend on the medium in which it is dissolved. Infrared spectra of the glycerol-containing mixtures were plotted in the present work to check the findings of S. J. Goddard.

The relatively low concentration of the ions present prevents the use of 'classical' infrared to determine the individual species. However this problem can be overcome by use of Fourier Transform infrared spectrophotometry.

The theory of classical and Fourier Transform infrared spectroscopies are described by Vogel (1989b), Griffiths (1972) and Colthop *et al* (1975). Absorption of infrared radiation occurs as a result of energy changes in a molecule caused by molecular vibrations, stretching and bending being the two main types of vibration. A stretching vibration is a vibration along a bond axis such that the distance between the two atoms is either increased or decreased. A bending vibration involves a change in bond angle. Each atom or group of atoms in a molecule oscillates about a point at which the attraction of nuclei for electrons balances the repulsion of nuclei by nuclei, and electrons by electrons. The length and strength of any one bond is controlled by the relative magnitudes of these attractions and repulsions. The oscillations have natural periods which depend on the masses of the atoms and the strengths of the bonds involved. The amplitude of the oscillations, but not the frequency, can be increased by supplying energy by means of electromagnetic radiation. Nuclei and electrons bear electric charges. If the frequency and phase of an electromagnetic wave match those of a particular molecular vibration, then a change in amplitude of the oscillation can occur. If this change in amplitude results in a change of molecular dipole moment, this

being a measure of the charge distribution, then transfer of energy can occur. Radiant energy is then absorbed and the intensity of radiation at this particular wavelength is reduced on passing through the compound. The intensity of any absorption depends on the magnitude of the change in the oscillating dipole moment during the transition, and is also directly proportional to the number of bonds in the molecule responsible for that particular absorption. Hence, hydrogen or carbon bonded to oxygen or nitrogen gives rise to a strong infrared absorption because of the polarity of these particular bonds. These bonds are termed infrared active. Some bonds, however, are infrared inactive. Homonuclear double or triple bonds which are symmetrically substituted are infrared inactive, as stretching vibrations produce no change in dipole moment.

For a diatomic molecule A–B, the only vibration that can occur is a periodic stretching along the bond. With polyatomic molecules many more fundamental vibration modes are possible, such as symmetric and asymmetric stretching and a variety of bending deformations. However, these fundamental vibrations cannot account for all observed infrared absorptions. This is because of the occurrence of combination, overtone and difference bands. The first arises when absorption by a molecule results in the excitation of two vibrations simultaneously, say ν_1 and ν_2, and the combination bands appear at the frequency of $(\nu_1 + \nu_2)$. The overtone band corresponds to a multiple (2ν, 3ν, etc.) of the frequency of the absorption band. Difference bands occur when absorption of radiation converts a first excited state into a second excited state. These bands are frequently of lower intensity than the fundamental absorption bands.

In any spectroscopic technique, a polychromatic beam of radiation must be acted upon so that each frequency of the radiation is

somehow differentiated from the other frequencies, in order that the intensity of each frequency can be measured. Conventionally, this differentiation is carried out by passing the beam through a prism or reflecting it from a grating so that each frequency is distinguished spatially. Hence, when producing a classical infrared spectra by scanning the range of wavelengths 2.5-50 μm, the sample is only exposed to a narrow band of radiation at any one time. The amount of absorption at any particular wavelength is determined by comparison to a reference beam. Water is not used as a solvent in this instance, because absorption bands due to any solute would be masked by the much greater size of absorption bands due to the solvent. Solid samples can be incorporated into KBr disks, the salt being adequately transparent to infrared radiation in the range 12-25 μm. For samples producing absorptions in the range 2-15 μm, polished plates of sodium chloride can be used as the support material for pure liquids or nujol mull's. In Fourier Transform infrared spectrophotometry there is no monochromator in the system, instead the beam of radiation is examined using an interferometer. Radiation of many frequencies passes through the sample, all frequencies not selectively absorbed reach the detector. The most commonly used instrument is the Michelson interferometer, a diagram of which appears in Fig.2.2.4. Radiation from the source is passed through a beam splitter which transmits half the beam to a movable mirror, A, and reflects the other half to a stationary mirror, B. These two beams are reflected from their respective mirrors and recombined either constructively or destructively at the beam splitter depending on the position of the movable mirror. As the path difference between the two beams is altered, the detector signal pattern obtained is the interferogram of the radiation entering the interferometer. For monochromatic

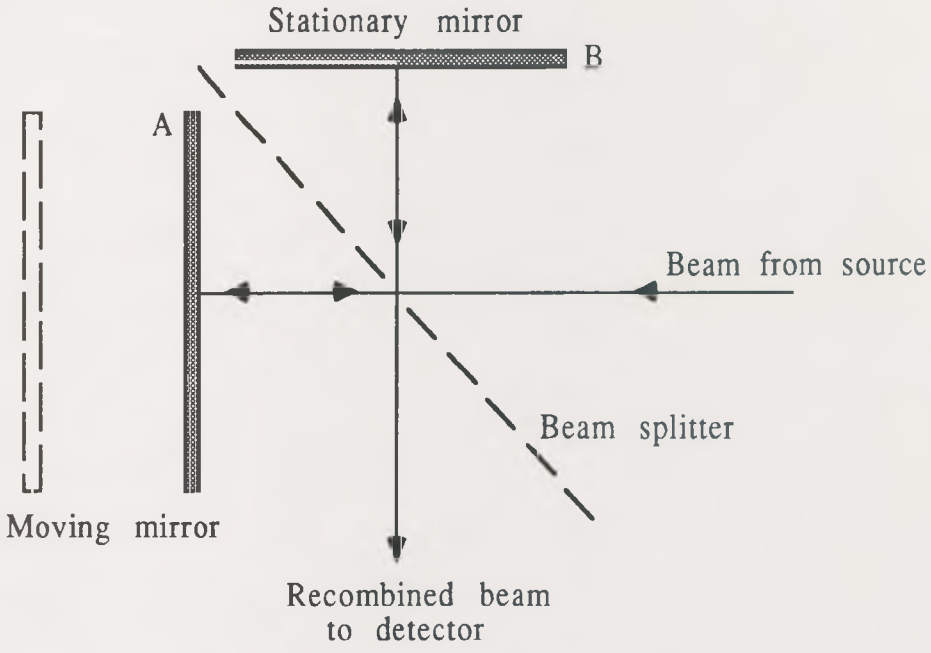


Fig.2.2.4. A schematic diagram of a michelson interferometer.

radiation the amplitude of the detector signal is a cosine function of mirror position. For polychromatic radiation the detector signal is a summation of all the constructive or destructive interferences of each wavelength interacting with every other wavelength and results in an interferogram. The interferogram contains information on the intensity of each frequency in the spectrum which can be calculated at each frequency by a mathematical operation known as the Fourier transformation, to yield the infrared spectrum. The sample is scanned a designated number of times and the spectra summed to improve the signal to noise ratio. In the same manner spectra of the pure solvent and air contained in an empty sample compartment are obtained. These absorptions are then eliminated from the initial spectra by "ratioing out", thus leaving any absorptions due to the solute. As a result of the elimination of absorption bands due to the solvent, it is possible to obtain spectra of samples dissolved in water.

Solutions containing S(IV) were standardised iodimetrically.

Infrared spectra were recorded from an ATR assembly (Zinc selenide crystal) attached to a Nicolet Instruments 510 FTIR spectrometer and data processing computer. In all cases, FTIR data were accumulated from 20 scans. An FTIR spectrum of air contained in the sample compartment was recorded. This process was repeated for both the pure solvent and the sample (*c* 10 ml) containing S(IV) oxospecies. Spectra due to S(IV) oxospecies was obtained in the manner described above.

CHAPTER 3: RESULTS AND PRELIMINARY DISCUSSION.

3.1. A critical review of the kinetic model for the Sulphite-Inhibited Maillard reaction.

3.1.1. Preliminary appraisal of the model system containing glucose, glycine and S(IV).

3.1.1.1. Investigation of reaction mechanism by means of chemical kinetics.

Chemical kinetics is concerned with how a system gets from one state to another and the time required to achieve this. Reaction rate is defined as the rate of change of concentration of a substance in the reaction. By studying the relationship between reaction rate and concentration it is possible to obtain a functional equation, the rate expression, equating these variables for a particular reaction.

A typical rate equation is of the form:

$$-dc_1/dt = k\ c_1^{n1}\ c_2^{n2}\ c_3^{n3}$$

where c_1, c_2 and c_3 are the concentrations of components 1, 2 and 3, n1, n2 and n3 are integers and k is the rate constant for the reaction. The overall order of reaction, n, is given by:

$$n = n1 + n2 + n3 ....$$

Each exponent is called the order with respect to that component. Analysis of the empirical relationship between reaction rate and reactant concentration allows the order of reaction with respect to each reactant to be obtained. The rate expression describes the mechanism of a reaction, but may be a somewhat simplified view as the expression only concerns the steps leading up to the rate

determining-step. Essentially the order of reaction with respect to each component reflects the number of molecules of that component used to make up the reaction intermediate formed in the rate determining-step.

The component being measured to obtain the reaction rate may or may not be involved in the rate determining step of the reaction. For example, in the reaction with the stoichiometric equation:

$$A + B + C \longrightarrow D$$

the rate expression could well be:

$$\text{Rate} = k[A][B]^2$$

but reaction rate may be obtained by measuring the rate of loss of A, B, or C, or the rate of formation of D. Measurement of the rate of change of concentration of a component not involved in the rate determining step offers the simplest way of obtaining reaction rate, as its concentration will not affect the rate of reaction.

In the example above rate depends on the concentrations of A and B. If both reactants are at comparable concentrations, then the rate will decrease as the concentration of the reagents falls during the course of the reaction. However, if $[B] >> [A]$, then the concentration of B is effectively constant throughout the reaction, and one can write a simplified rate equation as,

$$\text{Rate} = k'[A]$$

Thus, to establish the kinetic order with respect to A one could measure with time the concentration of an appropriate species, e.g. A or D and observe that the overall reaction was of first order. Such kinetic behaviour is referred to as a pseudo first order reaction to emphasise the fact that the overall kinetic behaviour is of a higher order.

To enable accurate measurement of reaction rate there must be

a substantial change in the concentration of the component being measured. Measurement of the concentration of a product is convenient as it increases from zero, to a given concentration. When the concentration of a reactant is measured, the concentration of other reactants should ideally be in excess.

3.1.1.2. Measurement of reaction rate in the system glucose-glycine-S(IV).

As described in section 1.4.2, S(IV) present in mixtures of reducing sugar and amino acid is converted to reversibly and irreversibly bound forms. Preliminary kinetic evidence suggests that apart from a catalytic involvement of S(IV) in the early stages of the S(IV)-inhibited Maillard reaction, the additive reacts irreversibly with 3,4-dideoxyhexosulos-3-ene, DDH, in a fast process. Before any kinetic investigation is carried out it is necessary to decide which reactants are to be analysed to measure the progress of the reaction. As explained in section 3.1.1.1 above, it is normally desirable to simplify the apparent kinetic behaviour by creating a pseudo order reaction. This requires that the concentration of one of the reactants be much smaller than those of the others and this brings about a significant change in the concentration of the minor component which can be accurately measured with time. The concentration of the other reactants are assumed to remain constant throughout a kinetic run. The composition of the S(IV)-inhibited Maillard reaction is such that S(IV) is a minor component, which suggests that it is S(IV) concentration which should be measured with time. Apart from the reducing sugar: S(IV) ratio in, say, a sulphited dehydrated vegetable being such that [reducing sugar] >> [S(IV)], the use of such conditions in model experiments is desirable if one is to study the mechanism of

the part of the S(IV)-inhibited Maillard reaction which is not catalysed by the additive. Measurement of S(IV) concentration during a kinetic run is also advantageous as the methods are relatively straight forward and well documented; also it is possible to measure the concentrations of free and reversibly bound S(IV).

As shown by Wedzicha and Vacalis (1988), the rate of the constant rate phase of the S(IV)-inhibited Maillard reaction is given by:

$$\text{Rate} = -\frac{d[S(IV)]}{dt} = f\{[\text{glucose}][\text{glycine}][S(IV)].....\}$$

where f represents some function of the concentrations shown and those of other species not yet identified as being rate determining, e.g. H^+, anions, cations, H_2O.....

The concentrations of free and reversibly bound S(IV), in the system glucose-glycine-S(IV) were plotted as a function of time and a typical example is shown diagrammatically in Fig.3.1.1.

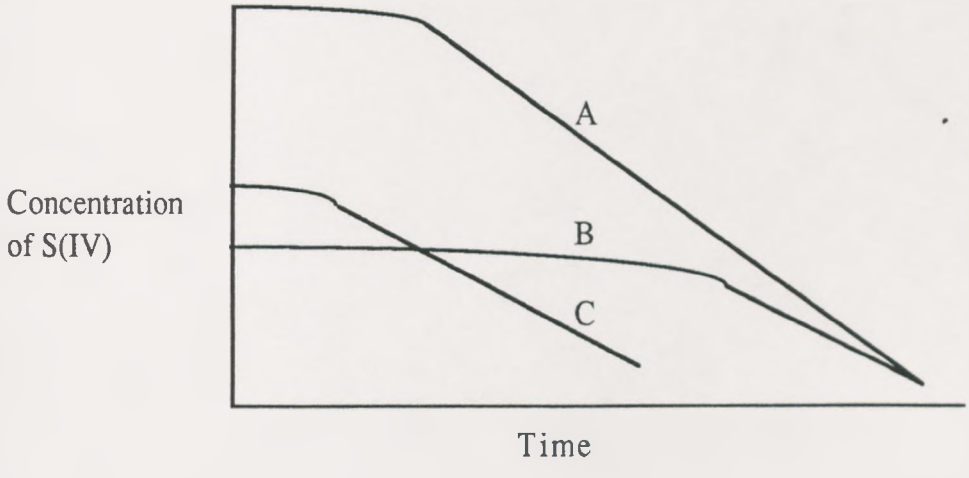


Fig.3.1.1. A typical reaction profile observed by by McWeeny *et al* (1969) for the system glucose-glycine-S(IV). A=Total S(IV), B=Reversibly bound S(IV), C=Free S(IV).

Total recoverable S(IV), that is the sum of that which is free and that which is reversibly bound, decreases at a constant rate over the major part of the reaction after an initial induction period, during which the rate of irreversible combination of the additive with components of the mixture is increasing from zero; this has been observed by McWeeny *et al* (1969) and Wedzicha and Vacalis (1988).

If S(IV) combined directly with glucose and glycine to form stable products which did not brown, then S(IV) would be irreversibly lost from time zero; the induction phase suggests that rate determining formation of an intermediate in a series of consecutive reactions takes place. If the first intermediate, I1, were to react with S(IV) in a fast step to produce products directly, no induction period would be seen as I1 is expected to be produced as soon as the reaction commences. Therefore, to explain the observed kinetics, the minimum number of steps requires that the intermediate must be converted to a second intermediate, I2, at a rate that is of a similar order of magnitude to the rate of production of I1. Then I2 may react with S(IV) in a fast step to produce DSH. At time zero the concentrations of I1 and I2 are zero; after a small increment of time a small amount of I1 will be present, but the rate of formation of I2, which depends on the concentration of I1, is very slow and hence the rate of loss of S(IV) will be negligible. The reaction rate gradually increases as the concentrations of I1 and hence I2 increase, until eventually the rate of loss of I1 equals its rate of formation; at this point a constant rate of loss of S(IV) is obtained. In this manner it is possible to account for the initial induction and constant rate phases of the reaction.

It is thought that I1 may be 3-deoxyhexosulose, DH, because

Wedzicha and Kaban (1986) showed that if the rate constant for DH loss, measured in the system DH-glycine-S(IV) under the same conditions of temperature and pH, was substituted into the kinetic model for the system glucose-glycine-S(IV), a good fit of experimental results was obtained. Intermediate I2 would be DDH as it is formed by dehydration of DH and is more reactive towards browning than DH; it may, therefore, be expected to react rapidly with S(IV).

The reactivity of intermediates in non-enzymic browning reactions is dependent on the presence of the carbonyl group, the most reactive components being those with an α–dicarbonyl function. Therefore, reaction of carbonyl compounds with S(IV) to form the hydroxysulphonate adducts is expected to inhibit effectively the browning reaction, the extent of inhibition being a function of the amount of free carbonyl compound present in equilibrium with hydroxysulphonate.

The most elementary reaction which could lead to the control of Maillard browning is that between glucose and S(IV) to form glucose hydroxysulphonate. The equilibrium constant for the reaction is close to unity over the pH range 2-6 at 20 ^{o}C (Vas, 1949), a value which may be compared with equilibrium constants of the order of 10^{5}-10^{6} M^{-1} for adduct formation for simple aldehydes or ketones. However, the only effect of combination of S(IV) with glucose is a reduction of the amount of the former available for other purposes. In model systems the aldose-hydrogen sulphite reaction will not inhibit the browning reaction of glucose, as the concentration of S(IV) will not be sufficiently high to lead to a significant reduction in glucose concentration (Wedzicha, 1984b).

One of the rate-determining steps in the S(IV)-inhibited Maillard reaction of glucose and glycine is production of DH.

Reversible binding of DH as a hydroxysulphonate may contribute more to inhibition of browning than does the formation of glucose hydroxysulphonate. It has been shown that browning does not commence until free S(IV) concentration reaches a low level (McWeeny *et al*, 1969). Once this has occurred any hydroxysulphonate adducts present will dissociate releasing free carbonyl compound. It is possible that such a carbonyl compound is DH and that formation of hydroxysulphonates of DH or related intermediates is important in inhibition of browning.

A kinetic model which includes all these considerations for the system glucose-glycine-S(IV) is shown in Fig.1.4.6 This also illustrates the findings of Wedzicha and Vacalis (1988) and Wedzicha and Kaban (1986) regarding the catalysis of the conversion of DH to DSH by glycine, these points being discussed in the introduction (section 1.4.3). When the rate of DH formation equals its rate of loss, S(IV) is irreversibly bound at a rate equal to that of DH production; therefore, the rate of DH production can be obtained by measuring the rate of irreversible S(IV) loss.

3.1.1.3. Analysis of S(IV) in model systems.

The simplest method of analysis of S(IV) in foods and model Maillard systems involves iodimetry. This has the advantage that it can be adapted to measure free and reversibly bound S(IV) and is described in section 1.3.2. The known trends in concentration of free and bound S(IV) in a model Maillard system are reviewed in sections 1.4.2 and 1.4.3 and it is seen that measurement of the reversibly bound S(IV) offers a critical method of checking conclusions derived from kinetic studies in which total S(IV) concentration is used as a measure of the progress of the reaction. Thus, any proposal for the

formation of carbonylic intermediates and products which could bind S(IV) reversibly could be checked by measurement of dissociation constants in isolation and predicting the extent of reversible binding in model Maillard systems. A full kinetic study of the S(IV)-inhibited Maillard reaction thus requires measurement of free and reversibly bound additive.

Eichner (1975) demonstrated that in a freeze dried glucose-lysine-Avicel model system at a_w 0.23 held at 40 °C for 70 h, reducing power was exclusively attributed to the Amadori rearrangement products α–,ε-difructoselysine, α-fructoselysine and ε-fructoselysine. Reducing power was calculated as reducing equivalents related to mol of glucose initially present in the reaction mixture, measured by the potassium ferricyanide test using cysteine as a standard (Crowe *et al*, 1948). At higher a_w, total reducing power can only be partly attributed to the fructoselysine derivatives. In the model system glucose-glycine-S(IV), monofructoseglycine and difructoseglycine are formed. Reductones are known to react with iodine and it is necessary to validate that iodimetric analysis of the S(IV)-inhibited Maillard reaction provides a true indication of the S(IV) content and there is negligible interference from other reducing agents. It should be noted that in most situations in which the fate of S(IV) is studied, there is essentially no browning over the major part of the reaction and the only known reducing agents are, therefore, Amadori products (Eichner, 1975) and the reducing sugar.

Wedzicha and Kaban (1986) demonstrated that total S(IV) concentration measured iodimetrically in a model system initially containing [DH] = 0.0239 M, [S(IV)] = 0.0250 M, [glycine] = 0.5 M, at pH 5.5 and 55 °C is in excellent agreement with values obtained spectrophotometrically using 5,5'-dithiobis (2-nitrobenzoic acid),

DTNB. Under the mildly alkaline conditions (pH 8.0) used for the spectrophotometric analysis, hydroxysulphonate adducts are relatively labile and the reaction of S(IV) with DTNB is quantitative. It is expected that measurement of S(IV) using DTNB gives the concentration of total S(IV). This was confirmed by analysis of a reaction mixture after 120 h reaction, for total S(IV) iodimetrically and spectrophotometry. Duplicate iodimetric determinations gave total [S(IV)] = 0.0134 and 0.0131 M whilst the determination using DTNB reagent gave [S(IV)] = 0.0131 and 0.0133 M.

After all the S(IV) has been irreversibly bound in a glucose-glycine-S(IV) mixture ([glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0423 M), there remains a very small reproducible titre corresponding to a maximum concentration of reducing species of 0.7 mM.

3.1.1.4. Reproducibility of results obtained for the analysis of free and reversibly bound S(IV).

Hydroxysulphonate adducts were decomposed in a single, rather than a double, step when measuring free and reversibly bound S(IV). This was because after obtaining a typical titre of, say, 15 ml of iodine in the determination of reversibly bound S(IV), a second decomposition only yielded a titre of *c.* 0.1 ml or less. As this is less than the combined error for two readings of the burette, i.e. titre $\pm$ 0.1 ml, a two step decomposition was thought unnecessary.

Duplicate reaction mixtures of composition [glucose] = 0.4-1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0078-0.0423 M were prepared to test the reproducibility of results obtained for the analysis of free and reversibly bound S(IV), Figs.3.1.2-3.1.5. Reaction rate is obtained by measuring the gradient during the constant rate of loss of total S(IV),

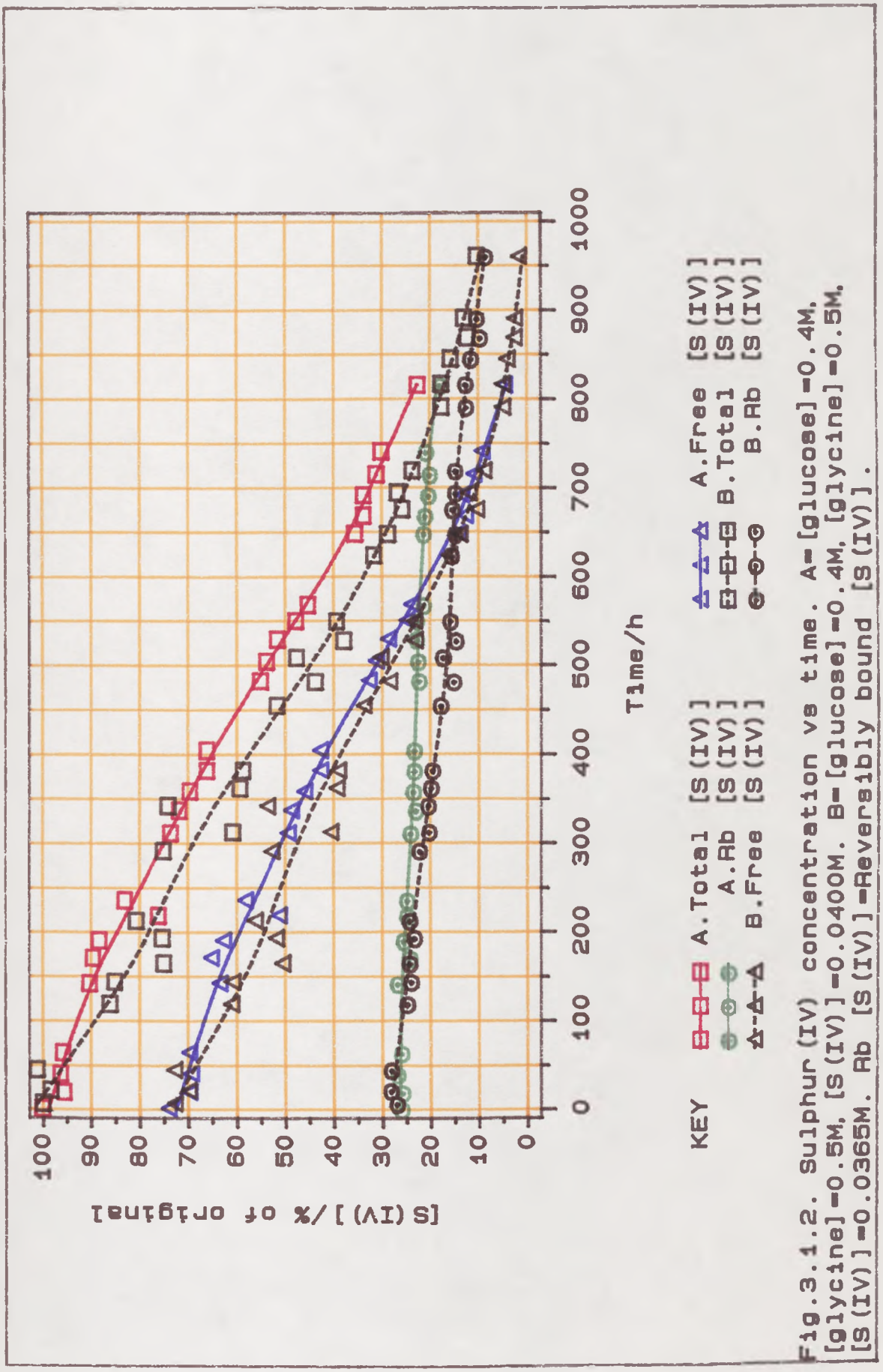


Fig.3.1.2. Sulphur(IV) concentration vs time. A=[glucose]=0.4M, [glycine]=0.5M, [S(IV)]=0.0400M. B=[glucose]=0.4M, [glycine]=0.5M, [S(IV)]=0.0365M. Rb [S(IV)]=Reversibly bound [S(IV)].

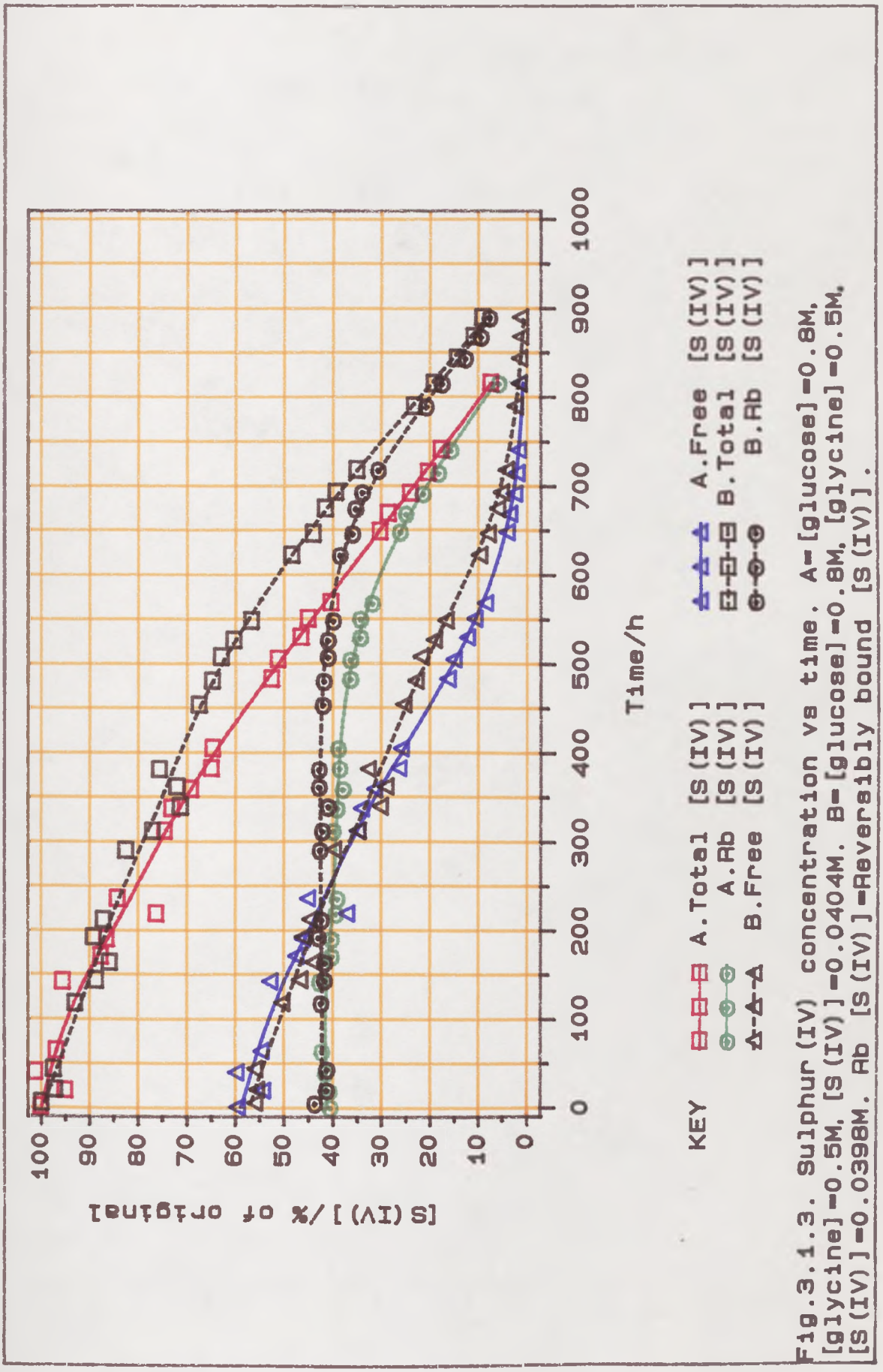


Fig.3.1.3. Sulphur(IV) concentration vs time. A=[glucose]=0.8M, [glycine]=0.5M. [S(IV)]=0.0404M. B=[glucose]=0.8M. [glycine]=0.5M. [S(IV)]=0.0398M. Rb [S(IV)]=Reversibly bound [S(IV)].

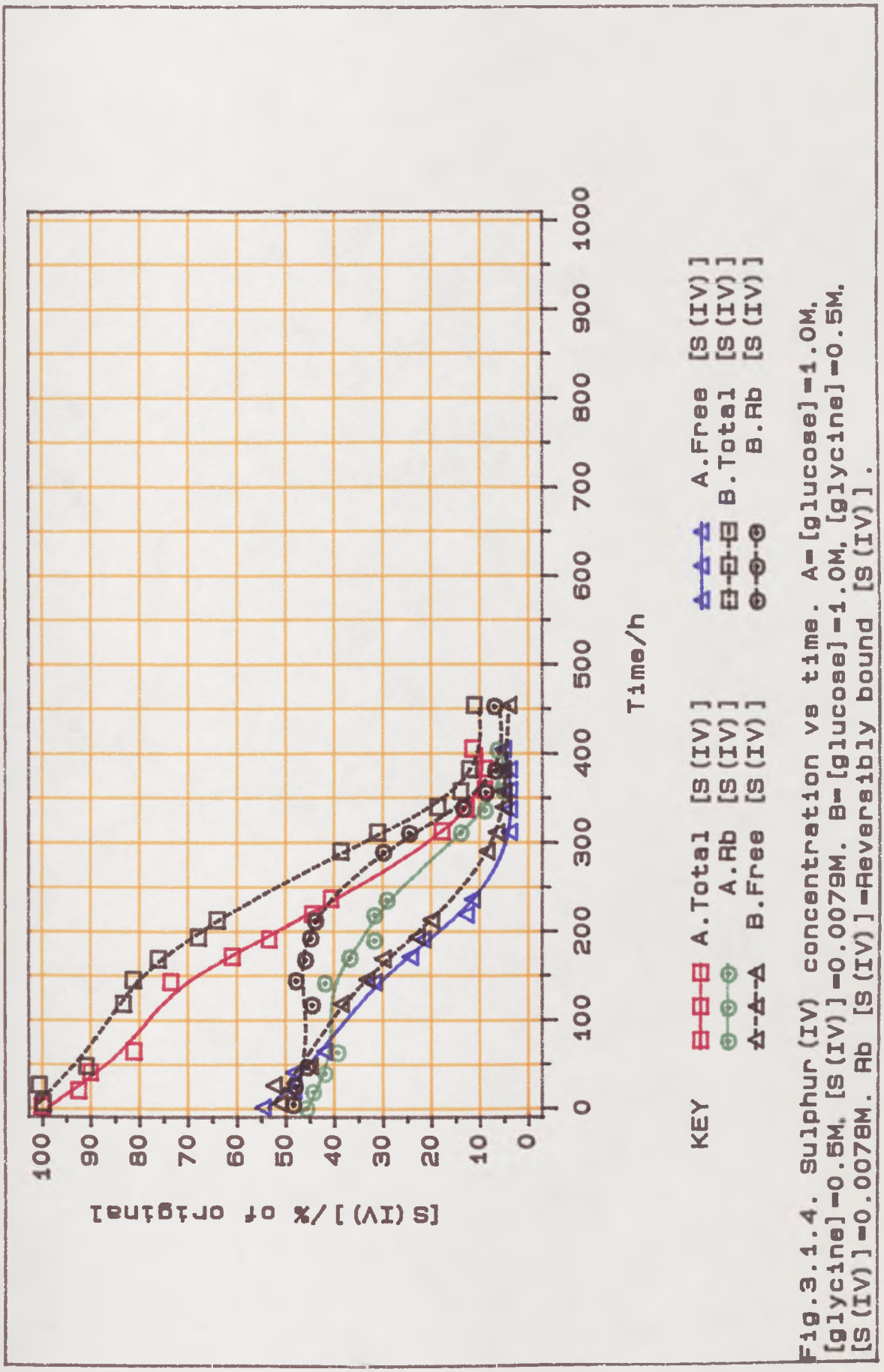


Fig.3.1.4. Sulphur(IV) concentration vs time. A=[glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0079M. B=[glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078M. Rb [S(IV)]=Reversibly bound [S(IV)].

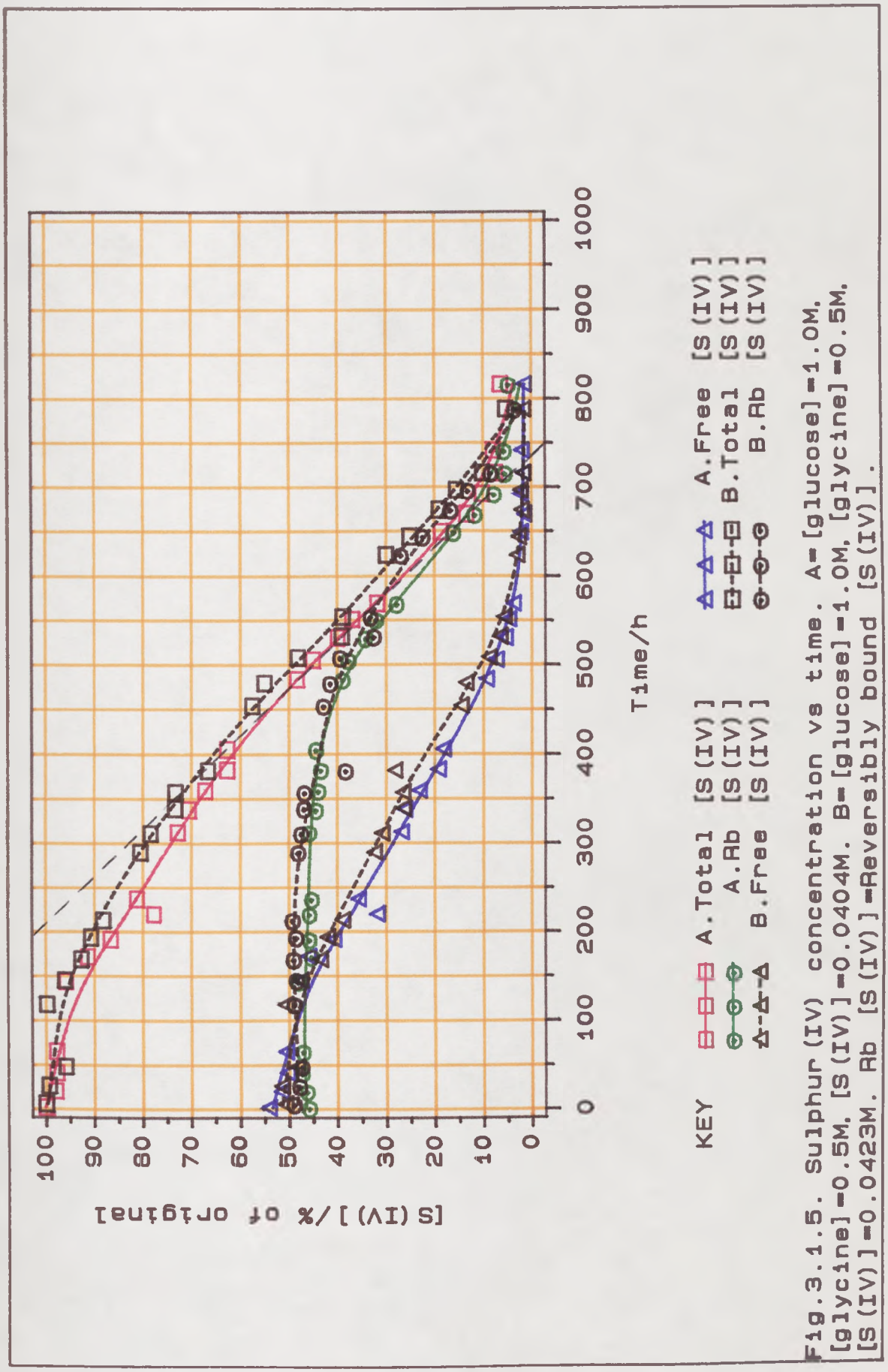


Fig.3.1.5. Sulphur(IV) concentration vs time. A=[glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0404M. B=[glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0423M. Rb [S(IV)]=Reversibly bound [S(IV)].

as illustrated for the mixture of composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0404 M. Reaction mixtures with the same glucose and glycine concentrations had differing initial S(IV) concentrations, because the mixtures were made up fresh for each experiment.

Glucose reacts with HSO_3^- to form glucose hydroxysulphonate as follows:

$$\underset{\text{(glucose)}}{RCHO} + \underset{\text{(S(IV))}}{HSO_3^-} \rightleftharpoons \underset{\text{(glucose hydroxysulphonate)}}{RCHOHSO_3^-}$$

and the dissociation constant for the adduct is given by:

$$K = [RCHO][HSO_3^-] / [RCHOHSO_3^-]$$

McWeeny *et al* (1969) demonstrated that at pH 4.3 and pH 5.5, equilibrium has been established by 24 h. Values of the constant rate during the linear portion of the concentration-time curve and the apparent equilibrium constant, K, for the formation of glucose hydroxysulphonate, after 24 h, are shown in table 3.1.1. Vas (1949) showed that the value of the apparent equilibrium constant is independent of glucose and S(IV) concentration at 20 °C and only slightly dependent on pH; its value is least between pH 3 and 5.5 and increases rapidly on the acid or alkaline side. This is in agreement with McWeeny *et al* (1969), who found that at pH 5.5 and 4.3 the values of K were 1.30 and 1.58 M respectively at 55 °C. The average value of the dissociation constant of glucose hydroxysulphonate, K, measured here over a 2.5 fold range of glucose and a 5.4 fold range of S(IV) concentration at pH 5.5 and 55 °C is 1.05 ± 0.04 M, n=8; this is

[glucose] /M	[glycine] /M	Initial [S(IV)] /M	K /M	pH	Rate of irreversible S(IV) binding /10^{-5} M h^{-1}
0.4	0.5	0.0365	0.96	5.43	4.01
0.4	0.5	0.0400	1.04	5.36	4.54
0.8	0.5	0.0398	1.05	5.47	5.96
0.8	0.5	0.0404	1.04	5.34	5.70
1.0	0.5	0.0078	1.08	5.33	2.69
1.0	0.5	0.0079	1.09	5.27	2.65
1.0	0.5	0.0423	1.05	5.48	7.18
1.0	0.5	0.0404	1.09	5.37	7.48

Table 3.1.1. Dissociation constant, K, for glucose hydroxysulphonate, reaction mixture pH after measurement of K and rate of irreversible S(IV) binding during the constant rate phase for duplicate reaction mixtures at 55 ^{o}C.

lower than the literature value of 1.30 M (Mcweeny *et al*, 1969). The reproducibility of the value for K between duplicate mixtures and mixtures of differing composition in the present work is to be expected, because when K was measured the pH of the reaction mixtures was similar, see table 3.1.1. In addition to there being little variation in pH between duplicate reaction mixtures, it can be seen that such mixtures also produce very similar values of reaction rate.

Wolfrom *et al* (1953) showed that acid is liberated during the reaction of xylose with glycine. However, he also showed that the rate of this Maillard reaction is independent of pH in the range pH 3-7. Vacalis (1986) showed that variation of the initial pH in the range 4.5-5.5 had no effect on the constant rate of reaction in the system glucose-glycine-S(IV), but there is an increase in the rate of loss of S(IV) above pH 5.5. However, the glucose-glycine reaction is catalysed by S(IV). At pH 5.5 the concentration of the predominant

S(IV) species, HSO_3^-, will be relatively unaffected by a small change in pH, but, such a change in pH will significantly affect the amount of the minor S(IV) species, e.g. SO_3^{2-}. A change in the amount of SO_3^{2-} may also change the extent to which the Maillard reaction is catalysed and, therefore, the reaction rate. To test whether or not this is the case, it was decided that reaction mixture pH would be held constant. This should be by addition of NaOH in a pH-stat arrangement rather than the use of buffers, as buffers may lead to acid/base catalysis of the Maillard reaction (Wedzicha and Vacalis, 1988).

Reaction mixture pH was recorded and adjusted to pH 5.5 on each occasion the mixture was analysed for S(IV). The maximum variation in the pH during the operation of the pH-stat is 0.82 pH units, (typically the variation was between 0.1 and 0.3 pH units) Fig.3.1.8 (a). This change in pH during the course of an experiment is much smaller than permitted by Vacalis (1986) who used no pH control and represents a compromise between frequency of adjustment of the pH-stat and rate of acid production. Any effect of pH on the reaction should be somewhat smaller than in previously reported work.

From Fig.3.1.4 it can be seen that for repeat measurements of the maximum deviation between values of free or total S(IV) was 16%. The mixture of composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0404 M, Fig.3.1.5 gave S(IV) concentrations that were more reproducible than mixtures with lower glucose or S(IV) concentrations. For this mixture the maximum deviation is 5%. Glucose and glycine can inhibit S(IV) autoxidation (Sapotnikskii and Glushchenko, 1962; Wedzicha, private communication). In mixtures containing low concentrations of glucose or glycine the extent of S(IV) autoxidation may vary due to natural variation in the concentration of dissolved

transition metal ions, that could catalyse S(IV) autoxidation, in solution. Clearly, autoxidation of S(IV) is less likely in mixtures of high concentration. With the exception of the mixture of composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0404 M, the mixtures tested for reproducibility represent the least concentrated systems studied during kinetic experiments in the present work. It is expected, therefore, that results from more concentrated mixtures will be less likely to include contributions from S(IV) autoxidation. It should be noted that the most important information obtained from these mixtures is the rate of irreversible S(IV) loss during the constant rate period; as shown these rates are reproducible.

3.1.2. Investigation of the model system glucose-glycine-S(IV).

3.1.2.1. Measurement of reaction mixture pH.

It was observed at an early stage that the pH of reaction mixtures prepared as described by previous workers (Mcweeny *et al.*, 1969; Wedzicha and Vacalis, 1988) changed with time. This is illustrated for reaction mixtures of composition [glucose] = 0.4-2.0 M, [glycine] = 0.2-1.0 M, [S(IV)] = 0.0886-0.0988 M in Figs.3.1.6 and 3.1.7. It can be seen that over the timescale of S(IV) loss the pH changes by up to 2.3 units. The observed rate of pH change is a result of the opposing effects of:

(a) The rate of acid production.

(b) The buffering capacity of the reaction mixture components.

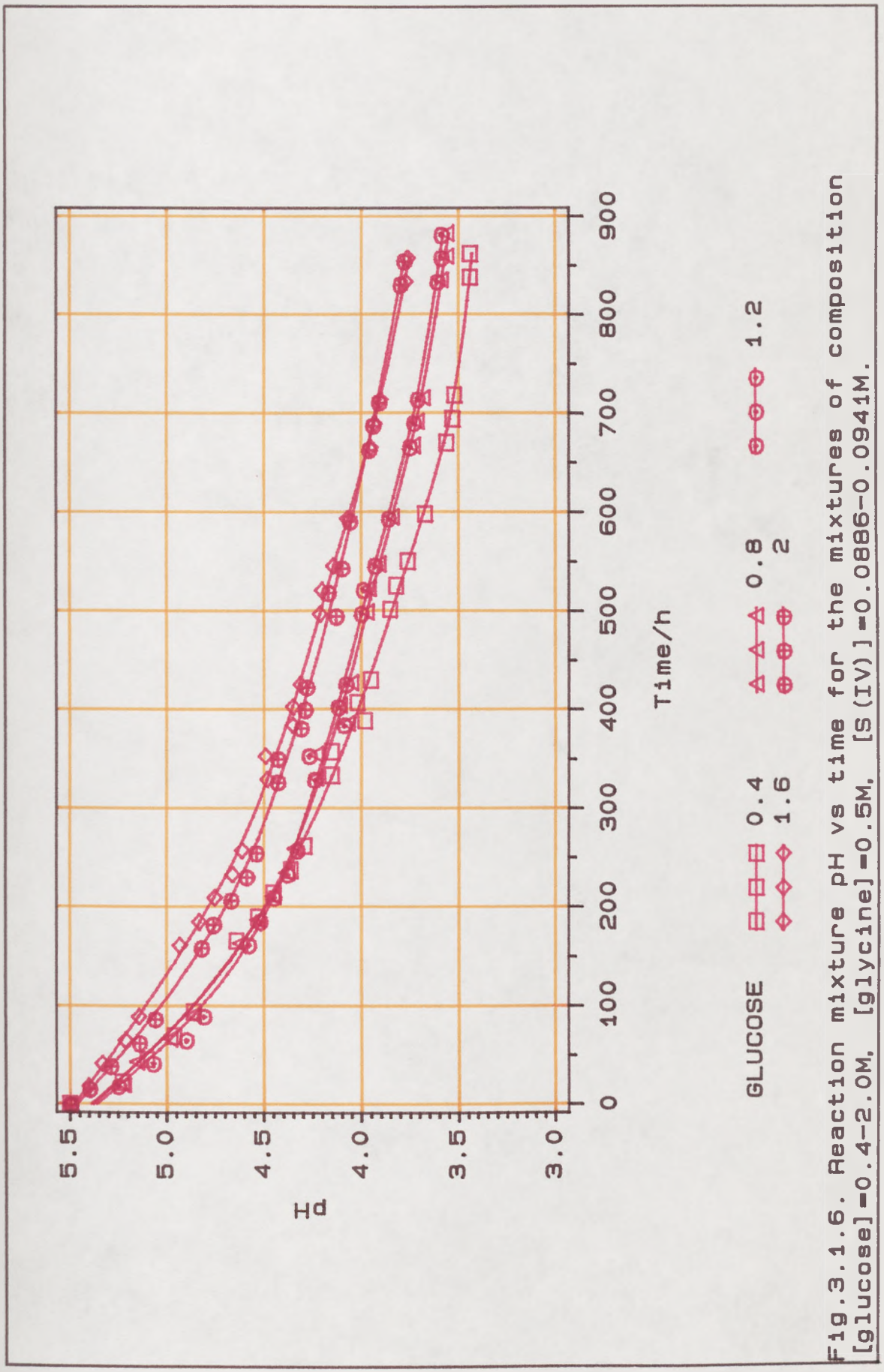


Fig.3.1.6. Reaction mixture pH vs time for the mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0886-0.0941M.

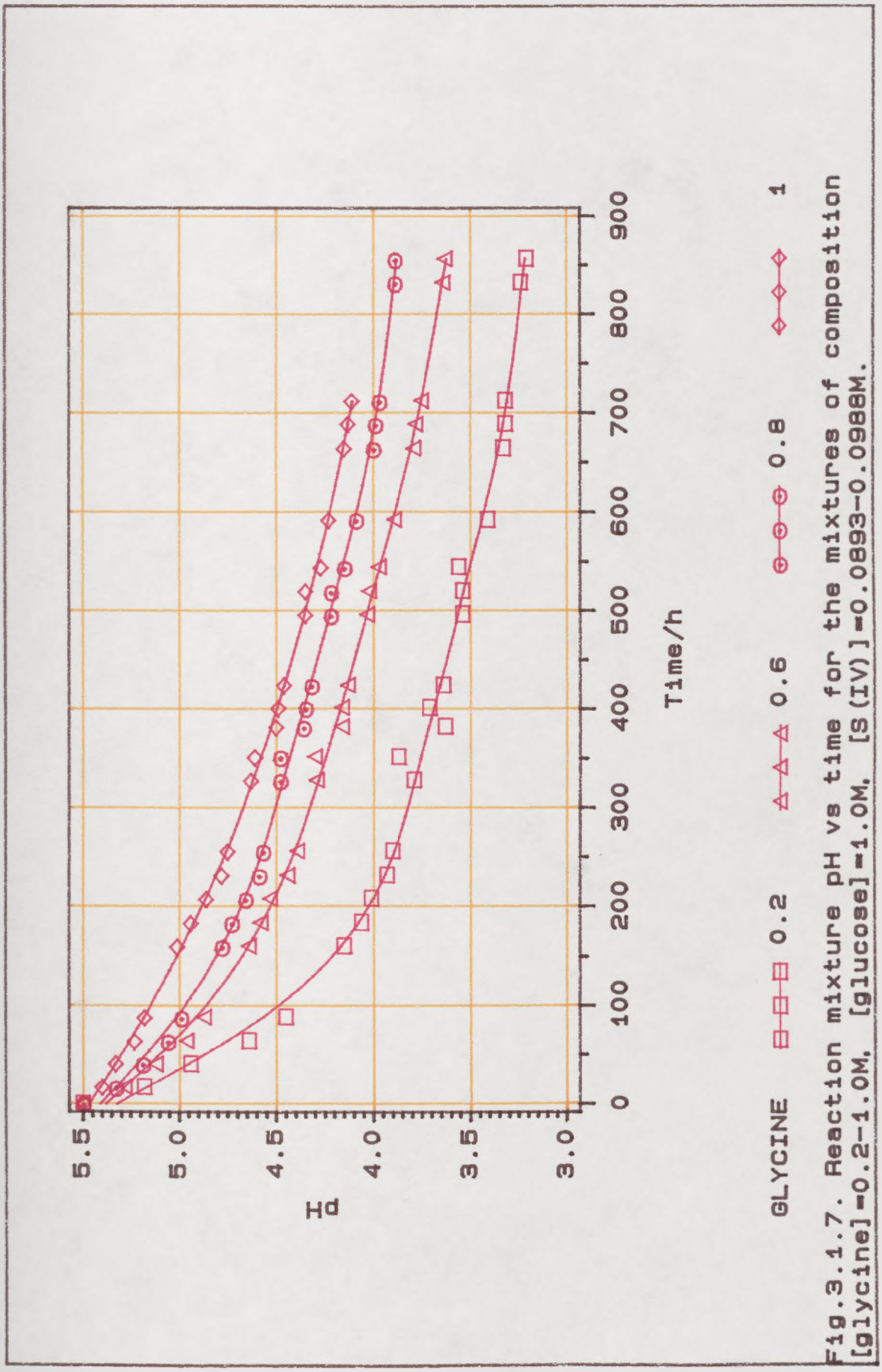


Fig.3.1.7. Reaction mixture pH vs time for the mixtures of composition [glycine]=0.2-1.0M, [glucose]=1.0M, [S(IV)]=0.0893-0.0988M.

An increase in glucose and particularly glycine concentration reduces the magnitude of pH change. This may be the result of a buffering action of glycine which has a $pK_a = 2.35$. Thus, at pH 5.5 glycine has little buffer capacity, but this will increase with decreasing pH and will contribute to the reduction in the rate at which the pH falls with increasing time. This may be demonstrated quantitatively by calculating the proportion of ionised carboxylic acid at pH 5.5 (the initial pH) and the pH at a given reaction time, say, 700 h where there is a clear difference in pH between reactions. The fraction of acid ionised is given by:

$$[A^-] / C = K / (K + [H^+])$$

Where C is the total concentration of glycine, i.e. ionised + unionised forms, K is the dissociation constant for the carboxyl group of glycine, $[A^-]$ is the concentration of the carboxylate anion and $[H^+]$ is the hydrogen ion concentration.

Thus, for the run shown in Fig.3.1.7 one obtains the following data at *c.* 700 h.

Initial [glycine]/M	1.0	0.8	0.6	0.2
pH at 700h	4.13	3.99	3.78	3.32
$[A^-]$/M at pH x	0.98	0.78	0.58	0.18
$[H^+]$ formed /M	0.016	0.017	0.021	0.019

where $[H^+]$ formed = $[A^-]_{\text{at pH 5.5}} - [A^-]_{\text{at pH x}}$; pH x is the pH at 700 h reaction.

We see that the amount of acid formed is relatively independent of glycine concentration and the reason why the pH differs from run to run is the buffer action of glycine. This calculation, of course, assumes that no other acids with pK_a different from glycine are formed and that all the carboxyl groups which are present at t=0 are available after 700 h. Whilst this approach is limited as some

decarboxylation of glycine is possible during the reaction it at least provides a justification for saying that buffer action is possible. Glucose does not show acid base behaviour in the range pH 3.5-5.5. The effect of glucose concentration on pH change must be due to other interactions.

Previous workers investigating the S(IV)-inhibited Maillard reaction (Mcweeny *et al*, 1969; Wedzicha and Vacalis, 1988) did not control pH, although the pH at the start and end of the reaction was measured. Wedzicha and Vacalis (1988) investigated the effect of initial reaction pH on the rate of reaction, but it seems that their conclusion that "the rate of S(IV) loss, in the reaction between glucose, glycine and S(IV), is essentially independent of pH between pH 4.5 to 5.5, and that there is an increase in rate of S(IV) loss above pH 5.5", cannot be strictly applied. Therefore, it is important that the effect of pH control on the rate of irreversible S(IV) loss be studied.

3.1.2.2. Effect of pH control on reaction rate.

A pH-stat is an apparatus set up to maintain the pH of a solution constant by addition of acid or alkali in response to a change in pH. The long timescale involved in the reaction studied meant that it was not appropriate to dedicate a single piece of purpose-built apparatus to each run. Instead reactions were checked for pH at frequent intervals and alkali (NaOH) added to restore the pH to the initial value.

Figs.3.1.8 and 3.1.9 show:

(a) Reaction mixture pH*vs* time during operation of the pH-stat.

(b) $[H^+]$ liberated*vs* time obtained from the amount of NaOH added to control the pH.

(c) [S(IV)]*vs* time during operation of the pH-stat.

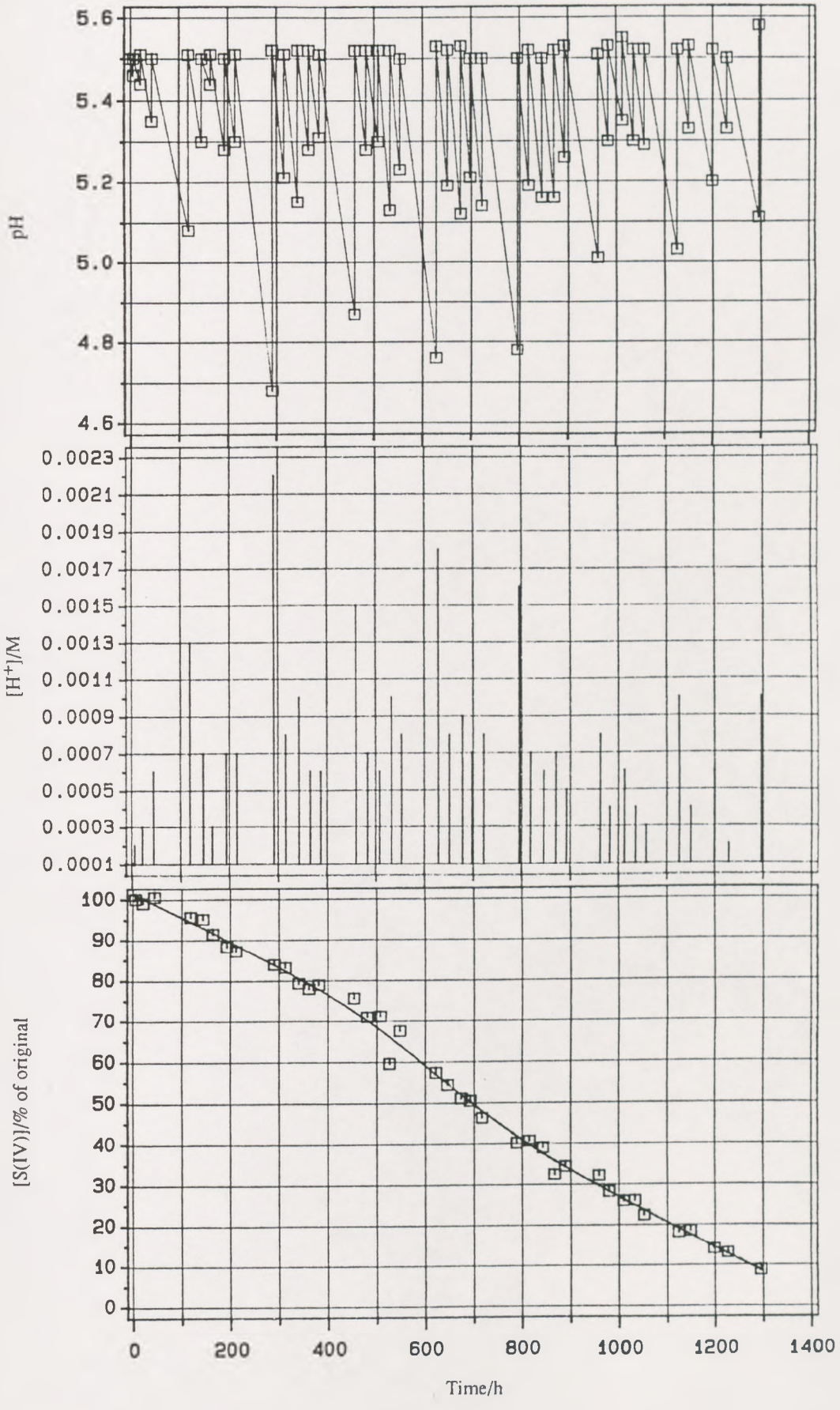


Fig.3.1.8. For the reaction mixture of composition [glucose] = 1.0 M, [glycine] = 0.2 M, [S(IV)] = 0.0406 M the following plots are shown: (a) reaction mixture pH *vs* time during operation of the pH-stat; (b) $[H^+]$ liberated *vs* time measured using the pH-stat; (c) [S(IV)] *vs* time during operation of the pH-stat.

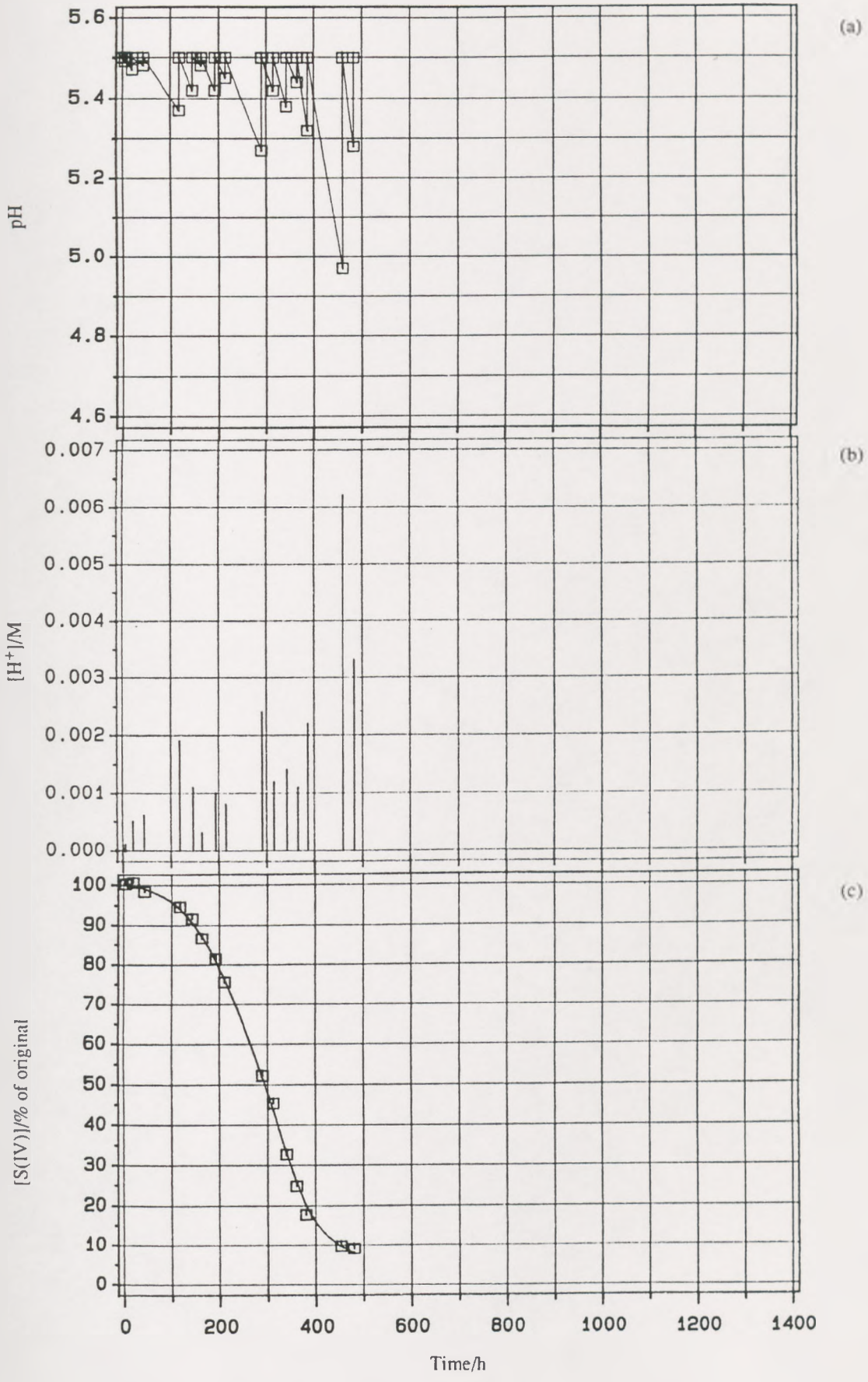


Fig.3.1.9. For the reaction mixture of composition [glucose] = 1.0 M, [glycine] = 1.0 M, [S(IV)] = 0.0423 M the following plots are shown: (a) reaction mixture pH *vs* time during operation of the pH-stat; (b) $[H^+]$ liberated *vs* time measured using the pH-stat; (c) [S(IV)] *vs* time during operation of the pH-stat.

for the mixtures containing 0.2 and 1.0 M glycine. From these figures it can be seen that during operation of the pH-stat, the reaction mixture pH oscillates as acid is liberated and the pH is corrected to 5.5; also, the lowest pH values during operation of the pH-stat correlate to the highest acid concentrations measured. The pH was normally adjusted at intervals of 24 h, however, the largest changes in pH always occur after the intervals of 72 h. These concentrations are a measure of the H^+ concentration liberated over this period of time and are, therefore, somewhat larger than the values obtained on a daily basis. The effect of maintaining the pH on the rate of irreversible S(IV) loss is examined below.

In order to examine whether or not pH control has an effect on the rate of S(IV) loss, it is necessary to compare data from mixtures with and without pH control. It was decided to choose mixtures of composition [glucose] = 2.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0926-0.1004 M and the data are summarised in Fig.3.1.10. It can be seen that when reaction mixture pH is maintained at pH 5.5 there is a significant increase in reaction rate indicated by irreversible S(IV) loss during the constant rate period. In addition, the length of the induction phase is reduced, leading to S(IV) being irreversibly lost at an earlier time.

Wedzicha and Garner (1991) showed that the steady state concentration of DH can be obtained from the expression:

$$[DH]_{steady\ state} = [S(IV)]_{int} - [S(IV)]_o$$

where $[S(IV)]_{int}$ is the concentration of S(IV) obtained when the linear constant rate phase is extrapolated back to time zero and $[S(IV)]_0$ is the measured S(IV) concentration at time zero. From Fig.3.1.10 $[S(IV)]_{int}$ can be obtained for the mixtures with and without pH

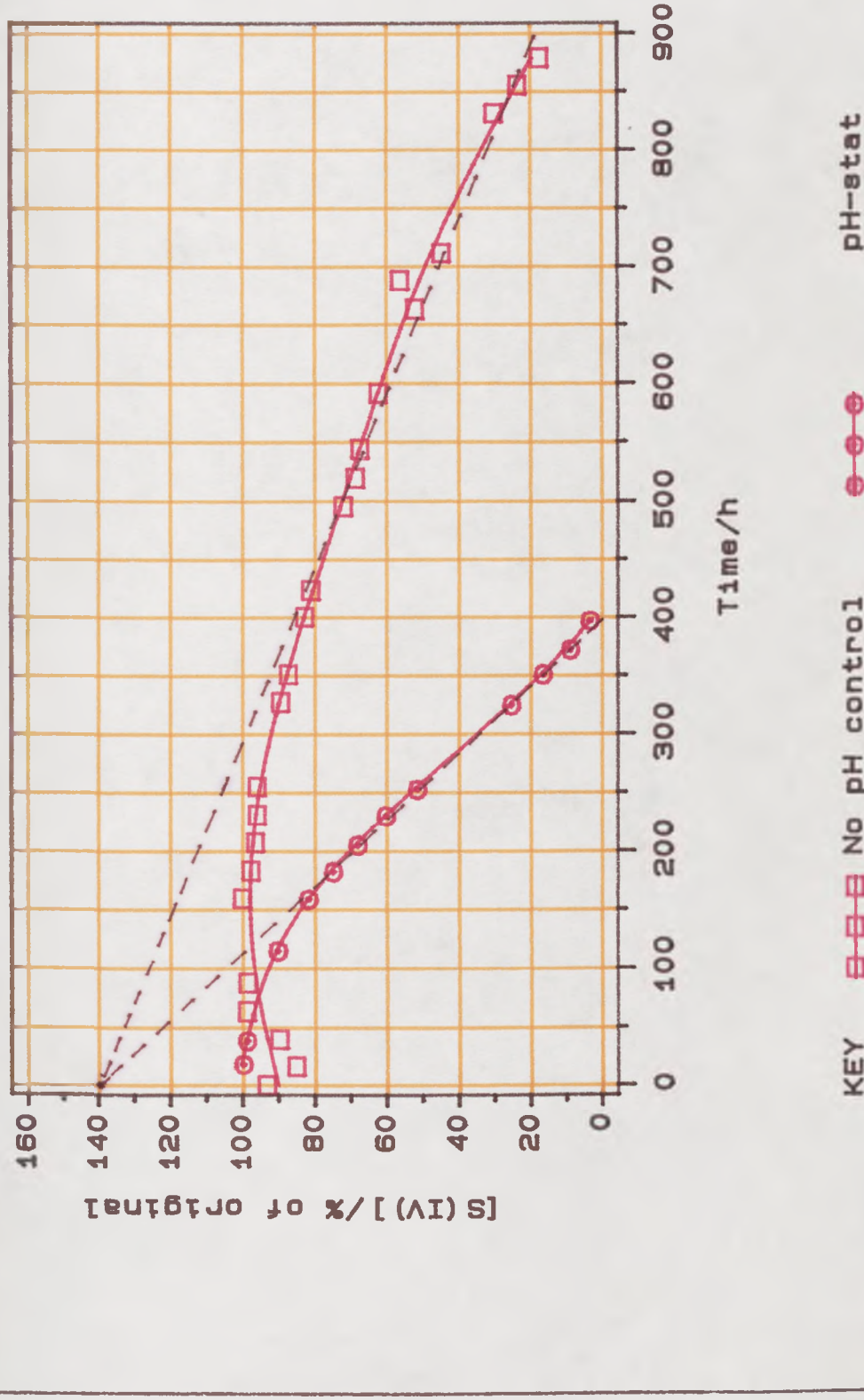


Fig.3.1.1.10. Effect of pH-stat on the rate of irreversible S(IV) loss for the mixtures of composition [glucose]=2.0M, [glycine]=0.5M, [S(IV)]=0.0926-0.1004M.

control and the calculated steady state DH concentrations are thus:

	$[S(IV)]_{int}$ /M	$[S(IV)]_o$ /M	$[DH]_{steady\ state}$ /M
pH-stat	0.1406	0.1004	0.0402
No pH control	0.1296	0.0926	0.0370

A fall of over 1.5 pH units has only a small effect on the steady state DH concentration. Wedzicha (1984a) showed that the steady state DH concentration can also be calculated as the ratio of the rate constants for DH formation and its conversion to I2; the small effect of pH may be the result of both of these processes being affected to a similar extent by pH. If only DH formation or loss were sensitive to pH, then the ratio of the rate constants and hence the steady state DH concentration would be affected by pH.

The initial step of the reaction between glucose and glycine involves formation of an addition compound; this is seen either as a glycosylamine or a Schiffs base. Wedzicha and Kaban (1986) also suggested the involvement of a Schiffs base to account for the catalysis by glycine of DSH formation from DH. A drop in pH of the model system glucose-glycine-S(IV) may lead to protonation of the amino group of glycine, thus inhibiting nucleophilic attack on a carbonyl group of DH or glucose. This would account for the increase in reaction rate when the pH is maintained as compared to the rate obtained when the pH is allowed to fall. If DH formation and loss both involve Schiffs base production, then the rates of these processes may be affected by pH to the same extent. Since DH concentration is the ratio of the rate constants for its formation and loss, the steady state DH concentration may remain unaffected despite the different rates of S(IV) loss. Alternatively, the first step of the facile irreversible rearrangement of the aldosylamine formed from glucose and glycine

to produce the ketoseamine, involves reversible formation of a cation of the Schiffs base by protonation of the aldosylamine, this step will be pH dependent. Suppose this species is rate determining. The reaction of DH with S(IV) proceeds by rate-determining transformation of DH which does not involve S(IV), but which is speeded up in the presence of glycine (Wedzicha and Kaban, 1986). A possible involvement of the amino acid can be discussed by reference to the well known reaction of DH with amines to form N-substituted pyrroles (Kato and Fujimaki, 1970). It is speculated that reversible protonation of the Schiffs base of DDH occurs as part of the rate-determining step. Thus both the formation of DH and its loss are similarly affected by pH.

Wedzicha and Garner (1991) obtained a steady state DH concentration of 6 mM when the initial S(IV) concentration was 43.2 mM. The large difference between the results of Wedzicha and Garner (1991) and those reported here may be due to the different glucose and initial S(IV) concentrations used, as the rate of the S(IV)-inhibited Maillard reaction has been shown to be dependent on glucose, glycine and initial S(IV) concentration (Wedzicha and Vacalis, 1988).

3.1.2.3. Order of reaction.

Kinetic order with respect to the reactants has been determined from the empirical relationship between reaction rate and concentration. Rate, for the sulphite-inhibited Maillard reaction, is obtained by measuring irreversible S(IV) loss during the constant rate period. Control of pH has been shown to significantly affect the rate of irreversible S(IV) loss during this period. It is important, therefore, that the kinetic order of reaction be determined for each of the

reactants under conditions of "constant" pH, in contrast with the work carried out by Vacalis (1986).

From the kinetic model in Fig.1.4.6 it can be seen that glycine reacts with glucose during formation of DH and is subsequently released. The production of melanoidins requires the presence of an amino compound (Wedzicha, 1984b). However, while sufficient S(IV) is present to inhibit melanoidin formation the concentration of glycine is expected to be constant provided that Schiffs base adducts with products such as DSH are not formed in significant amounts. When [glucose] >> [S(IV)] and one assumes that formation of DSH is the result of loss of one molecule of glucose and one molecule of S(IV), only a small fraction of the glucose present reacts to form DSH. Therefore, glucose concentration may be taken as constant provided that a significant concentration is not converted into Schiff's base or reaction intermediates such as MFG or DFG. Hydrogen sulphite ion binds irreversibly to DH via DDH to form DSH; consequently the concentration of S(IV) gradually falls to zero. Reaction rate is shown as a function of glucose, glycine, and initial S(IV) concentrations in Figs.3.1.11 to 3.1.13 respectively. Convincing first order behaviour is shown in each case. Correlation coefficients of 0.9896, 0.9944, and 0.9889 respectively were obtained; these results are a significant improvement on previously reported data; Vacalis (1986) obtained correlation coefficients of 0.9700, 0.9673 and 0.9683 respectively.

An intercept was obtained when rate was plotted*vs* initial S(IV) concentration. It is inferred that production of DH occurs by two parallel pathways, one of which is catalysed by S(IV) (Wedzicha and Vacalis, 1988). No such intercept was obtained when rate was plotted*vs* glucose or glycine concentration, indicating that glucose and glycine are involved in both pathways for DH formation. The rate

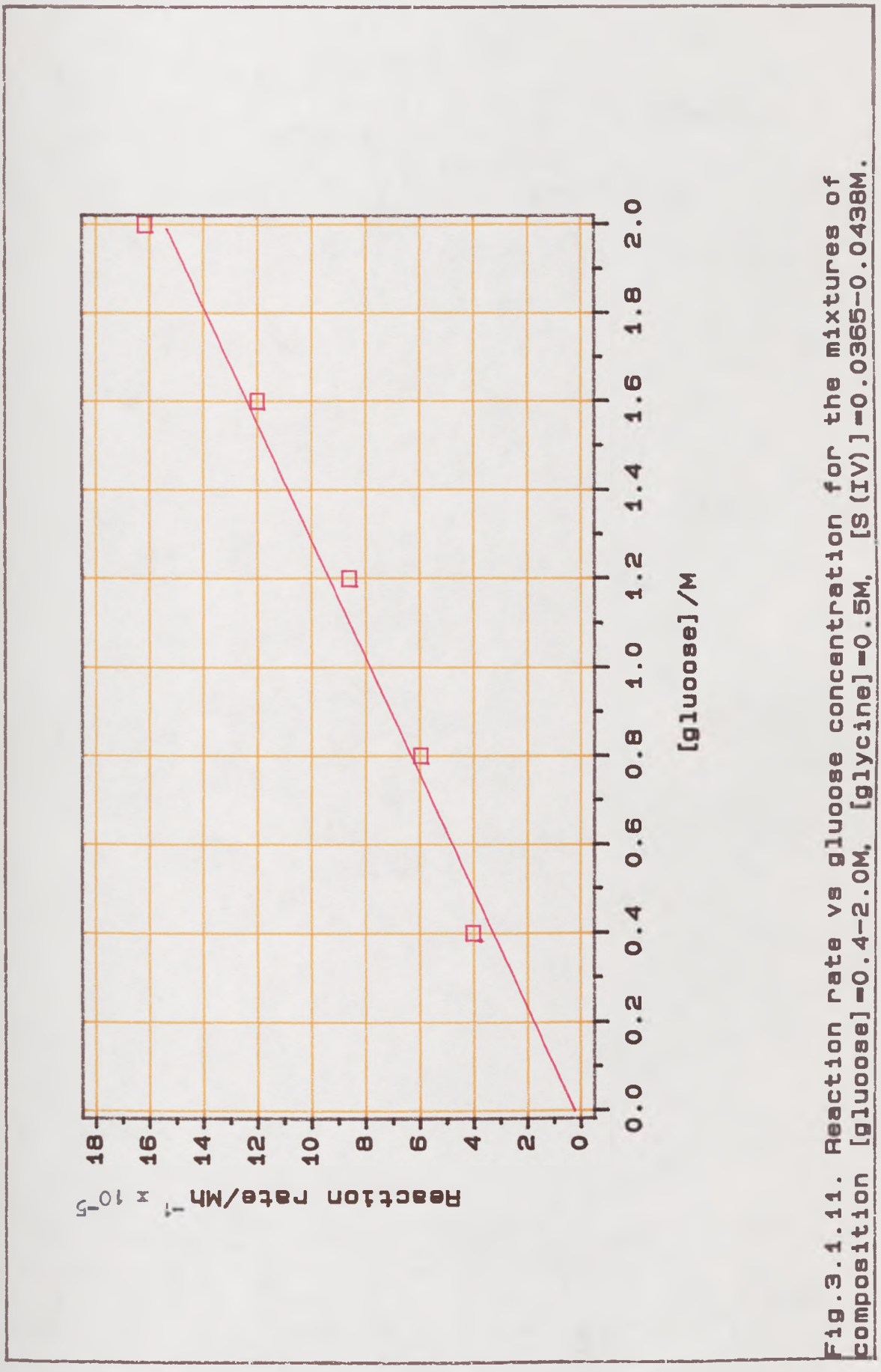


Fig.3.1.11. Reaction rate vs glucose concentration for the mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M.

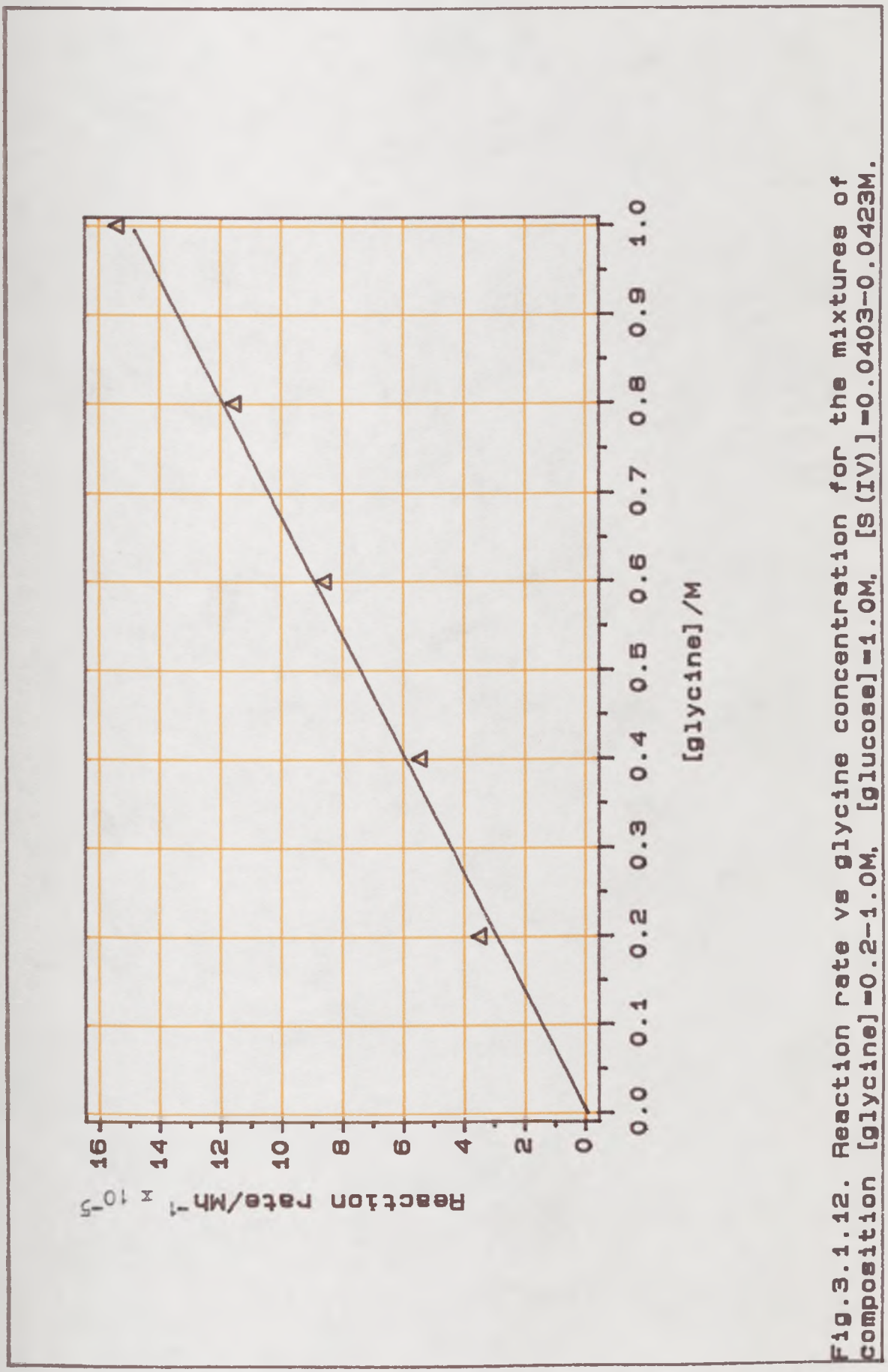


Fig.3.1.12. Reaction rate vs glycine concentration for the mixtures of composition [glycine]=0.2-1.0M, [glucose]=1.0M, [S(IV)]=0.0403-0.0423M.

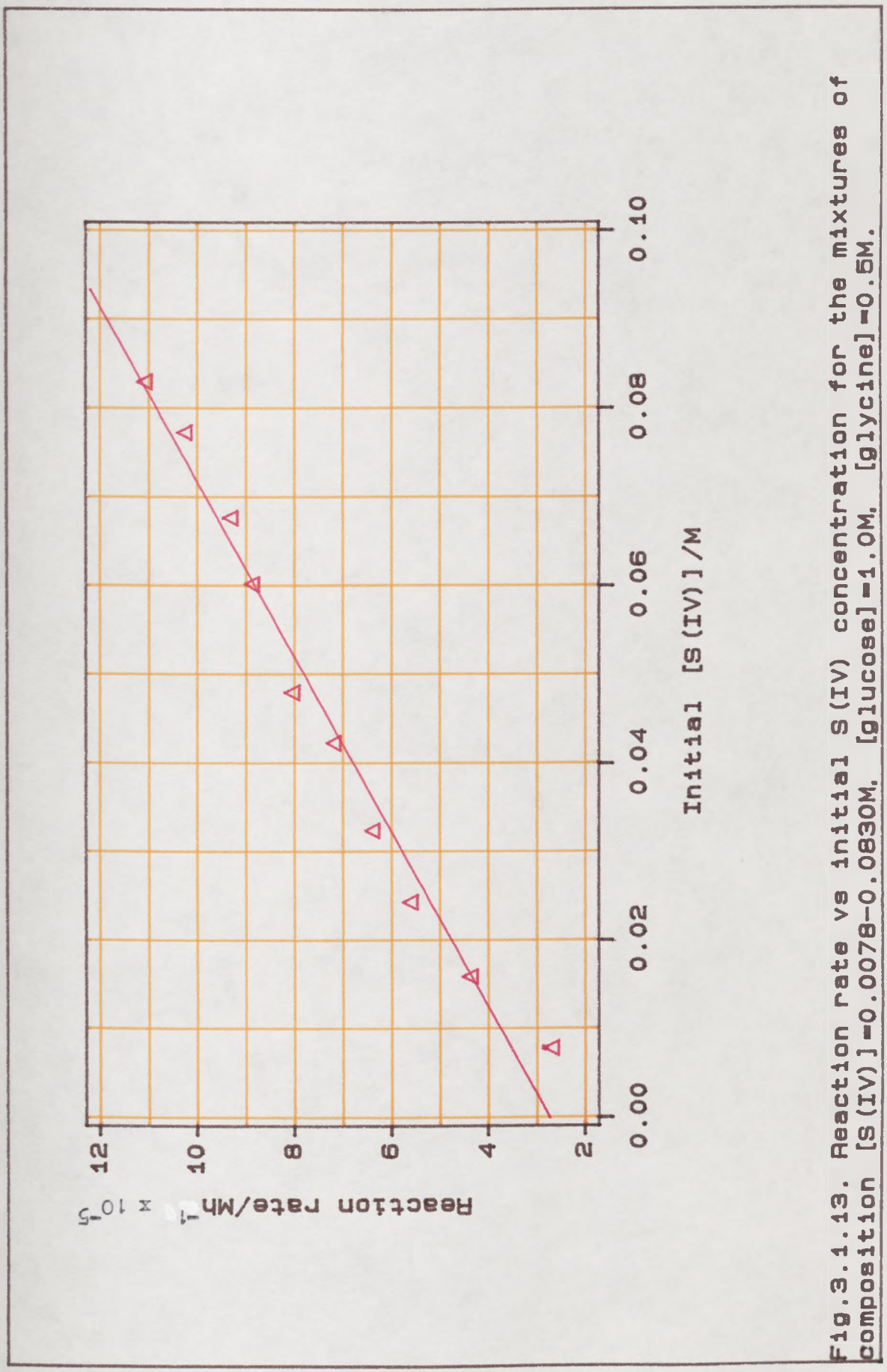


Fig.3.1.13. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0078-0.0830M, [glucose]=1.0M, [glycine]=0.5M.

equation for the constant rate phase of the reaction between glucose,glycine and S(IV) is, therefore, as shown in equation 3.1.1:

$$\text{Rate} = k_1[\text{glucose}_f][\text{glycine}] + k_2[\text{glucose}_f][\text{glycine}][\text{S(IV)}_f] \quad ...(3.1.1)$$

where the subscript f denotes the free species. It is assumed that only free glucose and S(IV) are kinetically significant. The initial step of the Maillard reaction is nucleophilic attack by the amino group of glycine on the carbonyl group of glucose. It is reasonable, therefore, to assume that free glucose is the kinetically significant species, because hydroxysulphonate formation removes the reactive carbonyl group. Free S(IV) is thought to be kinetically significant because it is not bound as part of any hydroxysulphonate adducts and is, therefore, available for reaction. A proportion of the free S(IV) may be involved in catalysis. Glycine reacts reversibly with glucose to form a Schiff's base, which undergoes an Amadori rearrangement to form monofructoseglycine (MFG); see section 1.1.4.1. Further reaction of MFG with glucose yields difructoseglycine (DFG), DH is formed either by decomposition of MFG or DFG; glycine is regarded as free as it as released when DH is formed.

The initial stages of the reaction occurring between glucose and glycine are summarised in Fig.3.1.14.

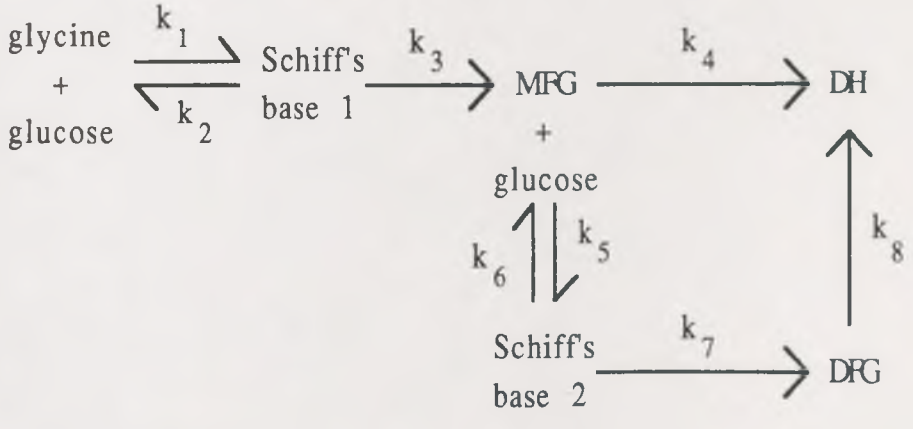


Fig.3.1.14. The initial stages of the glucose-glycine reaction.

It was shown in section 3.1.1.2 that there must be at least two consecutive steps occurring at rates with the same order of magnitude, in order to account for the induction period in the glucose-glycine-S(IV) reaction. It is interesting to examine the reaction scheme shown in Fig.3.1.14 by, in turn, considering each of the steps as rate-determining. The overall kinetics predicted may then be compared to the observed kinetics, calculated from measurements made during the constant rate phase of the reaction, to deduce the likelihood of a particular combination of steps occurring.

If $k_1 << (k_2\text{-}k_8)$ the rate of the overall reaction would be given by:

$$\text{Rate} = k_1[\text{glucose}][\text{glycine}]$$

assuming that formation of a Schiff's base is a second order kinetic process. The rate expression shown is consistent with the observed kinetics, the second of the two consecutive slow steps being conversion of DH to DSH.

The same kinetics will be observed if $k_3 << (k_1,\ k_2,\ k_4\text{-}k_8)$ but now the rate of reaction depends on the concentration of Schiff's base, i.e:

$$\text{Rate} = k_3[\text{Schiff's base}_1]$$

Since the Schiff's base is formed as a result of rapid equilibrium:

$$k_1 / k_2 = [\text{Schiff's base}_1] / [\text{glucose}][\text{glycine}]$$

and $[\text{Schiff's base}_1] = ([\text{glucose}][\text{glycine}]\ k_1) / k_2$

$$\therefore \quad \text{Rate} = (k_3\ k_1[\text{glucose}][\text{glycine}]) / k_2$$

$$= k[\text{glucose}][\text{glycine}]$$

where $k = (k_3\ k_1) / k_2$

The situation which exists when $k_4 << (k_1\text{-}k_3,\ k_5\text{-}k_8)$ is different from that above, because the conversion of Schiff's base to MFG is

irreversible.

$$\text{Rate} = k_4[\text{MFG}]$$

If the processes leading to the formation of MFG are fast compared with the rate of formation of DH from MFG, then there would be very rapid and, in theory, quantitative formation of MFG. Therefore, the concentration of MFG will equal the concentration of the limiting component, i.e. glucose or glycine. Hence, the derived rate expression may be rewritten:

$$\text{Rate} = k_4[\text{limiting component}]$$

When the order of reaction with respect to glucose was obtained in the present experiments, the concentrations of glucose and glycine were 0.4-2.0 M glucose and 0.5 M glycine respectively. If k_4 was the rate-determining step, then, we would not expect to see an increase in reaction rate above 0.5 M glucose, because the concentration of glycine would be rate-limiting. The first order behaviour of the glucose-glycine-S(IV) reaction with respect to glucose demonstrates that this is unlikely to be the case. In addition, in a reaction mixture containing 1 M glucose and 0.5 M glycine, hydrogen ion would be produced very rapidly at 0.5 M due to the quantitative binding of the NH_2 group of glycine. However, it can be seen from Figs.3.1.20-3.1.24 that the highest concentration of liberated H^+, by the end of the reaction, from unbuffered reaction mixtures where the concentration of glycine was in the range 0.2-1.0 M was 0.0331 M. It seems unlikely that the reaction of glucose with glycine will proceed by a scheme in which k_1-k_3 are fast. Rather, it appears that the rate-determining step could involve either k_1 or k_3. Present knowledge of the Maillard reaction points to the rate-determining step being part or the whole of the Amadori rearrangement, i.e. k_3. However this requires confirmation.

Having discussed the possibilities for the steps involving k_1-k_4 being rate-determining followed by slow conversion of DH to DSH, it is now possible to suggest combinations of slow steps within the scheme shown in Fig.3.1.14 to give the observed kinetics. Here we begin with the premise that the rate of MFG formation is rate-determining. One such combination of slow steps would be if $(k_3 \approx k_4) << (k_1,\ k_2,\ k_5\text{-}k_8)$; i.e. where the formation of MFG and its decomposition were at comparable rates. In such a situation the rate of MFG formation is given by:

$$\text{Rate} = k_3[\text{Schiff's base}_1]$$

and the rate of MFG loss is given by:

$$\text{Rate} = k_4[\text{MFG}]$$

The kinetic behaviour of such a reaction is that at t=0 the rate of formation of DH is zero and this will build up to a steady state rate of formation of DH, such that:

$$k_4[\text{MFG}] = k_3[\text{Schiff's base}_1]$$

i.e. $[\text{MFG}]_{\text{steady state}} = [\text{MFG}]_{ss} = k_3[\text{Schiff's base}_1] / k_4$

At the steady state:

$$d[\text{DH}]_{ss} / dt = -d[\text{MFG}] / dt = k_4[\text{MFG}]_{ss}$$

Substituting for $[\text{MFG}]_{\text{steady state}}$ gives:

$$d[\text{DH}]_{ss} / dt = k_3[\text{Schiff's base}_1]$$

However, as $[\text{Schiff's base}_1] = ([\text{glucose}][\text{glycine}]\ k_1) / k_2$

$$d[\text{DH}]_{ss} / dt = (k_3k_1[\text{glucose}][\text{glycine}]) / k_2$$

This equation is consistent with the observed kinetics, because in this new model DH is rapidly converted to DSH with loss of S(IV), i.e.,

$$-d[\text{S(IV)}] / dt = -d[\text{DH}] / dt$$
$$= K'[\text{glucose}][\text{glycine}]$$

where $K' = k_3k_1 / k_2$.

Alternatively, if k_1 was rate-determining and $(k_1 \approx k_4) << (k_2,$

k_3, k_5–k_8), then the rate of formation of Schiff's base 1, SB1, is given by:

$$d[SB1] / dt = k_1[glucose][glycine]$$

This is comparable to the rate of MFG loss, which is given by:

$$-d[MFG] / dt = k_4[MFG]$$
$$= d[DH] / dt$$

Therefore, at the steady state:

$$k_4[MFG] = k_1[glucose][glycine]$$
$$[MFG]_{ss} = k_1[glucose][glycine] / k_4$$
$$\therefore \quad d[DH] / dt = k_1[glucose][glycine]$$

This is consistent with the observed kinetics of the reaction.

Another combination would be if $(k_1 \approx k_3) << (k_2, k_4, k_5–k_8)$. Hence, the rate of formation of Schiff's base 1 is given by:

$$d[SB1] / dt = k_1[glucose][glycine]$$

This is comparable to the rate of loss of Schiff's base, which is given by:

$$-d[SB1] / dt = k_3[SB1]$$

at the steady state:

$$k_3[SB1] = k_1[glucose][glycine]$$
$$[SB1] = (k_1[glucose][glycine]) / k_3$$
$$d[DH]/ dt = -d[SB1] / dt$$
$$= k_1[glucose][glycine]$$

This equation is also consistent with the observed kinetics. It should be appreciated that the intermediates have been given specific names to illustrate the kinetics but in fact one is always referring to the unknown transition state. Thus, in the reaction considered here, Schiff's base 1 is really an intermediate formed reversibly from the reactants, whilst MFG denotes a subsequent irreversible step. This predicted kinetic behaviour is consistent with the measurements

which were made during the steady state part of the reaction.

Another reaction scheme involves DFG and a reasonable kinetic model might have $\{(k_1 \text{ or } k_3) \text{ and } (k_5 \text{ or } k_7)\} << (k_4 \text{ and } k_8)$. Under these conditions, MFG would be rapidly converted to DH by-passing DFG. If, however, k_5 were large and the reaction of MFG with glucose favoured as far as equilibrium is concerned, then a route via DFG can be envisaged with k_7 representing the rate-determining step. In this situation the rate of formation and loss of MFG are given by,

$$d[MFG] / dt = k''[glucose][glycine]$$

$$-d[MFG] / dt = k'''[MFG][glucose]$$

where k'' and k''' are combinations of constants depending on which of the stages in formation of ketoseamine is rate-determining. At the steady state rate, the rate of formation of MFG equals its rate of loss, so,

$$k''[glucose][glycine] = k'''[MFG][glucose]$$

and the steady state concentration of MFG is

$$[MFG]_{ss} = (k''/k''')[glycine]$$

Thus, under steady state conditions the rate of formation of DFG is,

$$-d[MFG] / dt = d[DFG] / dt$$

$$= k''[glycine][glucose]$$

If the process controlled by k_8 is relatively fast and DH is rapidly converted to DSH, the model is also consistent with the observed kinetics. However, the published evidence (Wedzicha and Garner, 1991) points to a significant steady state concentration of DH being present and there is a need to propose a rate-determining step after the formation of DH. Hence, of the options presented here, the most likely is a mechanism with $(k_1 \text{ or } k_3) << (k_4\text{-}k_8)$. If the formation of DH required a mechanism via DFG, then the rate of this must be fast.

The reaction is also of first order with respect to S(IV), despite

the fact that there is no sulphur present in the reaction intermediate DH. It has been shown by Wedzicha and Garner (1991) that if the reaction mixture is spiked with ^{14}C-DH, then, DH and DSH are the only ^{14}C radioactive species at 23 days, by which time the concentration of S(IV) had fallen significantly and the S(IV)-inhibited Maillard reaction had gone almost to completion. The effect of S(IV) is, therefore, possibly one of catalysis as suggested by Wedzicha and Vacalis (1988). After an initial induction period the rate of loss of total S(IV) is essentially constant over the main part of the reaction. This presents us with a problem in kinetics because the concentration of the species alleged to be a catalyst falls with time and yet the rate of reaction is constant. During much of this time the level of reversibly bound S(IV) is essentially constant. It may be, therefore, that the Maillard reaction is catalysed by a reversibly bound S(IV) species, as this would account for the first order dependence of reaction rate on S(IV) concentration. Consider the following equilibria. Glucose and glycine react to produce a Schiff's base (3.1.2) and this base reacts with hydrogen sulphite ion to produce the Schiff's base hydroxysulphonate (3.1.3):

$$G + A \underset{}{\overset{K_1}{\rightleftharpoons}} GA \quad \text{.....(3.1.2)}$$

$$GA + S \underset{}{\overset{K_2}{\rightleftharpoons}} GAS \quad \text{.....(3.1.3)}$$

where G = glucose, A = glycine, GA = Schiff's base, S = S(IV) and GAS is the Schiff's base hydroxysulphonate. The formation constants for the adducts are given by:

$$K_1 = [GA] / [G][A] \quad \text{..... (3.1.4)}$$

$$K_2 = [GAS] / [GA][S] \quad \text{..... (3.1.5)}$$

rearrangement of (3.1.5) gives an expression for [GA] which can be substituted into (3.1.4) yielding:

$$K_1 = [GAS] / K_2[G][A][S]$$

which can be simplified to:

$$K_1' = [GAS] / [G][A][S]$$

where $K_1' = K_1K_2$. The two reactions may, therefore, be represented by the overall equation:

$$\underset{(g-x)}{G} + \underset{(a-x)}{A} + \underset{(s-x)}{S} \overset{K_1'}{\rightleftharpoons} \underset{x}{GAS}$$

This assumes that formation of GAS is the only reaction. The equilibrium concentrations of G and A also depend on how much GA is formed, therefore, it is assumed that the amount of GA is small.

If, however, $g >> s \quad \therefore \quad g-x \approx g$

and, $a >> s \quad \therefore \quad a-x \approx a$

then: $K_1' = x / ga(s-x)$ ..... (3.1.6)

If GAS is the catalytic species, then:

$$\text{Rate} \propto [GAS] = [x]$$

rearrangement of (3.1.6) yields:

$$x = (K_1'ga / 1 + K_1ga)s$$

If K_1' is small, then:

$$x = K_1gas$$

reaction rate depends on the concentration of S(IV); this is consistent with the observed kinetics of the S(IV)-inhibited Maillard reaction. If K_1' is large, then:

$$x = s$$

the concentration of x is equal to that of total S(IV). However, as S(IV) is present in both free and reversibly bound forms this cannot

be true. If $K_1'ga$ is approximately 1, then the concentration of x is *c.* half that of total S(IV); this could be true and the approach above serves to illustrate that the kinetics are not inconsistant with the formation of a distinct adduct such as GAS.

Alternatively, the catalysis of the Maillard reaction by S(IV) may be accounted for as follows. It is still reasonable to expect that the chemically reactive form of S(IV) is free S(IV). In the kinetic run with [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0423 M a constant rate of loss of total S(IV) of 7.2×10^{-5} M h^{-1} is observed between 300 and 700 h, but the concentration of free S(IV) falls from 0.0338 to 0.0064 M. A possible explanation for the constant rate is that as free S(IV) falls another process compensates for the expected reduction in rate. This would require a process that becomes faster as S(IV) concentration becomes smaller because the reaction appears to be catalysed by S(IV). Wedzicha and Kaban (1986) showed that, in isolation, DH will react with S(IV) to form a hydroxysulphonate adduct, but Wedzicha and Vacalis (1988) found that in order to obtain a satisfactory correlation between theoretical and experimental results in kinetic modelling of the glucose-glycine-S(IV) reaction, there was no need to postulate a hydroxysulphonate adduct of DH. However, it has been shown that control of reaction mixture pH affects the rate of irreversible S(IV) loss in the S(IV)-inhibited Maillard reaction. This raises the possibility that a satisfactory fit between experimental and theoretical results under conditions of constant pH might now require a kinetic model which includes DH hydroxysulphonate. The model used by Vacalis used 4 rate and 3 equilibrium constants and it is difficult, therefore, to obtain unique solutions for these when concentration-time data are fitted to the rate equation. A small change in concentration-time behaviour could well have a significant

effect on these rate constants or require the introduction of additional terms to ensure a good fit. The means of fitting the kinetic model is merely a mathematical exercise based on reasonable expectation of reaction mechanism. As S(IV) concentration falls during the course of a kinetic run any DH hydroxysulphonate adduct present in the model system glucose-glycine-S(IV) will dissociate releasing DH. Thus, the rate of formation of DH would be greater than would be expected from the reaction of glucose and glycine at that stage. This may compensate for the otherwise expected reduction in the rate of irreversible S(IV) loss.

It was reported in section 3.1.2.2 that the rate of irreversible S(IV) loss was increased when reaction mixtures are maintained at *c.* pH 5.5, compared to the rate measured when the pH was not controlled. It appears that the rate expression, equation 3.1.1, may require the addition of at least a hydrogen ion term or the improvement in the description of processes already included in the equation. As it stands the expression appears to be simplistic because it does not allow for the effect of the stated fall in pH on the reaction rate. If the expression is simplistic, then, constants derived using the current description are likely to be pseudo order and might, therefore, not be the true values although they still offer an empirical fit to the data. This will become apparent when the derived constants are used to calculate theoretical concentration-time data for comparison with experimental results. The concentrations of free and bound S(IV) are related as follows.

$$\underset{(g-x)}{G} + \underset{(s-x)}{S} \underset{}{\overset{K_1}{\rightleftharpoons}} \underset{x}{GS}$$

Where G, S and GS are glucose, S(IV) and glucose hydroxysulphonate

respectively; g, s and x represent the initial concentrations of glucose, S(IV) and the concentration of glucose hydroxysulphonate at equilibrium, respectively. The formation constant for the adduct is given by: $K_1 = [GS] / [G][S]$

However, if $g >> x$, then $g-x \approx g$, therefore:

$$K_1 = x / g(s-x) \quad \text{..... (3.1.7)}$$

Rearrangement of equation 3.1.7 yields:

$$x = (K_1 g / 1 + K_1 g)s$$

Free S(IV) concentration is given by:

$$(s-x) = s - (K_1 g / 1 + K_1 g)s$$
$$= s(1 - (K_1 g / 1 + K_1 g))$$

This shows that the concentrations of free and reversibly bound S(IV) are proportional to that of total S(IV). For simplicity the rate equation for the first step in the reaction can be written as:

$$\text{Rate} = k_1[\text{glucose}][\text{glycine}] + k_2[\text{glucose}][\text{glycine}][S(IV)_f]_o$$

assuming that the concentration of glucose is constant and that the total S(IV) concentration at time zero is directly proportional to the concentration of free S(IV) at time zero.

To obtain values of rate constants k_1 and k_2 the rate expression is reduced further to the equation of a straight line:

$$\text{Rate} = k_1' + k_2'[S(IV)_f]_0$$

Where $k_1' = k_1[\text{glucose}][\text{glycine}]$ and $k_2' = k_2[\text{glucose}][\text{glycine}]$. By plotting rate *vs* initial S(IV) concentration, k_1' and k_2' can be obtained from the intercept on the y axis and the gradient of the plot respectively, hence values of k_1 and k_2 can be calculated. These constants are shown with the values derived from the data of Vacalis (1986), along with correlation coefficients obtained for the respective plots, in table 3.1.2.

Author	k_1	k_2	Correlation
	/M^{-1} hr^{-1} X 10^{-5}	/M^{-2} hr^{-1} X 10^{-3}	coefficient
Bellion	5.5	2.0	0.98888
Vacalis	3.3	0.6	0.96826

Table 3.1.2. Rate constants for early steps in the uncatalysed, k_1, and S(IV)-catalysed, k_2, Maillard reactions; correlation coefficients are for plots of reaction rate *vs* initial S(IV) concentration.

It can be seen that values of k_1 and k_2 obtained in this work where the reaction mixtures were maintained at *c.* pH 5.50, are 1.7 and 3.4 times larger than the respective constants derived from the data of Vacalis (1986) who did not control pH. The straight line obtained here also shows a slightly better linear correlation coefficient. Wolfrom *et al* (1953) found that the rate of the Maillard reaction between xylose and glycine is independent of pH in the range pH 3-7 and it is not unreasonable to expect this to apply to the glucose-glycine reaction. Wolfrom *et al* (1953) studied the effect of pH on the development of UV-Visible absorbing products and the reasons for the reported observations are, of course, complex. Thus, for example, pH could have different and opposing effects on the rates of formation of intermediates such as DP (in the case of xylose), the conversion of DP to other precursors of melanoidins and subsequent reactions and the extinction coefficient of the melanoidin. A fortuitous balancing of the effects of pH on there reactions could give rise to an overall pH-independent process. However, the data reported here show that the uncatalysed reaction is relatively unaffected by pH when our "constant" pH data are compared with the variable pH data

of Vacalis (1986). The larger difference in values obtained for k_2 may be a genuine pH effect. The pH of each reaction mixture prepared by Vacalis is expected to fall significantly during the course of the reaction, possibly by some two units of pH. This will affect the proportions of the individual S(IV) oxospecies in solution. Over the range pH 5.50-3.50 the predominant S(IV) species in solution is HSO_3^-, but as the pH falls from 5.50 to 3.50 the small amount of SO_3^{2-} initially present will be reduced by one hundred fold as a similar increase in $SO_2 \cdot H_2O$ takes place. Such a change may affect the extent to which the reaction is catalysed by S(IV) if the catalyst is either SO_3^{2-} or $SO_2 \cdot H_2O$. There are a small number of cases in which S(IV) species have been shown capable of acting as general acid or base catalysts (Wedzicha and Vacalis, 1988). An example of this is the investigation of the relative abilities of sulphite, hydrogen sulphite and disulphite ions as well as other anions to catalyse the deamination of 1-methyl-5,6-dihydrocytosine (Slae and Shapiro, 1978). Slae and Shapiro (1978) showed the order of effectiveness of the species to be:

$$SO_4^{2-} < H_2PO_4^- < CH_3CO_2^- < HSO_3^- < HPO_4^{2-} < SO_3^{2-} < S_2O_5^{2-}$$

It is clear that sulphite and, particuarly, disulphite ion are capable of acting as very effective catalysts. The reduction in concentration of SO_3^{2-} with pH may, therefore, reduce the extent to which the Maillard reaction is catalysed and this is consistent with a significantly larger value of k_2 being obtained when the pH is maintained at 5.50, than when the pH is allowed to fall.

3.1.2.4. Irreversible S(IV) loss and H^+ liberation.

A small proportion of S(IV) is bound irreversibly and H^+ liberation occurs at an early stage before the linear constant rate phase in the glucose-glycine-S(IV) reaction. These changes were

considered together to determine whether or not they are connected.

Figures 3.1.15-3.1.35 show concentrations of H^+ liberated and S(IV) irreversibly lost as a function of time for reaction mixtures of composition [glucose] = 0.4-2.0 M, [glycine] = 0.2-1.0 M, [S(IV)] = 0.0000-0.0830 M. Although the increase in acidity of reaction mixtures is thought of as liberation of H^+, a reduction in pH and the need to add OH^- to correct it, could be the result of the reaction using up OH^-. For simplicity, the effect will be described here as liberation of H^+. It is evident from Fig.3.1.25 that glucose and glycine alone react to liberate H^+. There is also a high degree of correlation throughout the reaction, between H^+ liberation and irreversible S(IV) loss at low glucose (0.4 M) and S(IV) (0.0078 M) concentration (Figs.3.1.15 and 3.1.26). This is deduced from the fact that $[H^+]$*vs* time and [S(IV)]*vs* time curves are superimposible. However, at higher concentration ([glucose] = 0.8-2.0 M, [glycine] = 0.2-1.0 M, [S(IV)] = 0.0159-0.0830 M) there is a less satisfactory correlation or no correlation at all between the two processes after the induction phase (i.e. $t > 200$ h), the rate of irreversible S(IV) loss being fastest.

The similar behaviour of irreversible S(IV) loss and H^+ liberation during the early stages of the reaction will now be examined in greater detail. Table 3.1.3 shows the initial rate, $t < 200$ h, of irreversible S(IV) loss and H^+ liberation for reaction mixtures of composition [S(IV)] = 0.0000-0.0830 M, [glucose] = 1.0 M, [glycine] = 0.5 M. It can be seen that both sets of initial rates are similar.

Using the data from tables 3.1.3, 3.1.4 and 3.1.5, initial rate of irreversible S(IV) loss is shown as a function of initial rate of H^+ liberation in Fig.3.1.36, a correlation coefficient of 0.8389 and

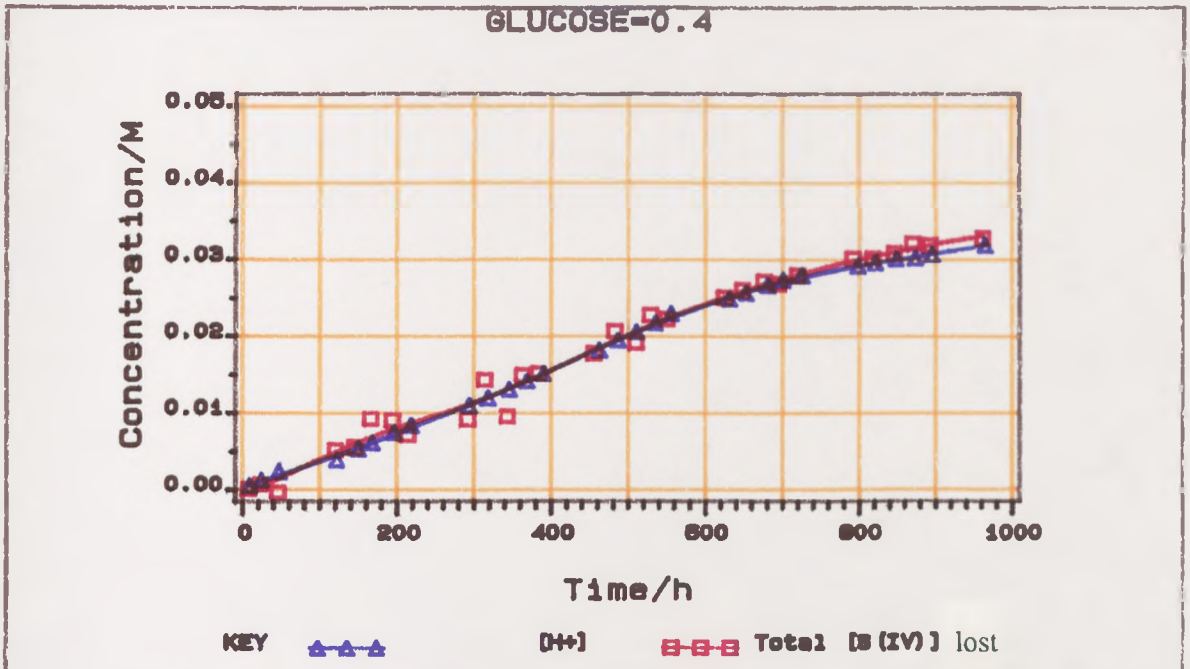


Fig.3.1.15. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=0.4-2.0M, [Glycine]=1.0M, [S(IV)]=0.0365-0.0438M.

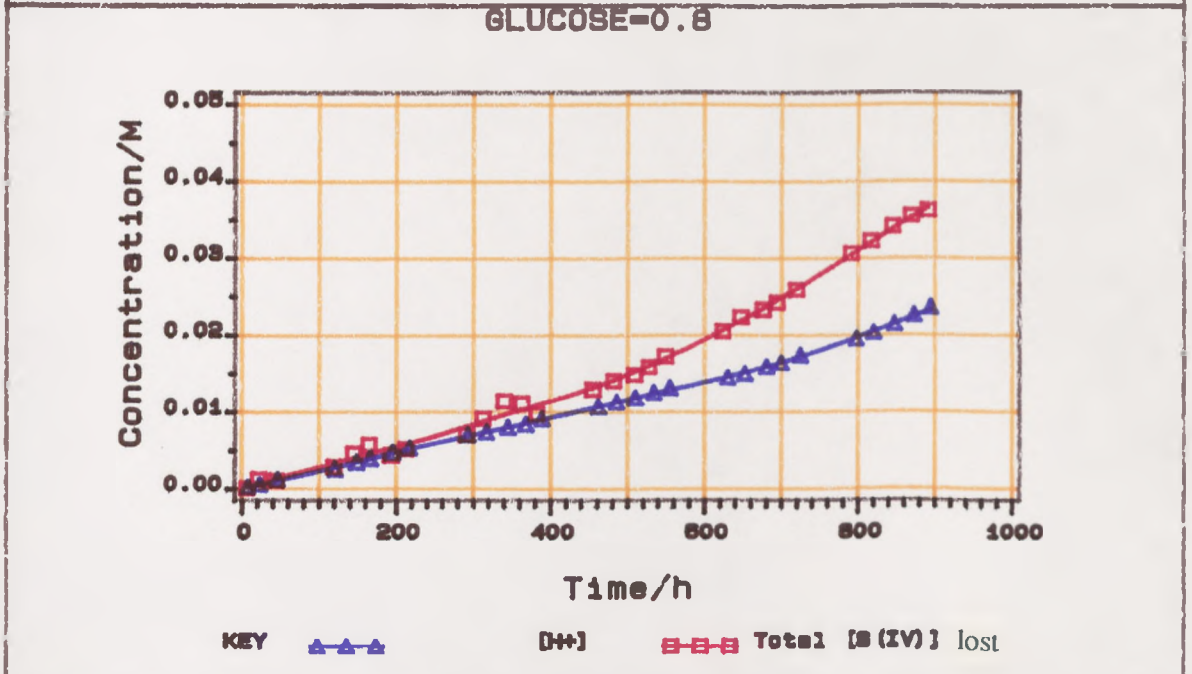


Fig.3.1.16. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=0.4-2.0M, [Glycine]=1.0M, [S(IV)]=0.0365-0.0438M.

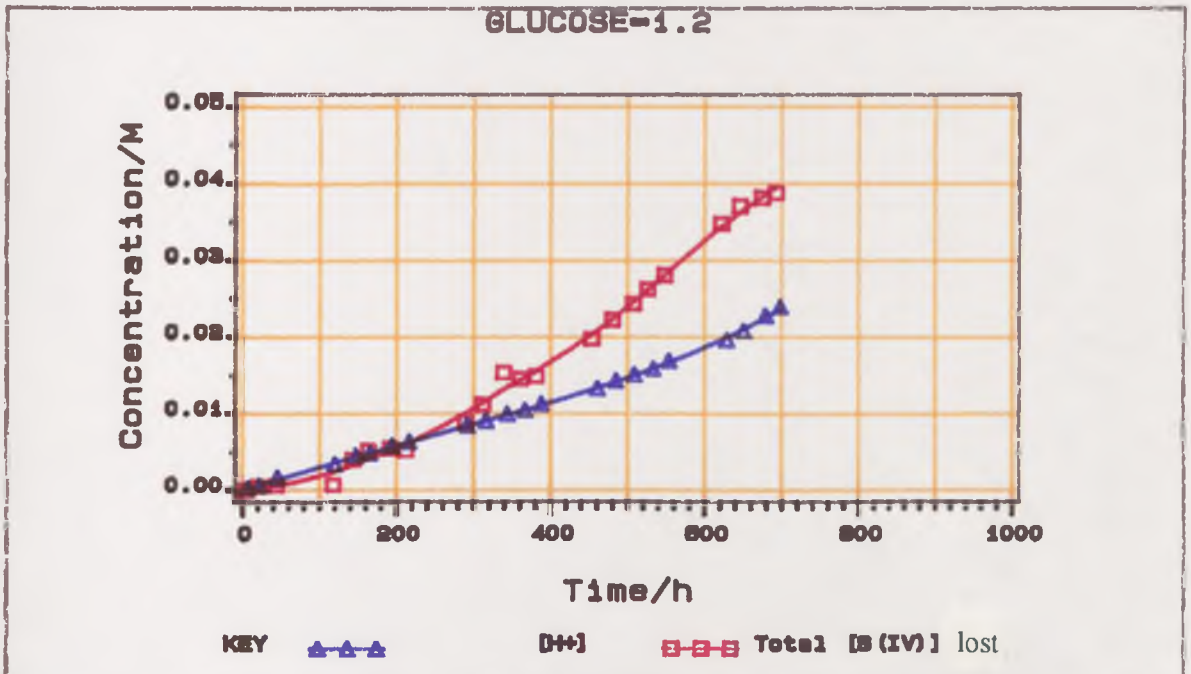


Fig.3.1.17. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=0.4-2.0M, [Glycine]=1.0M, [S(IV)]=0.0365-0.0438M.

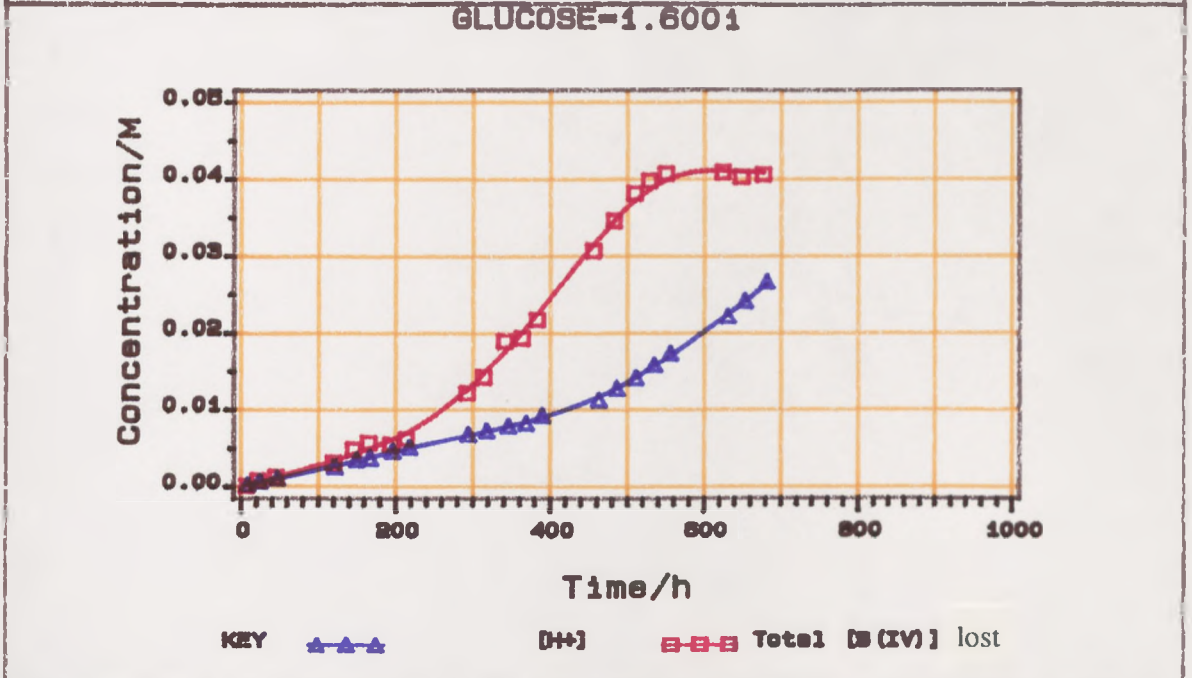


Fig.3.1.18. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=0.4-2.0M, [Glycine]=1.0M, [S(IV)]=0.0365-0.0438M.

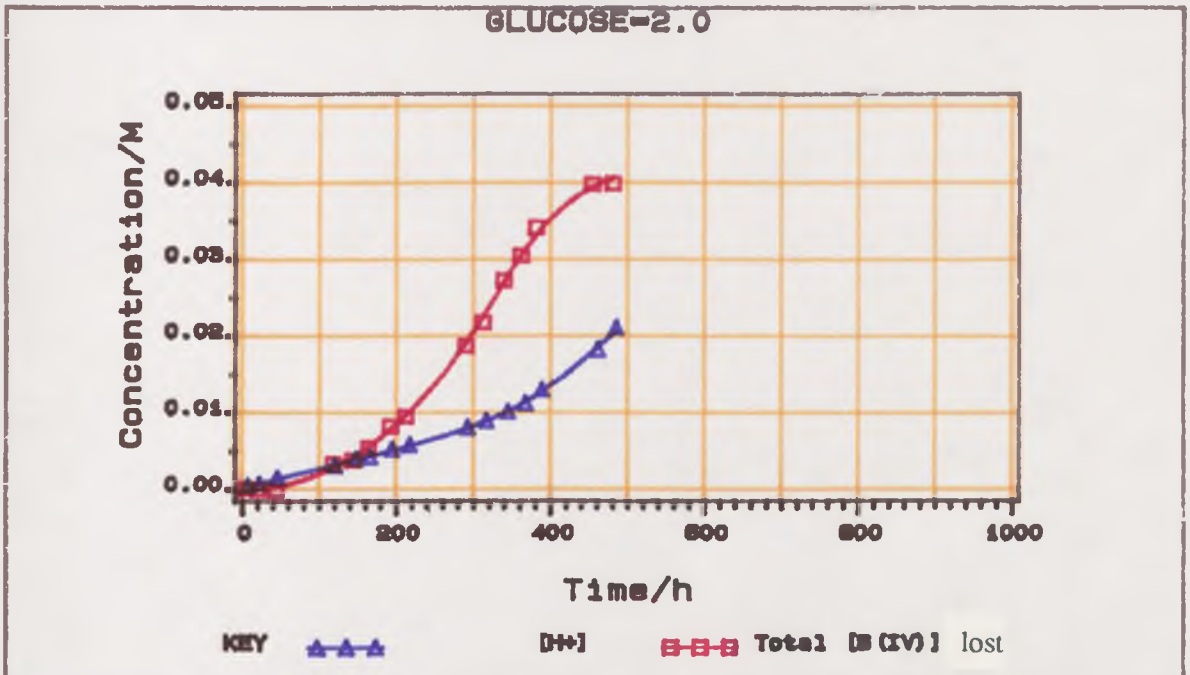


Fig.3.1.19. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=0.4-2.0M, [Glycine]=1.0M, [S(IV)]=0.0365-0.0438M.

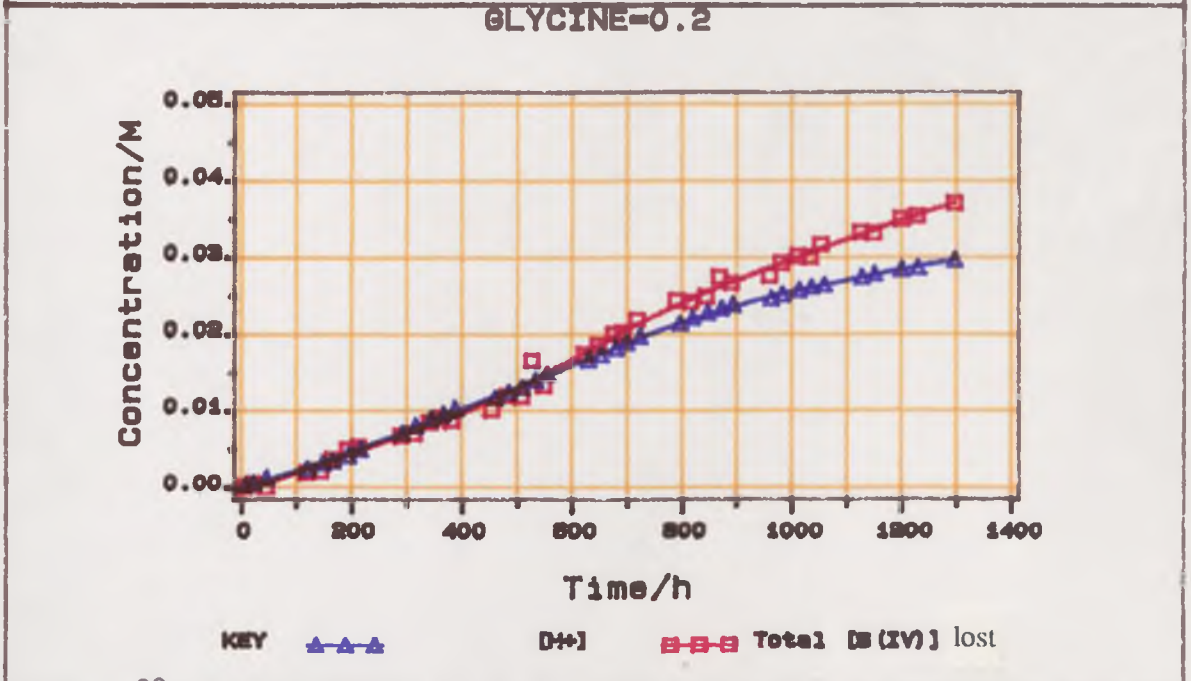


Fig.3.1.20. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M.

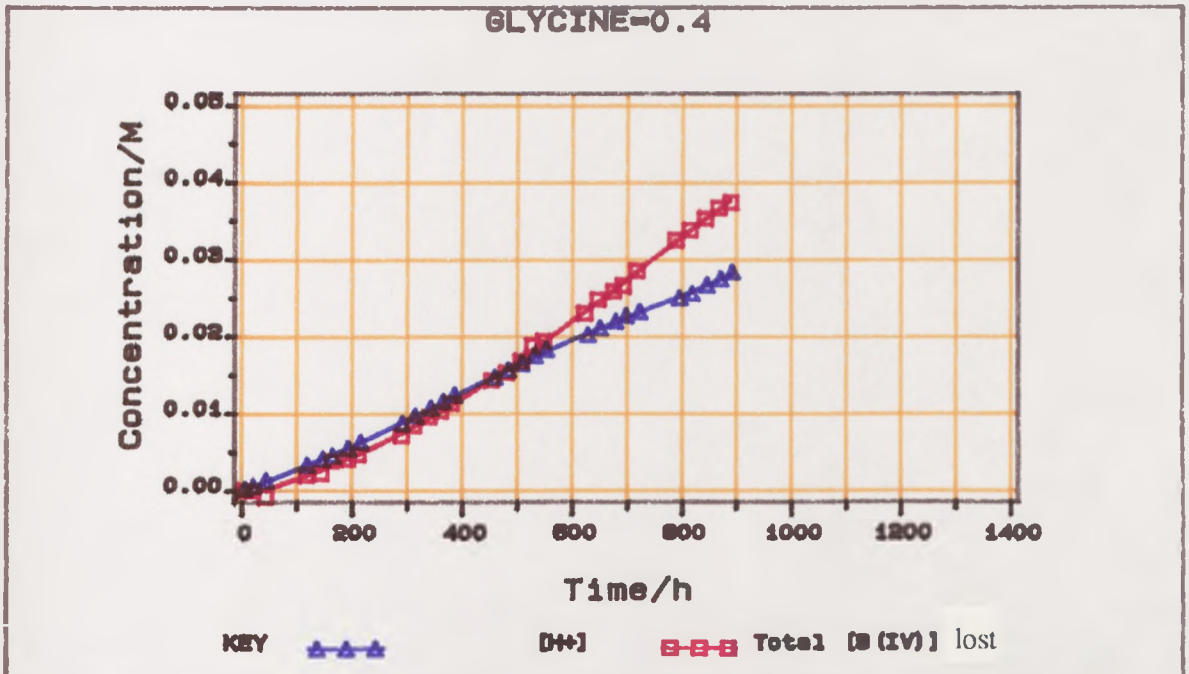


Fig.3.1.21. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M.

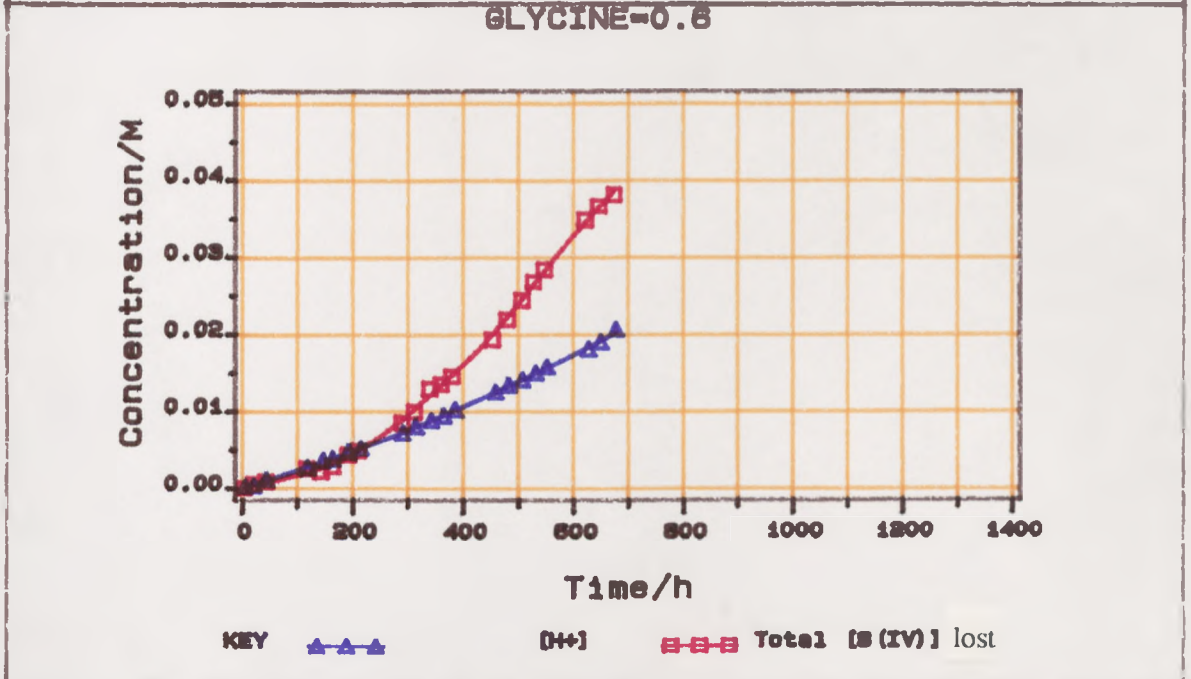


Fig.3.1.22. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M.

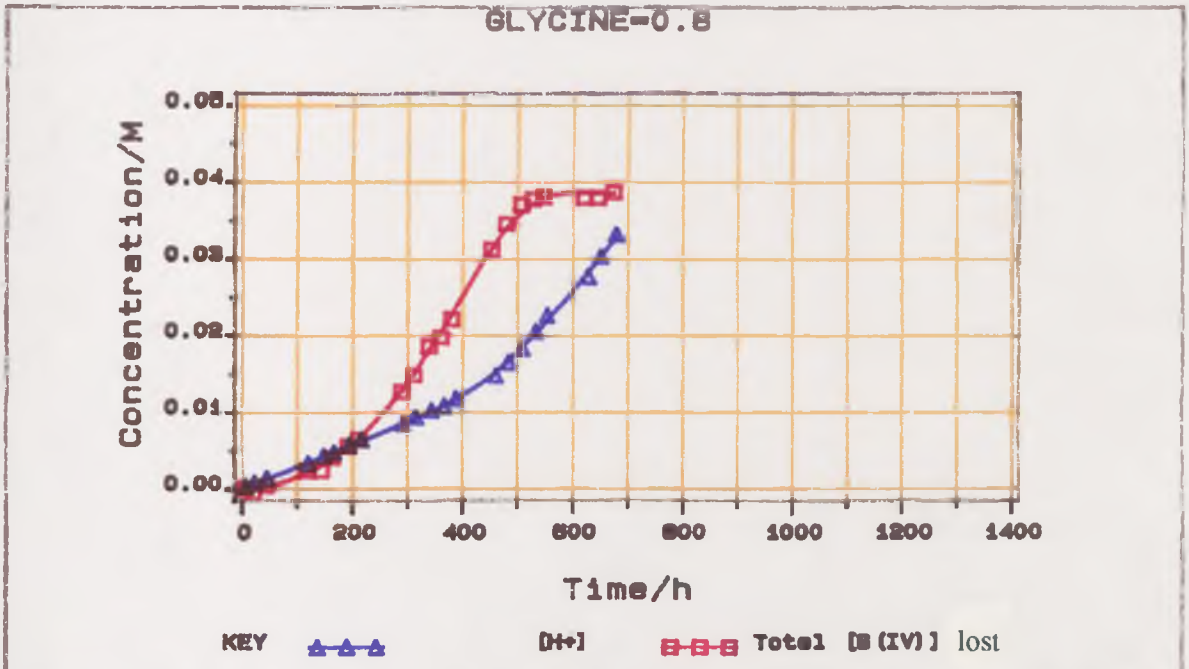


Fig.3.1. 23. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M.

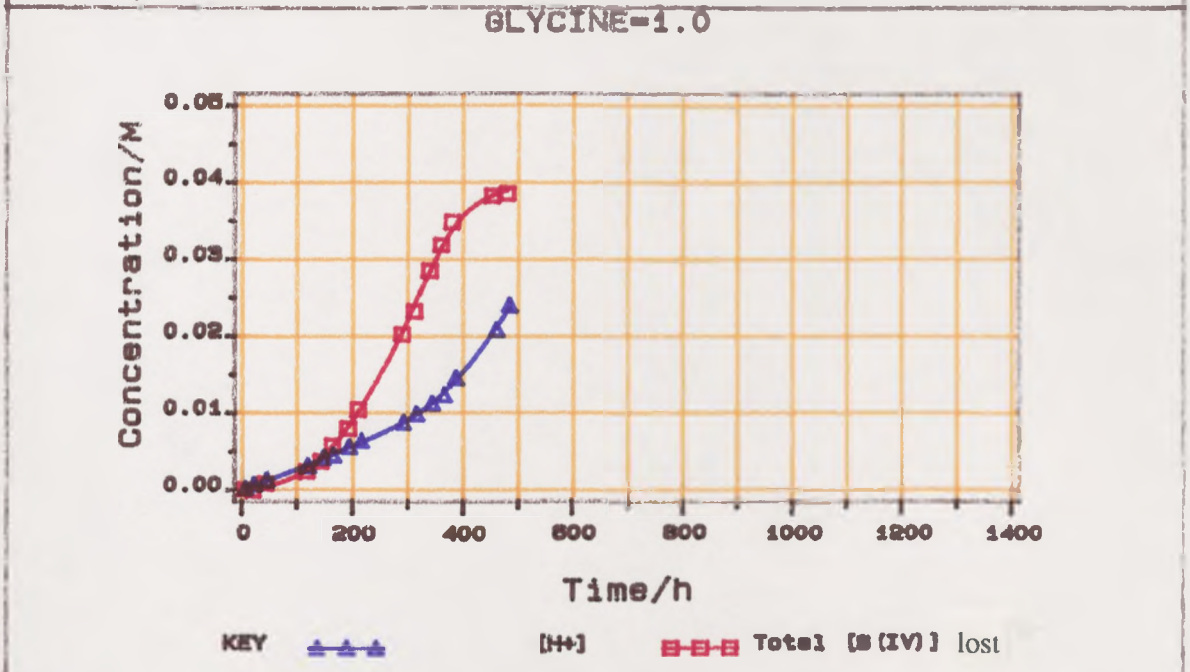


Fig.3.1. 24. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M.

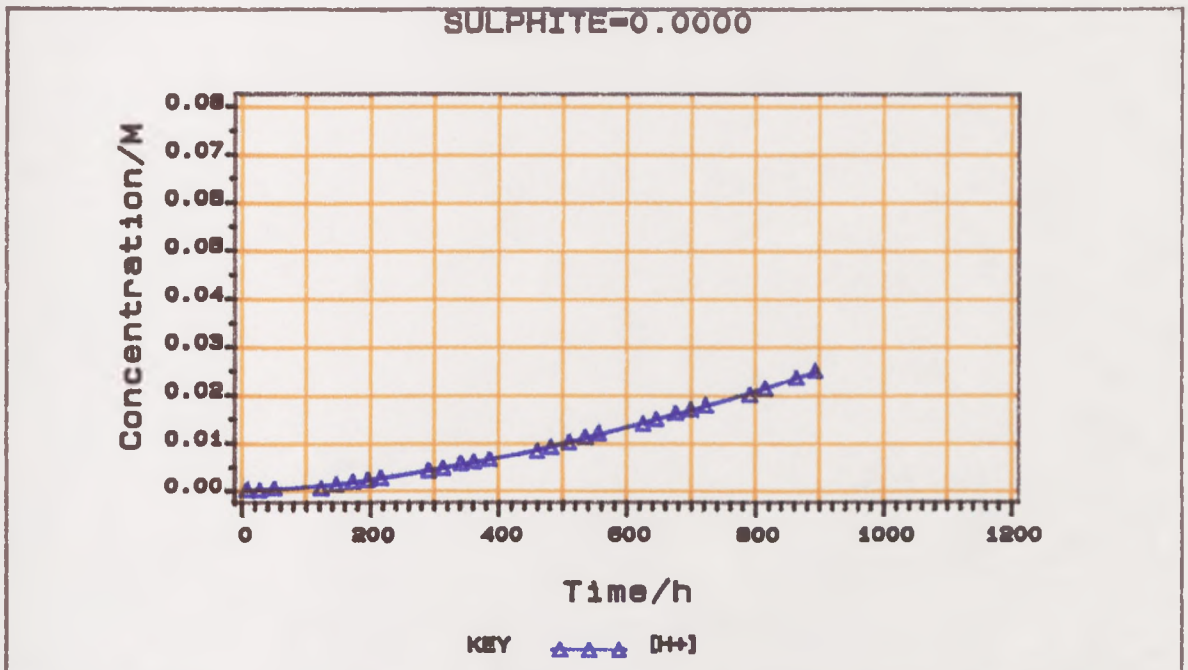


Fig.3.1.25. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

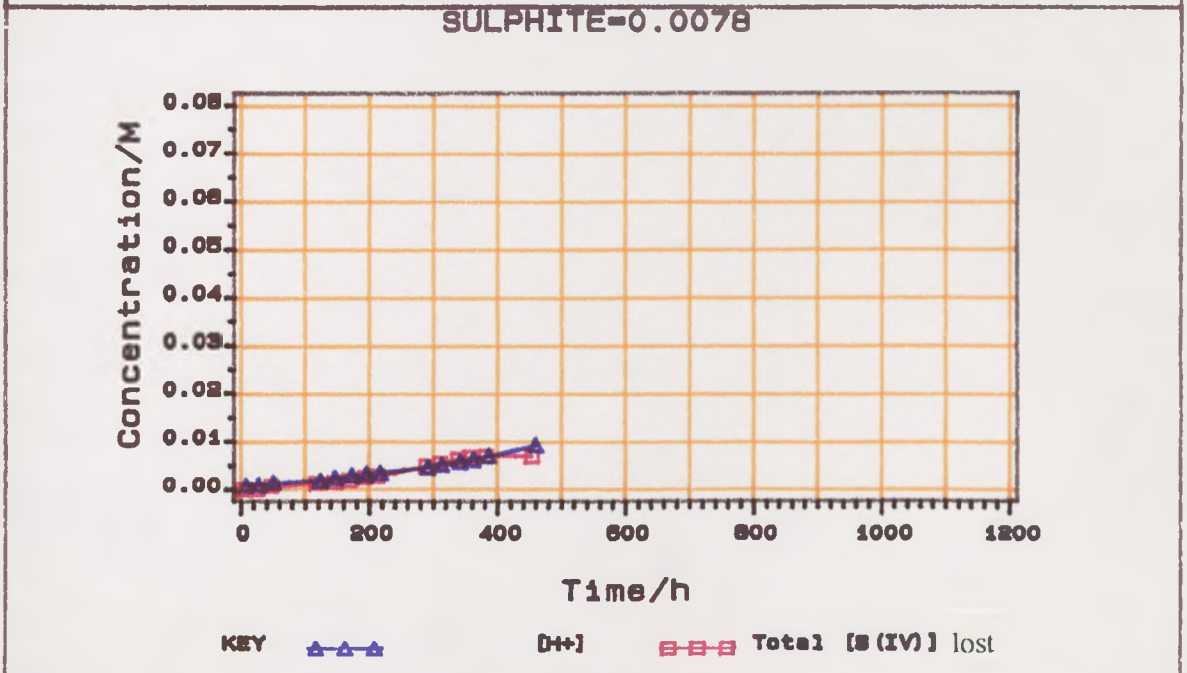


Fig.3.1.26. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

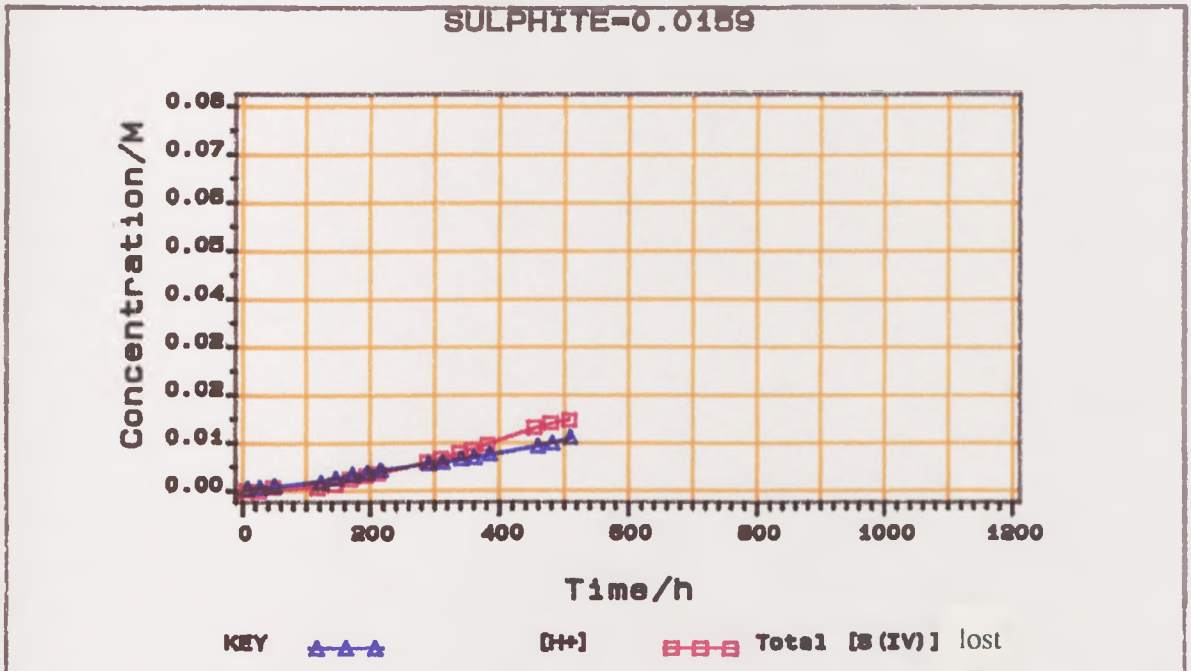


Fig.3.1.27. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

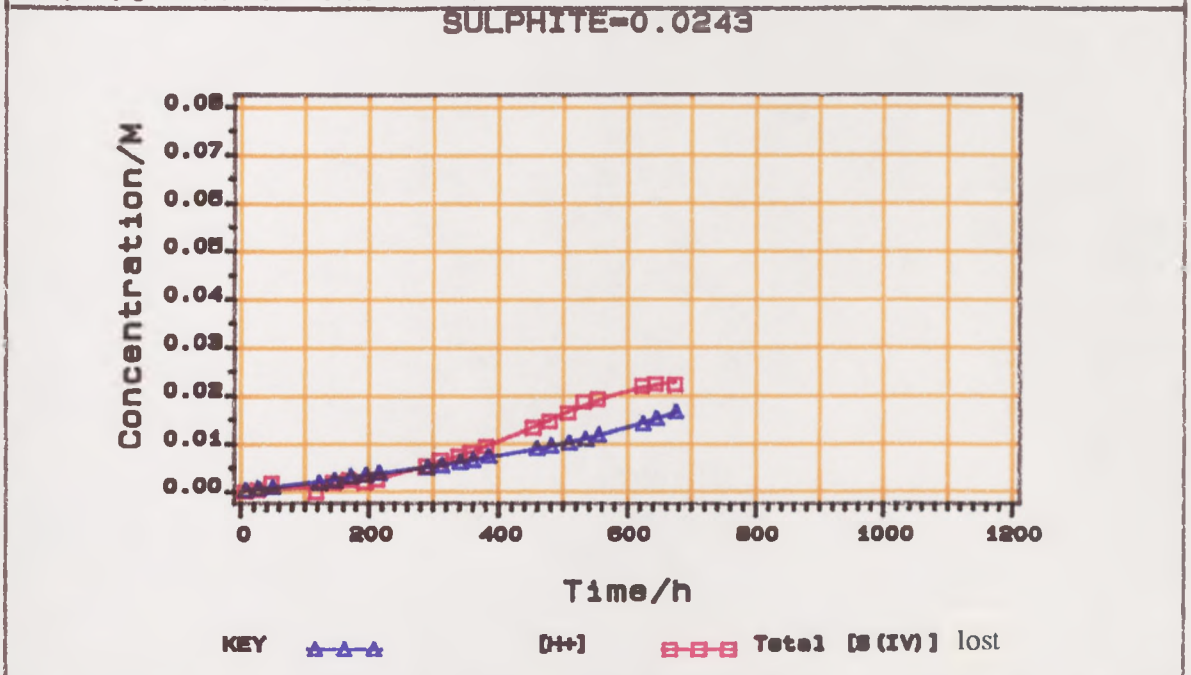


Fig.3.1.28. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

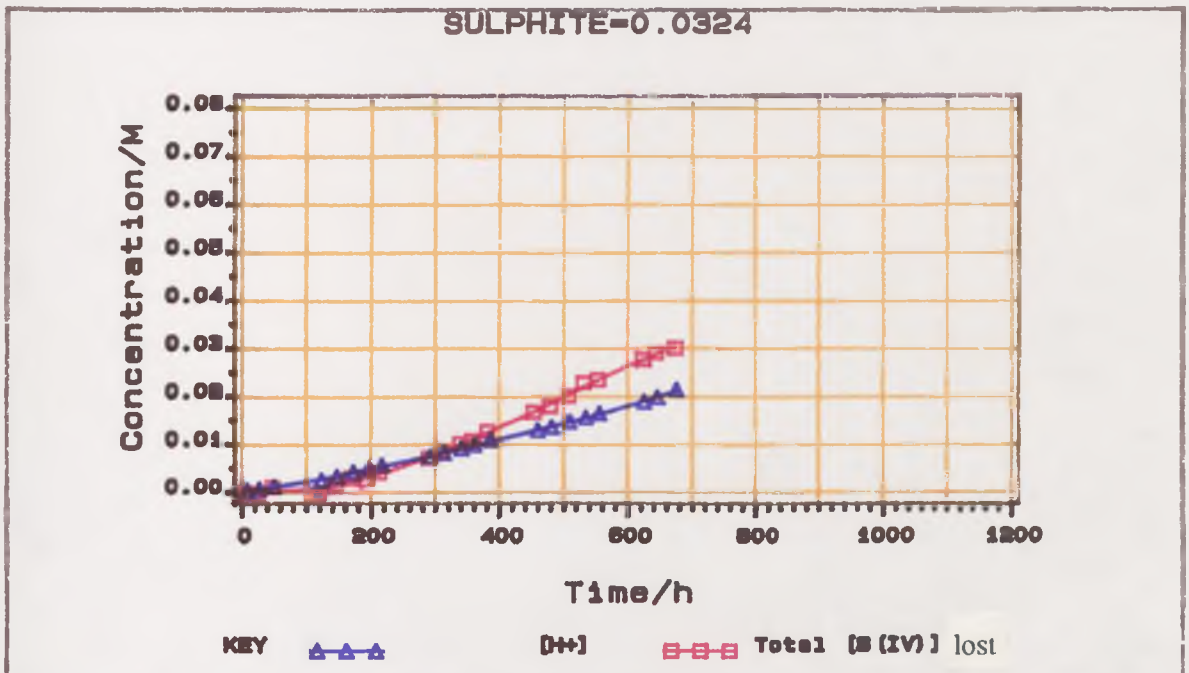


Fig.3.1.29. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

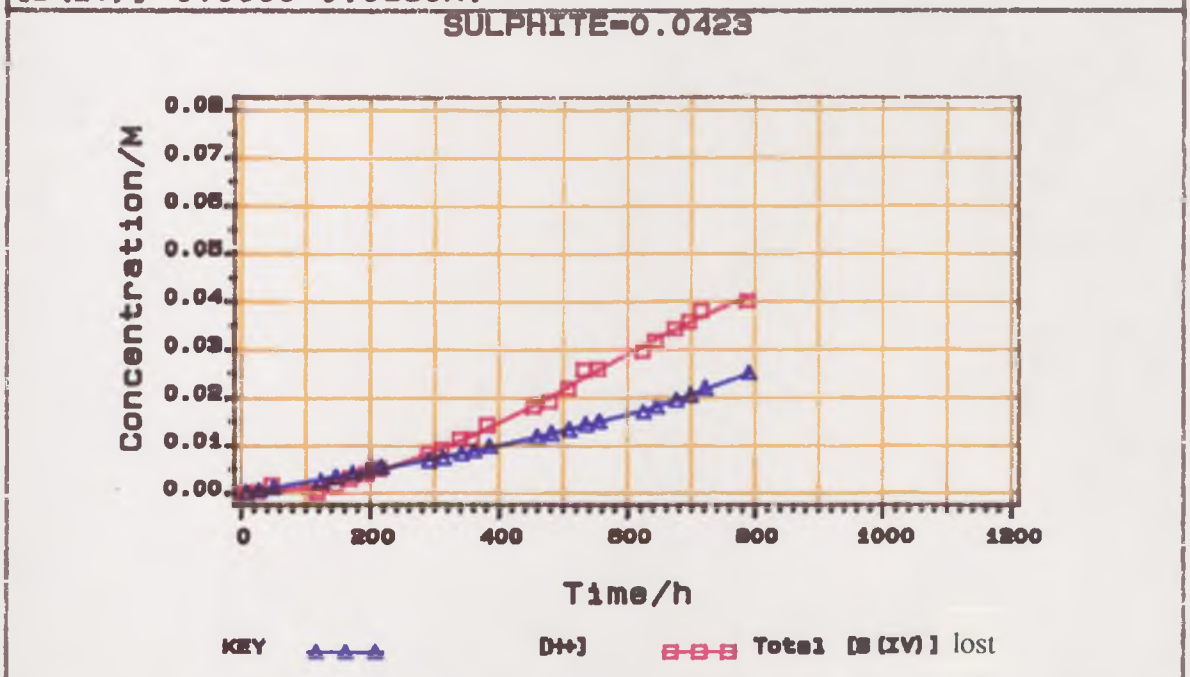


Fig.3.1.30. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

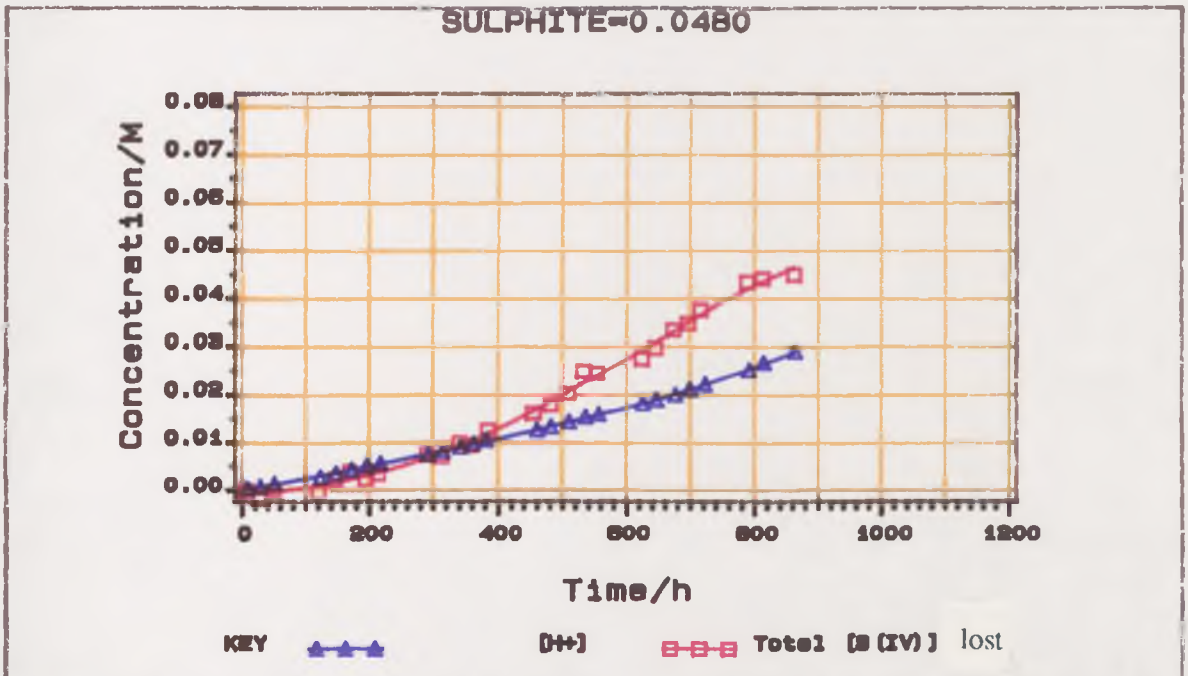


Fig.3.1.31. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

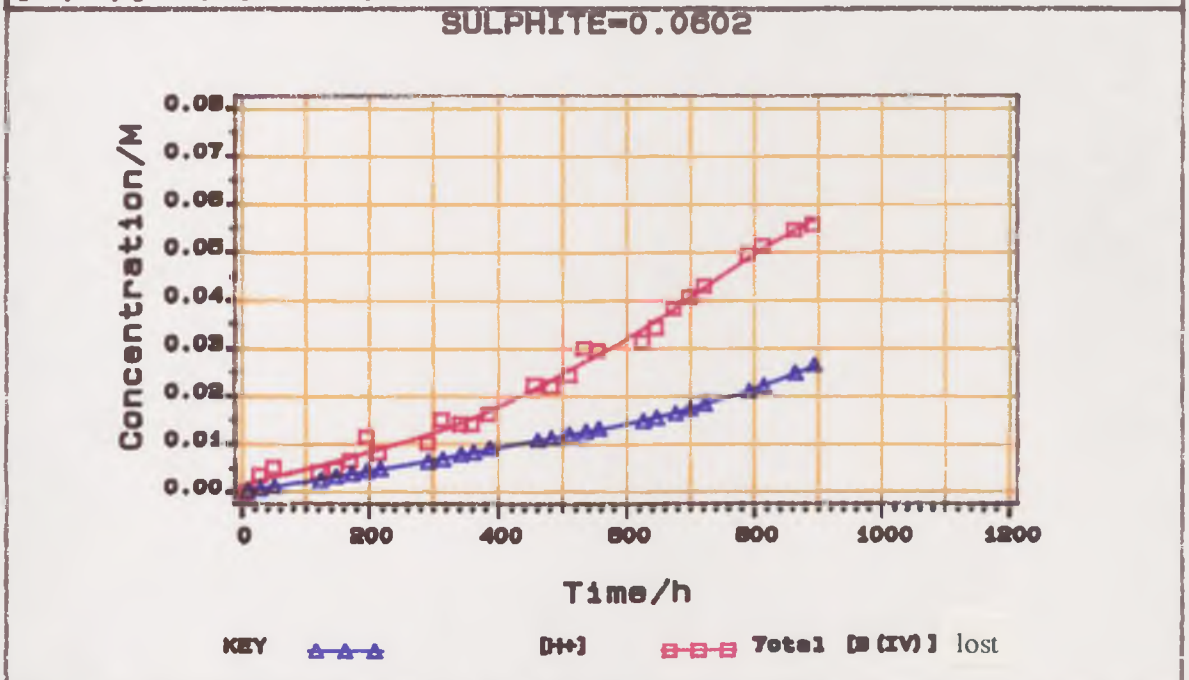


Fig.3.1.32. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

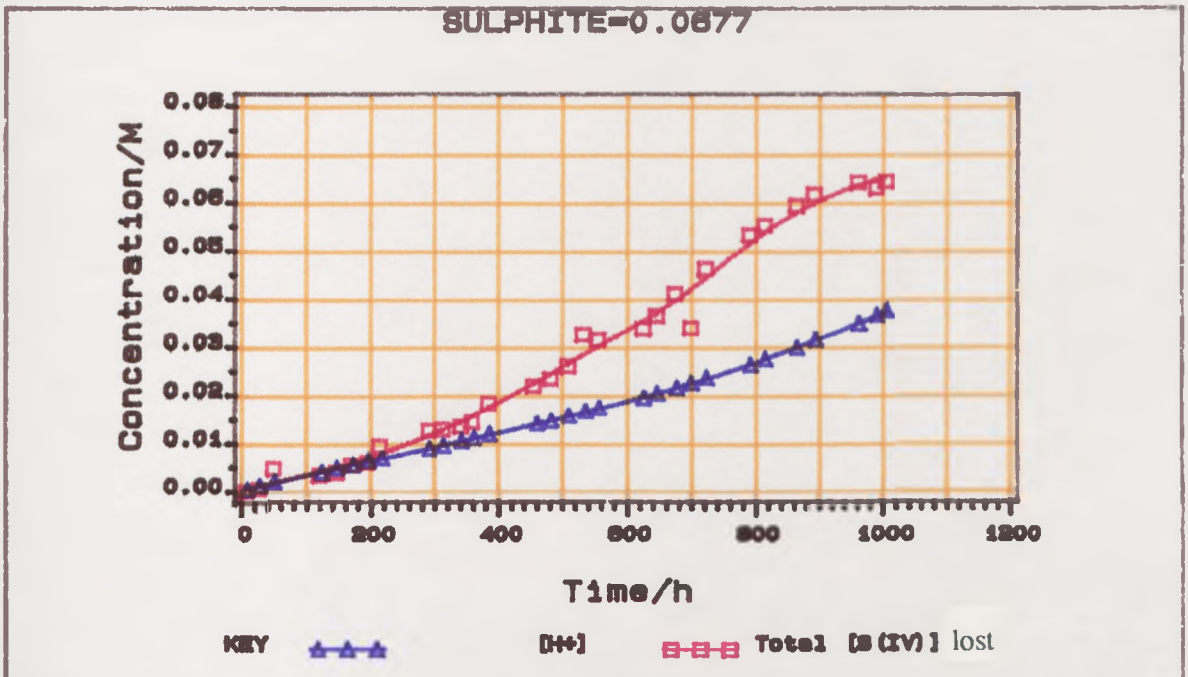


Fig.3.1.33. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

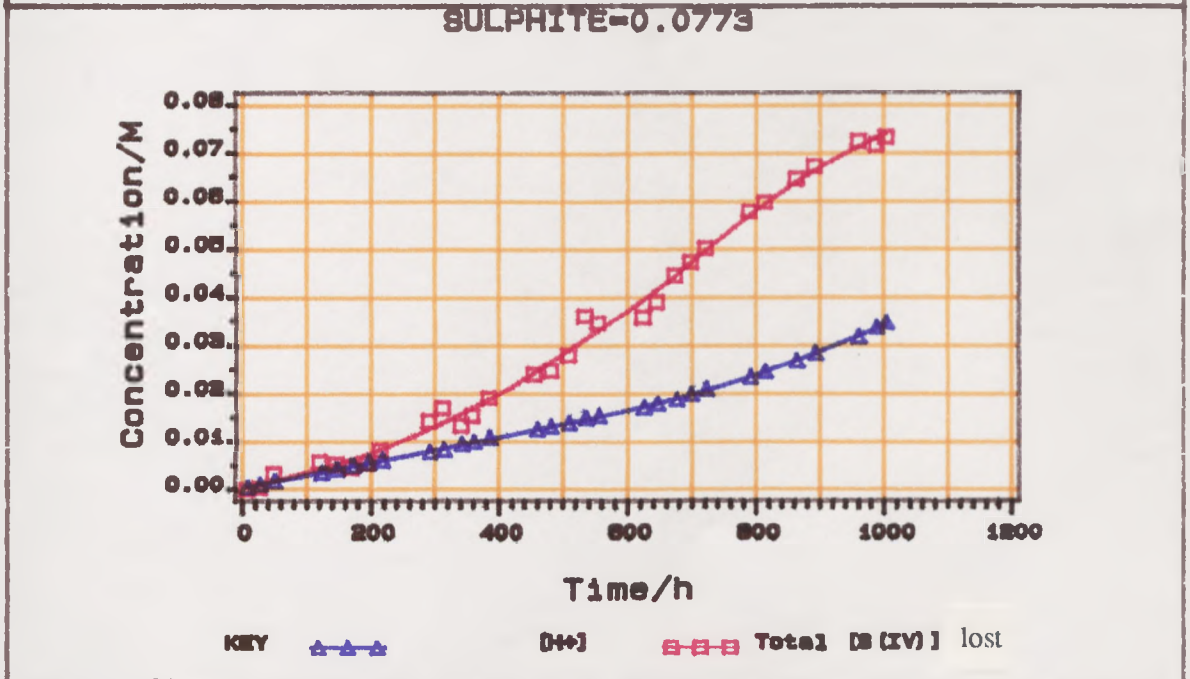


Fig.3.1.34. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

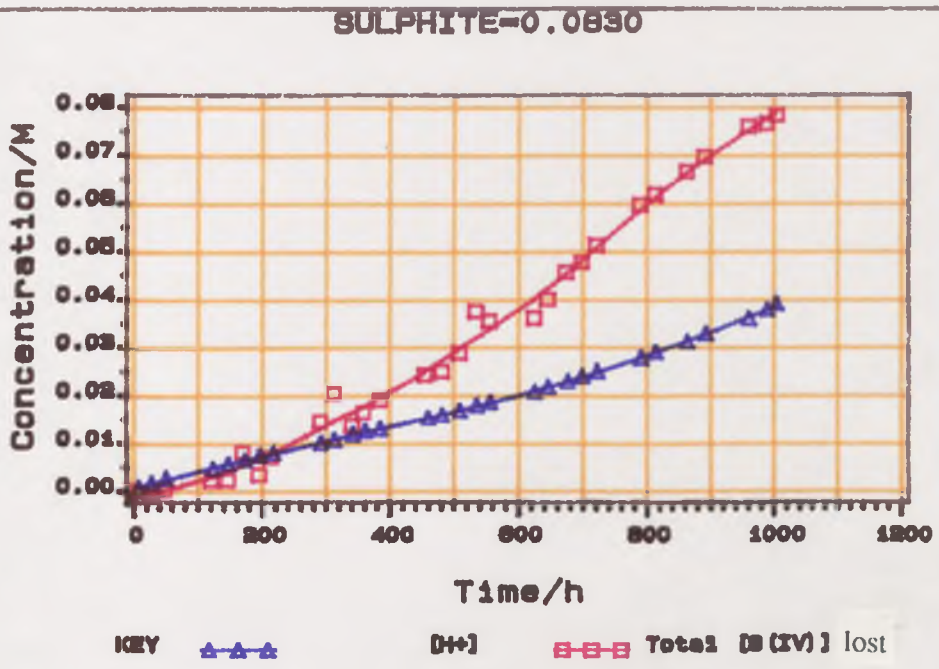


Fig.3.1.35. Concentrations of H+ liberated and S(IV) irreversibly lost vs time for the mixtures [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

[S(IV)] /M	Initial rate of Irreversible S(IV) loss /M h^{-1} X10^{-5}	Initial rate of H^+ liberation /M h^{-1} X10^{-5}	Deviation /X10^{-5}
0.0000	0.0	1.11	0.0
0.0078	1.26	1.25	0.01
0.0159	1.51	1.74	-0.23
0.0243	0.95	1.65	-0.70
0.0324	1.43	2.40	-0.97
0.0423	1.62	2.36	-0.74
0.0480	2.09	2.43	-0.34
0.0602	3.82	2.14	1.68
0.0677	2.69	3.12	-0.43
0.0773	2.86	2.68	0.18
0.0830	3.10	3.48	-0.38

Table 3.1.3. Initial rate, t < 200 h, of irreversible S(IV) loss and H^+ liberation for reaction mixtures of composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0000-0.0830 M. Deviation = initial rate of irreversible S(IV) loss - initial rate of H^+ liberation.

intercept equal to 1.6 x 10^{-6} M h^{-1} being obtained. Initial irreversible S(IV) loss, determined iodimetrically, and H^+ liberation, determined by titration with NaOH, appear initially to be kinetically linked.

Initial rate of H^+ liberation is shown as a function of glucose and initial S(IV) concentration in Figs.3.1.37 and 3.1.38, increasing glycine concentration showing no significant effect on this rate. Increasing glucose concentration, however, reduces the initial rate of H^+ liberation. Whereas, the initial rate of H^+ liberation is first order with

[glucose] /M	[S(IV)] /M	Initial rate of Irreversible S(IV) loss /M h^{-1} $X10^{-5}$	Initial rate of H^+ liberation /M h^{-1} $X10^{-5}$	Deviation /$X10^{-5}$
0.0	0.0396	7.74	7.86	-0.12
0.4	0.0365	5.35	3.46	1.89
0.8	0.0398	2.68	2.33	0.35
1.2	0.0414	2.93	2.89	0.04
1.6	0.0438	3.09	2.23	0.87
2.0	0.0434	4.13	2.50	1.63

Table 3.1.4. Initial rates, t < 200 h, of irreversible S(IV) loss and H^+ liberation for reaction mixtures of composition [glucose] = 0.0-2.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0365-0.0438 M. Deviation = initial rate of irreversible S(IV) loss - initial rate of H^+ liberation.

[glycine] /M	[S(IV)] /M	Initial rate of Irreversible S(IV) loss /M h^{-1} $X10^{-5}$	Initial rate of H^+ liberation /M h^{-1} $X10^{-5}$	Deviation /$X10^{-5}$
0.0	0.0397	2.59	3.06	-0.48
0.2	0.0406	2.37	1.97	0.40
0.4	0.0403	2.47	2.80	-0.33
0.6	0.0414	1.95	2.42	-0.46
0.8	0.0410	2.86	2.81	0.05
1.0	0.0423	3.92	2.78	1.13

Table 3.1.5. Initial rates, t < 200 h, of irreversible S(IV) loss and H^+ liberation for reaction mixtures of composition [glucose] = 1.0 M, [glycine] = 0.0-1.0 M, [S(IV)] = 0.0397-0.0423 M. Deviation = initial rate of irreversible S(IV) loss - initial rate of H^+ liberation.

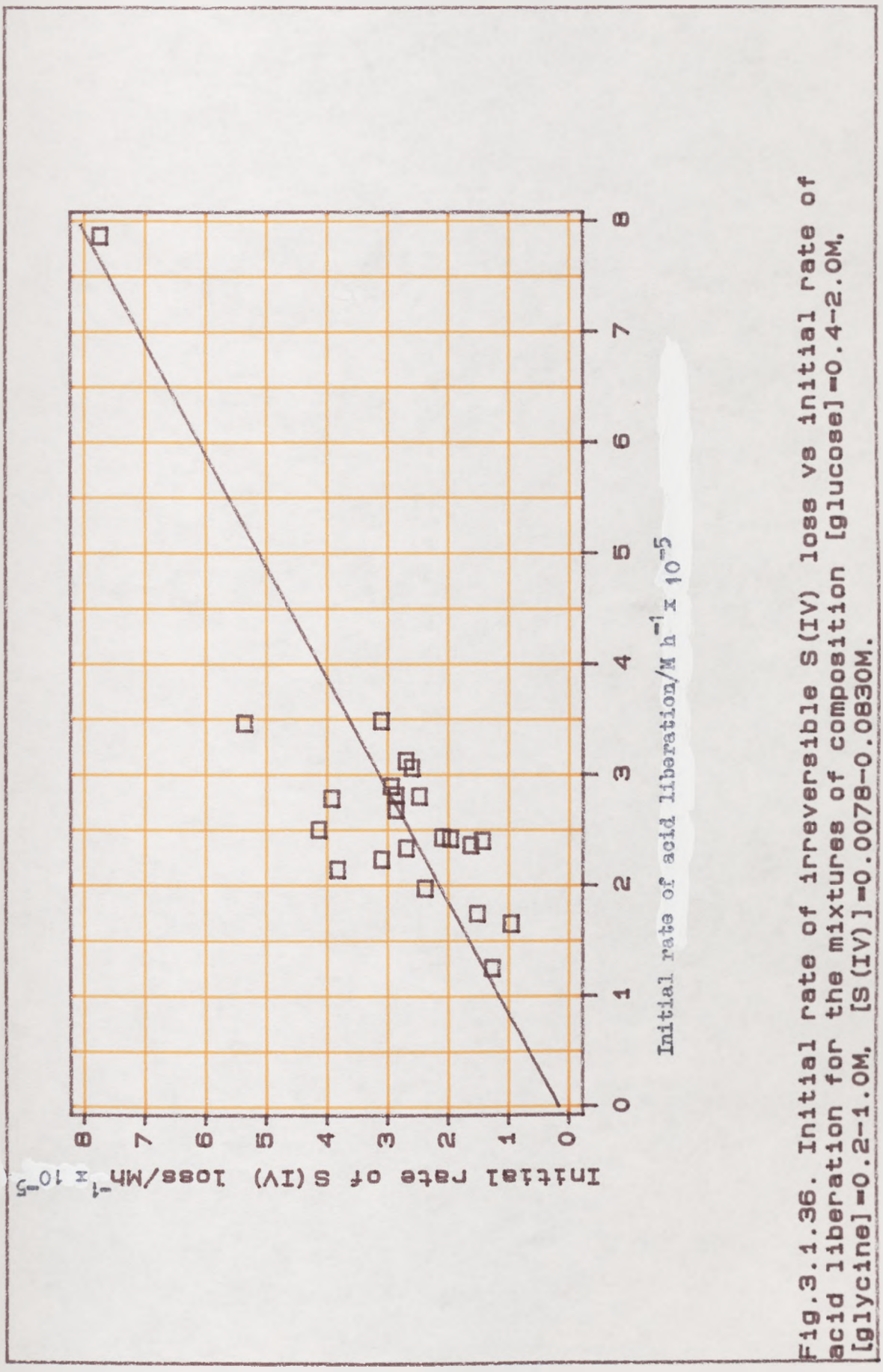


Fig.3.1.36. Initial rate of irreversible S(IV) loss vs initial rate of acid liberation for the mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.2-1.0M, [S(IV)]=0.0078-0.0830M.

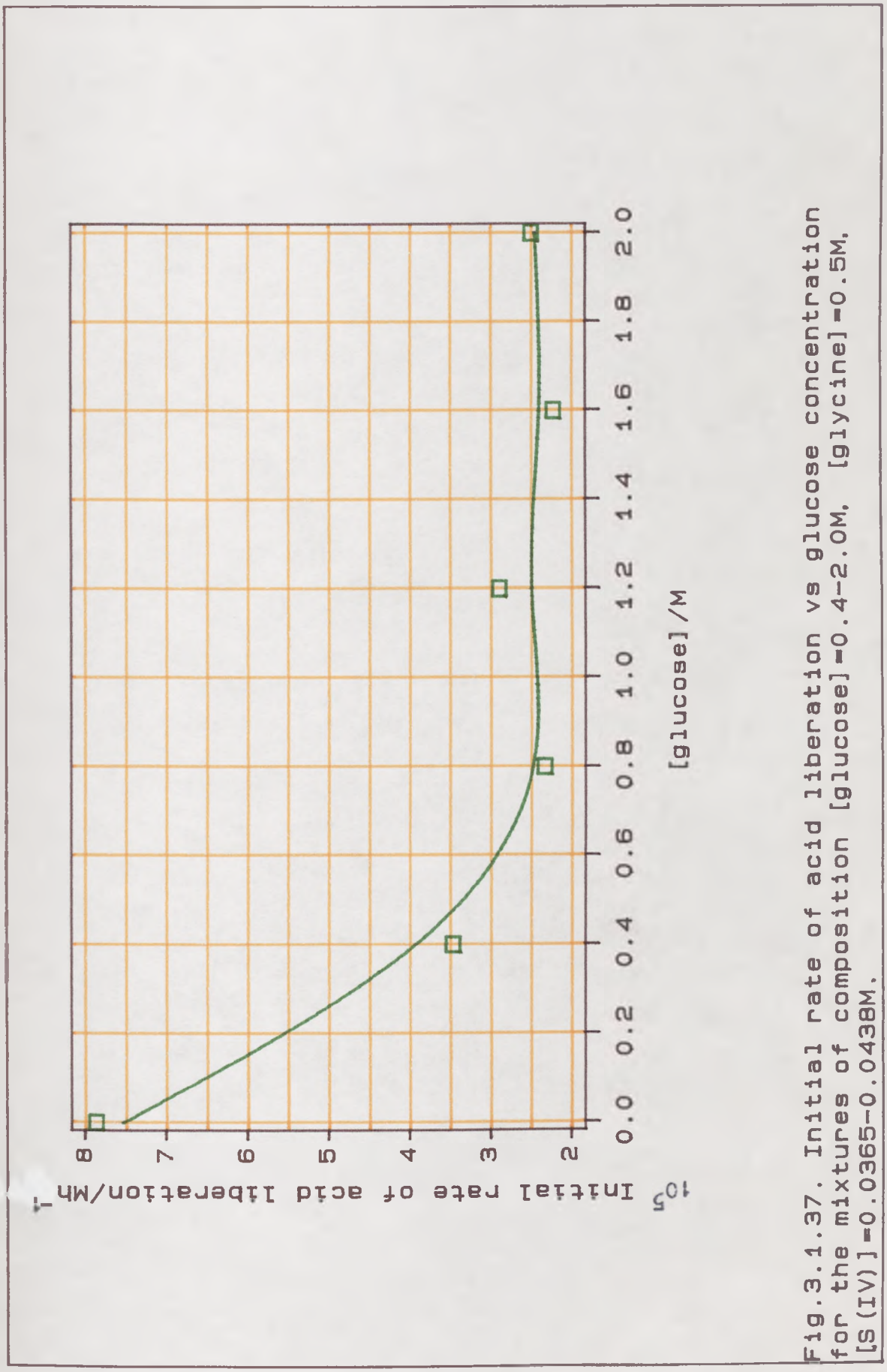


Fig.3.1.37. Initial rate of acid liberation vs glucose concentration for the mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M.

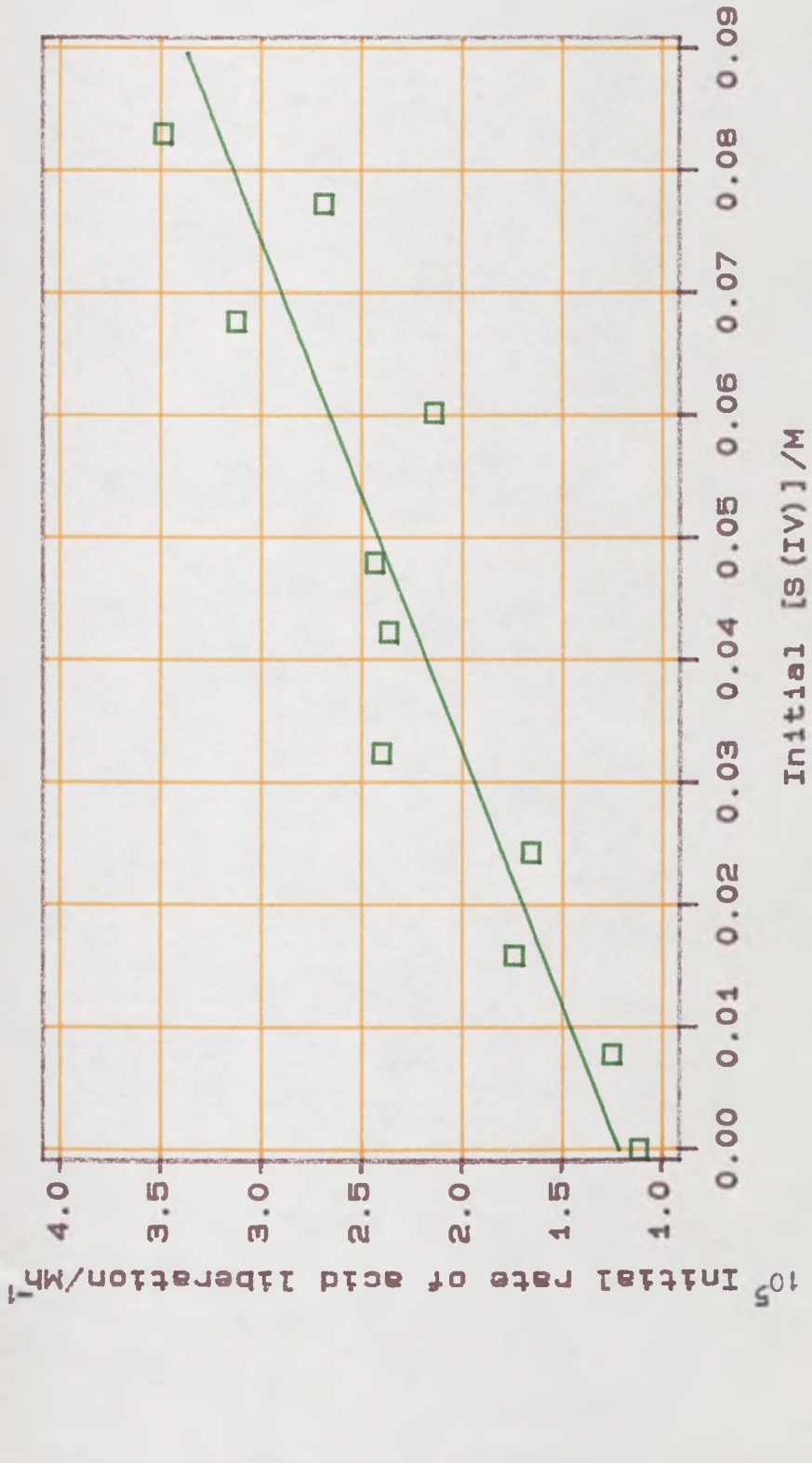


Fig.3.1.38. Initial rate of acid liberation vs initial S(IV) concentration for the mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0000-0.0830M.

respect to initial S(IV) concentration, correlation coefficient = 0.9196, and the occurrence of an intercept indicates that there are at least two processes for H^+ liberation.

The rate of H^+ liberation can, therefore, be described in terms of the following equation:

$$d[H^+] / dt = k_1 + k_2[S(IV)]$$

A mechanism to account for the observed kinetics may be postulated by considering the known steps in the S(IV)-inhibited Maillard reaction. Glycine is in equilibrium with its zwitterion form as shown:

$$NH_2CH_2COOH \rightleftharpoons NH_3^+CH_2COO^-$$

However, when the Schiff's base is formed the nitrogen forms part of the imino group thus:

$$\underset{\text{(glucose)}}{RHC{=}O} + \underset{\text{(glycine)}}{NH_2CH_2COOH} \rightleftharpoons \underset{\text{(Schiff's base)}}{RHC{=}NCH_2COOH} + H_2O$$

It is not possible for the H^+ from the carboxyl group of glycine to protonate its amino group. The pK_a for the carboxyl group of glycine is 2.35 at 25 °C (CRC, 1981-1982) and, therefore, at pH 5.5 it is likely that H^+ will be released to solution. This may account for the liberation of acid from time zero, when it is unlikely that any further intermediates or products will be present. A general reaction pathway for the formation of imines could be as shown in Fig.3.1.39 (Sykes, 1986; McMurry, 1984). The reaction can be acid catalysed at two stages. Firstly, weak nucleophiles often require acid catalysts to activate the C=O group as follows:

$$R_2C{=}O: \overset{H^+}{\rightleftharpoons} R_2C{=}O^+H \longrightarrow R_2C^+{-}OH$$

This increases the positive character of the carbon atom thereby facilitating nucleophilic attack upon it. Secondly, the hydroxyl group of the carbinolamine can be protonated, thus making the water a

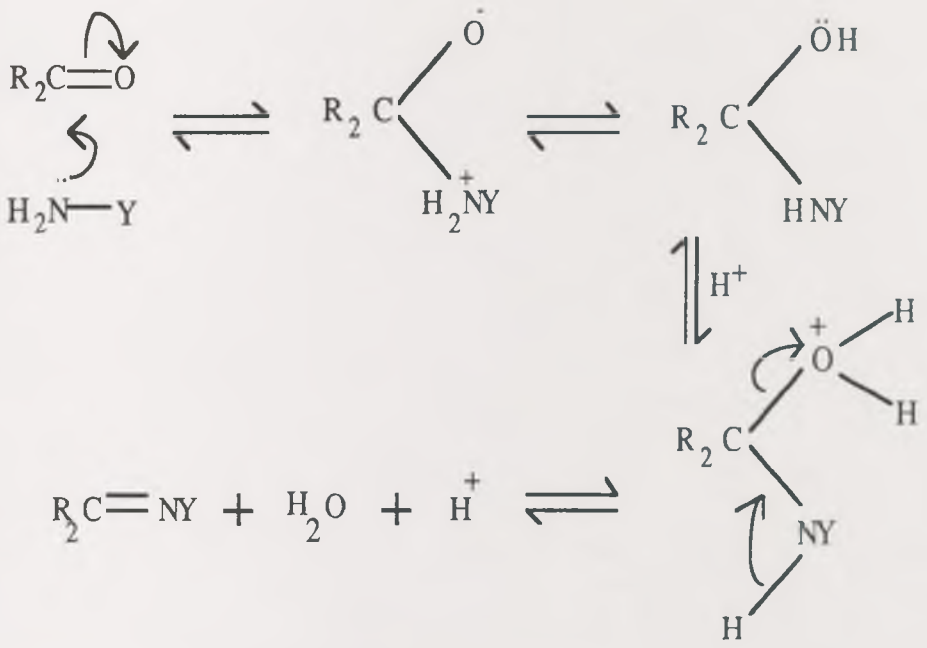
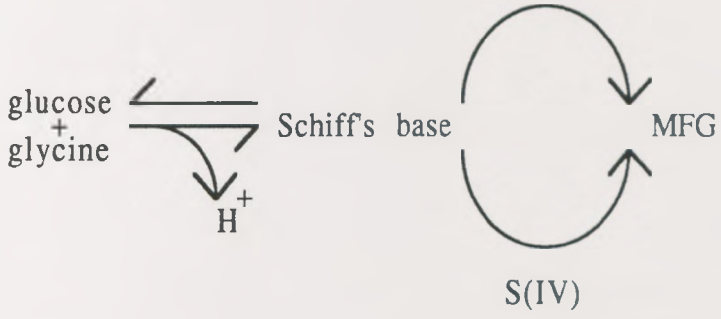


Fig.3.1.39. A general reaction pathway for the formation of imines.

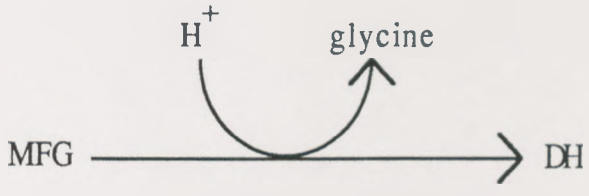
better leaving group. The iminium ion is formed by dehydration of the carbinolamine and this loses a proton to form the imine. The production of the Schiff's base and subsequent liberation of H^+ could proceed by two parallel pathways, one of which is catalysed by S(IV). However, acid catalysis by HSO_3^- is thought to be unlikely as the H–S bond is strong.

Alternatively, as the Schiffs base is unstable it may be formed only in small amounts and significant liberation of H^+ at this stage may not occur. Indeed according to Wedzicha (1984a), the apparent dissociation constant of glucose hydroxysulphonate (1.33 M) in the glucose-glycine-S(IV) system during the first 100 h of the reaction is very similar to that measured using glucose and S(IV) under similar conditions, but without glycine (McWeeny *et al.*, 1969). Therefore, the glucose is present in a free form rather than, say, in the form of aldosylamine or Schiff's base.

Rate-determining production of MFG could proceed by two parallel pathways, one of which is catalysed by S(IV). As the ketoseamine is formed irreversibly from the Schiffs base, a build up of MFG could occur thus liberating H^+ as shown below. The secondary amino group of MFG is much harder to protonate than the amine group of glycine and the conversion of glycine to MFG results in effective release of H^+ at pH 5.5.



The important steps (Hodge, 1955) in the conversion of glycosylamine to ketoseamine are (1) acid-catalysed protonation of the nitrogen followed by a likely opening of the oxide ring and (2) removal of a proton at C_2. The second step could well be base catalysed by SO_3^{2-}. However, glycine is released during the subsequent formation of DH; the amino group of glycine is thus released and H^+ would be recovered by the base, i.e.,



It appears that their is no net production of H^+ on going from glucose to DH.

The pH may also vary due to the following changes in concentration. At pH 5.5 the predominant S(IV) species is HSO_3^-, but small amounts of SO_3^{2-} and $SO_2 \cdot H_2O$ are also present. The product DSH

is formed from the stoichiometric reaction of DDH with HSO_3^-. Thus, each molecule of DSH formed results in the loss of one HSO_3^- ion. Whilst the pH of the system is maintained at pH 5.5, the ratio of $[SO_3^{2-}] : [HSO_3^-]$ remains constant. From the equation:

$$pH = pK + \log_{10}([SO_3^{2-}] / [HSO_3^-])$$

it can be seen that as HSO_3^- is lost during formation of DSH, the pH will tend to increase. In order to retain the $[SO_3^{2-}] : [HSO_3^-]$ ratio after removal of an HSO_3^- ion, it is necessary to form HSO_3^- and this may be achieved by protonation of SO_3^{2-}. As no H^+ is added to the system, such protons will be picked up from the mixture. Let the concentrations of SO_3^{2-} and HSO_3^- be m and n, p is the amount of HSO_3^- used in the formation of DSH and x is the amount of H^+ used to convert SO_3^{2-} to HSO_3^- to maintain the ratio. The ratio of $[SO_3^{2-}] : [HSO_3^-]$ at any time during the reaction may be given by:

$$m / n = (m - x) / (n - p + x)$$

This expression can be rearranged to:

$$x = p(m / m + n)$$

However, at pH 5.5 $[HSO_3^-] >> [SO_3^{2-}]$, i.e. $n >> m$, therefore:

$$x \approx p(m / n)$$

As expected the amount of H^+ added to maintain the pH is related to the amount of product formed. However, at pH 5.5 the fraction m/n will be small, therefore, the amount of H^+ used to maintain the ratio of $[SO_3^{2-}] : [HSO_3^-]$ and the pH, is only small. It would appear from this that formation of DSH from DDH should cause the pH to *increase*, but in practice, the pH of reaction mixtures has been observed to fall. However, the requirement for H^+ in the later stages of the reaction could be one contributor to the overall change in pH which is observed, but some other mechanisms which release H^+ are dominant.

The ratio $[SO_3^{2-}] : [HSO_3^-]$ could be maintained by dissociation of

$SO_2 \cdot H_2O$ releasing H^+. However, this idea can be discounted as the concentration of $SO_2 \cdot H_2O$ present in a 40 mM solution of S(IV) at pH 5.5 is 9.10×10^{-6} M; this is unlikely to account for the observed production of at least 0.02 M H^+, shown in Figs.3.1.15-3.1.24. The mechanism described above does not satisfactorily account for the kinetics of acid liberation and is, therefore, discounted.

The formation of hydroxysulphonate adducts may affect the reaction mixture pH in two ways. Firstly, loss of HSO_3^- as the adduct is formed will affect the proportions of SO_3^{2-} and HSO_3^- and hence the pH. However, as shown above that loss of HSO_3^- may only bring about a small increase in the pH. In addition, towards the end of the S(IV)-inhibited Maillard reaction these adducts decompose releasing HSO_3^-, hence there may be no overall change in pH. Secondly, any hydroxysulphonates formed may ionise:

$$R_1R_2C{=}O + HSO_3^- \rightleftharpoons R_1R_2C(OH).SO_3^- \overset{pK_a}{\rightleftharpoons} R_1R_2C(O^-).SO_3^- + H^+$$

For acetophenone, where $R_1=C_6H_5$ and $R_2=CH_3$, for which the pK_a of the hydroxysulphonate is known, $pK_a = 10.9$. Therefore, at pH 5.5 the ratio of $[R_1R_2C(O^-).SO_3^-] : [R_1R_2C(OH).SO_3^-]$ will be $3.98 \times 10^{-6} : 1$. The unionised form of the hydroxysulphonate of acetophenone will be predominant at pH 5.5. Unfortunately this value is not known for glucose hydroxysulphonate. If the pK_a of glucose hydroxysulphonate is, say, 7.5 or higher, then this adduct will essentially be undissociated and will, therefore, have no significant effect on pH.

First order rate constants for the initial rate of acid liberation, or for S(IV) loss during the constant rate phase of the reaction are obtained by dividing the rate by the concentration of the reactant varied. Graphically this is obtained as the slope of rate/concentration plots and, in both cases, the rate equation takes the form of:

$$\text{Rate} = k_1 + k_2[S(IV)]$$

when S(IV) concentration is varied. This equation can be conveniently rewritten as:

$$\text{Rate} = k_1 (1 + k_2/k_1[S(IV)])$$

where k_2/k_1 is a measure of the relative rate constant of the S(IV)-catalysed reaction. Values of k_1 and k_2/k_1 for the initial liberation of H^+ and S(IV) loss, in reaction mixtures containing 1 M glucose and 0.5 M glycine in the constant rate phase are given as follows:

	-d[S(IV)] / dt	**d[H^+] / dt**
k_1/M h^{-1}	2.73 X 10^{-5}	1.26 X 10^{-5}
k_2/h^{-1}	1.01 X 10^{-3}	2.32 X 10^{-4}
$(k_2/k_1)/M^{-1}$	37.0	18.4

It can be seen that values of k_1 are 2.2 times greater and k_2/k_1 2.0 times greater, for the loss of S(IV) than the initial liberation of H^+. It is interesting to compare the results in relation to the mechanistic model proposed. In this it is suggested that the rate of the initial slow step of the Maillard reaction, leading to the formation of DH is equal to the rate of DH loss in the constant rate phase of the reaction. If H^+ is formed exclusively as a result of the primary reaction of glucose and glycine in the rate determining step of the reaction leading to DH, then this rate of H^+ liberation should equal the rate of S(IV) loss in the constant rate phase. It is seen, however, that H^+ liberation occurs at approximately half the rate of S(IV) loss and that the catalytic effect (k_2/k_1) of S(IV) on H^+ liberation is also half that of the effect on S(IV) reactivity. On the other hand, the constant factor in the rates may be a coincidence.

An alternative mechanism to account for the observed kinetics of acid liberation and irreversible S(IV) loss could be as follows.

Sulphur(IV) is irreversibly lost by a mechanism *which is in addition* to the S(IV)-inhibited Maillard reaction and this additional mechanism liberates acid. It is suggested that this additional process may become a less significant route for S(IV) loss as the overall rate of reaction increases. This is because from Figs.3.1.15 and 3.1.26 it can be seen that when glucose or S(IV) are present at their lowest concentrations, acid liberation and irreversible S(IV) loss are very highly correlated. However, when the concentration of either reagent is increased and the rate of the Maillard reaction also increases, there is a less satisfactory correlation between the two processes, or no correlation at all after the induction period. It is proposed that this process is the autoxidation of S(IV). In addition, acid is liberated from the reaction of glucose and glycine, but at the lowest concentrations of glucose and S(IV) the predominant pathway for liberation of acid is by autoxidation of S(IV) oxospecies.

The predominant S(IV) species in solution at pH 5.5 is HSO_3^-. The stoichiometric equation for the oxidation of HSO_3^- is:

$$HSO_3^- + [O] \longrightarrow HSO_4^-$$

The product has a pK_a of 1.99 ± 0.01 at 25 °C (Smith and Martell, 1976) and at pH 5.5 will readily ionise as follows:

$$HSO_4^- \rightleftharpoons SO_4^{2-} + H^+$$

Thus, the conversion of HSO_3^- to oxidation products leads to the liberation of a stoichiometric amount of H^+. However, HSO_3^- is less susceptible to autoxidation than SO_3^{2-} (Wedzicha, 1984b) and in practice it is SO_3^{2-} that is oxidised. Therefore, S(IV) autoxidation at pH 5.5 is preceded by dissociation of HSO_3^- to SO_3^{2-} liberating H^+. At pH 5.5 SO_4^{2-} is the predominant S(VI) species in solution, therefore, there is again a net liberation of one proton for each HSO_3^- ion which is subsequently oxidised, equation 3.1.8.

$HSO_3^- \rightleftharpoons SO_3^{2-} + H^+$ (pK_a=7.18 ± 0.03 at 25°C and zero ionic strength)

Oxidation

$HSO_4^- \rightleftharpoons SO_4^{2-} + H^+$ (pK_a=1.99 ± 0.01 at 25°C., Smith and Martell, 1976)

Equation 3.1.8. Dissociation of HSO_3^- liberating H^+, followed by oxidation of SO_3^{2-} to SO_4^{2-}.

Sapotnikskii and Glushchenko (1962) demonstrated the ability of glucose to inhibit S(IV) autoxidation and this may, therefore, account for the reduction in the initial rate of H^+ liberation with increased glucose concentration shown in table 3.1.4.

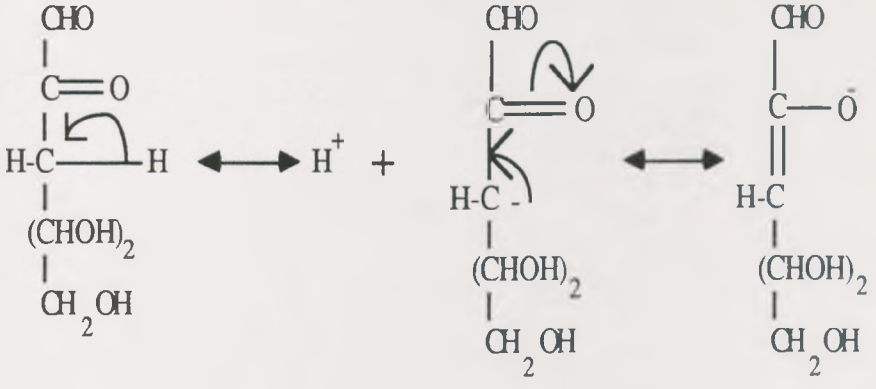
A reaction mixture containing glucose and glycine alone has been shown to liberate H^+, see Fig.3.1.25. This may be attributable to glucose autoxidation, which was described by Wolff and Dean (1987) in phosphate buffer at pH 7.4 over 6 days at 37 °C. Schiff's base or ketoseamine formation would liberate H^+ initially due to the quantitative binding of the NH_2 group of glycine. However, the stoichiometric equation shows the release of glycine during formation of DH with no net production of H^+. Alternatively, acid may be liberated as a result of some simple reactions undergone by DH or its subsequent products, these include:

(a) Hydration to the 2-hydroxy-acid by the benzilic acid rearrangement. However, kinetic experiments in which the intermediate DH was labelled with ^{14}C and reaction mixtures were spiked with this intermediate have shown DH and DSH to be the only ^{14}C-labelled species derived from glucose in the model system glucose-glycine-S(IV) after 23 days (Wedzicha and Garner, 1991). At this time significant acid liberation has occured and the

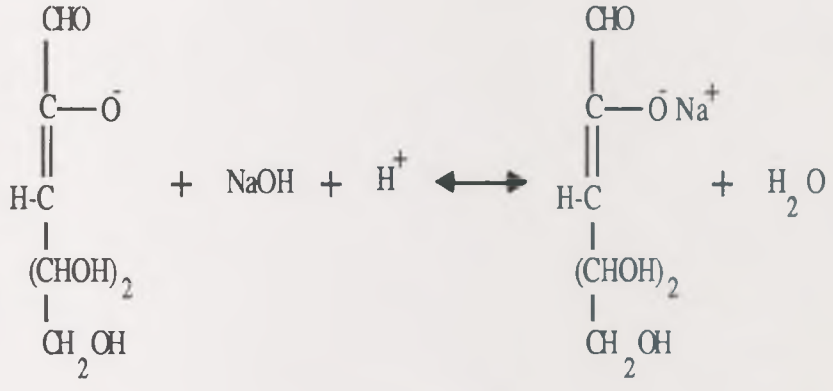
2-hydroxyacid is formed only at long reaction times (Wedzicha and Garner, 1991). Also the hydration reaction is most rapid in weakly alkaline media rather than weakly acid media.

(b) Schiff's base formation involving DH or DSH and glycine is possible. Evidence for a possible DH-glycine interaction stems from the fact that N-substituted pyrroles are formed as byproducts of Maillard browning. Also, glycine catalyses the DH-S(IV) reaction, and a covalent interaction between DH and glycine could be incorporated into the mechanism. One method by which glycine could become attached to the building up of the polymer is by condensation with monomer units; i.e. intermediates derived from DH. Furthermore, α-dicarbonyl compounds are involved in Strecker degradation. This requires the initial formation of a Schiffs base even though degradation leads to the complete "decomposition" of the amino acid with loss of CO_2. Ghiron *et al* (1988) studied reaction mixtures containing either DH, D-glucose, or 5-(hydroxymethyl)-2-furaldehyde, and combinations thereof, in conjunction with L-alanine at pH 3.5 and 100 °C. They concluded that DH participates in Strecker degradation reactions, this being confirmed by Feather (1989).

(c) Ionisation of the activated α-hydrogen of DH. The negative charge could be delocalised to some extent as follows:



As reaction mixture pH is adjusted with NaOH, a sodium salt of DH may be formed:



However, it is debateable whether or not the methylene group is sufficiently activated to form a stable enolate ion at pH 5.5. Hence, before any of these possibilities can be proposed further evidence is required.

Increasing S(IV) concentration increases the initial rate of H^+ liberation. $^{35}SO_3^{2-}$ labelling experiments in the model system glucose, glycine, S(IV), have shown DSH and SO_4^{2-} to be the only radioactive ^{35}S species after 42 days (Wedzicha and Garner, 1991). Therefore, SO_4^{2-} could be the product of S(IV) autoxidation liberating H^+ as shown above. The first order behaviour of the initial rate of acid liberation with respect to S(IV) concentration strengthens the idea that S(IV) autoxidation is occurring, because autoxidation of S(IV) is of first order with respect to sulphite concentration (Fuller and Crist, 1941). According to Hayon *et al* (1972), chain oxidation of sulphite ions in solution can lead to fast removal of oxygen from the system. It was decided, therefore, that the relative residual oxygen content of a series of reaction mixtures, with and without S(IV), would be measured as this may indicate whether or not S(IV) oxidation actually

occurs in the system glucose-glycine-S(IV).

3.1.2.5. Oxygen loss from reaction mixtures.

If autoxidation of reaction mixture components is occurring this may cause a significant reduction in the level of oxygen present in solution, which may be detectable using the Clark-type electrode.

At 55 °C a solution of sodium dithionite (10% w/v) was used to calibrate the meter at the zero oxygen response, whilst water saturated with air was used as the 100% standard. When certain reaction mixtures were placed in the Clark electrode vessel, the reading was found to decrease slowly with time. In such situations the O_2-content was measured as a function of time and the initial O_2-content obtained by extrapolation to zero time. The extrapolation is demostrated for the reaction mixtures of composition: (a) [glucose] = 0.4 M, [glycine] = 0.5 M and (b) [glucose] = 0.4 M, [glycine] = 0.5 M, [S(IV)] = 0.05 M in Fig.3.1.40. Table 3.1.6 shows the relative residual oxygen content, extrapolated to time zero of measurement, at 55 °C of pure water and a series of freshly prepared reaction mixtures.

Mixtures incorporating glucose and/or glycine but not S(IV) contain significant levels of oxygen, whereas any mixture incorporating S(IV) has a relative residual oxygen content below the limit of detection, this being 0.01 unit. The presence of S(IV) in reaction mixtures causes oxygen to be lost from solution and this may be due to S(IV) autoxidation.

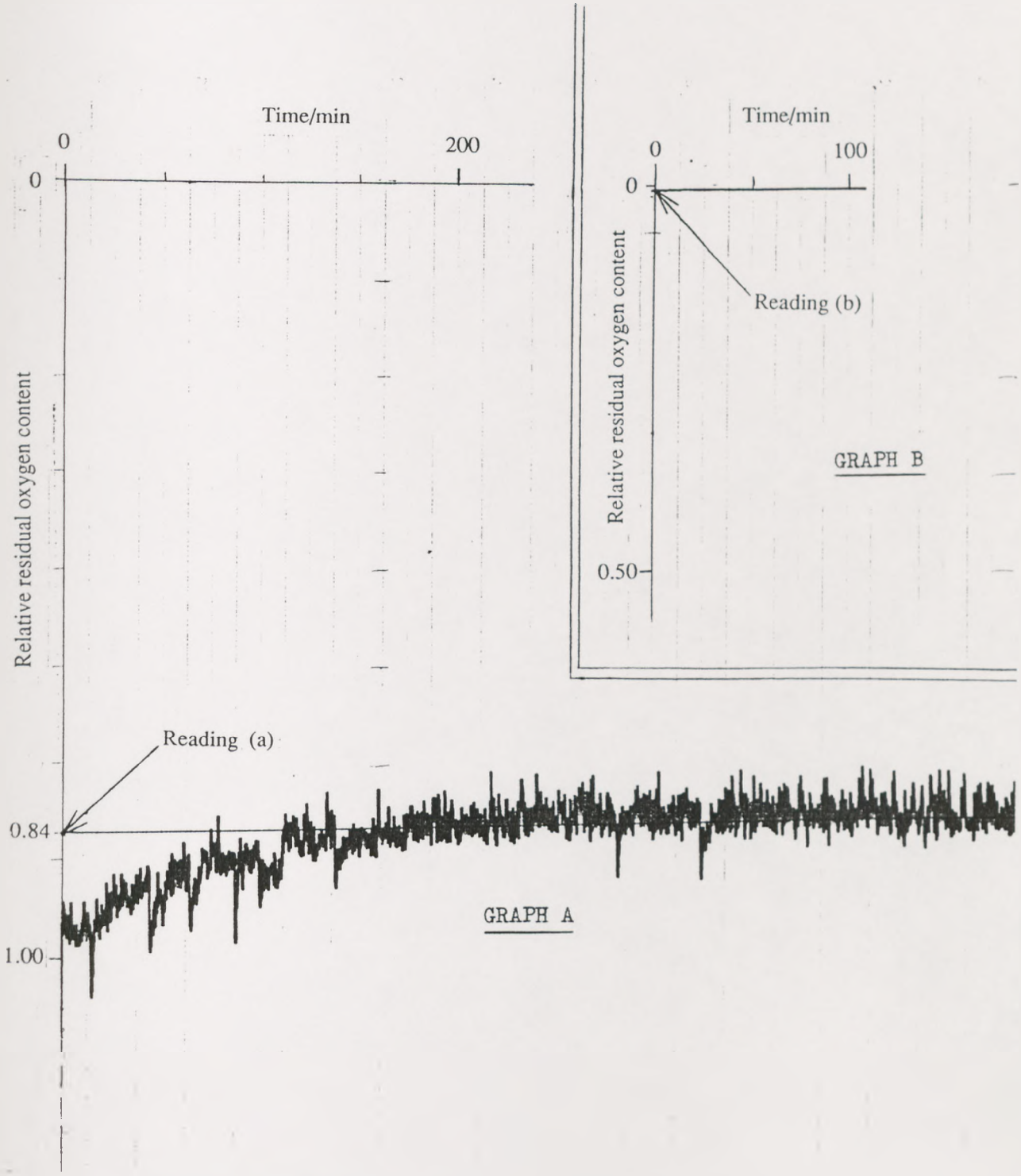


Fig.3.1.40. Relative residual oxygen content *vs* time for reaction mixtures of composition: (a) [glucose] = 0.4 M, [glycine] = 0.5 M; (b) [glucose] = 0.4 M, [glycine] = 0.5 M, [S(IV)] = 0.05 M at pH 5.5 and 55 °C.

[glucose] /M	[glycine] /M	[S(IV)] /M	Relative residual oxygen content.
0.0	0.0	0.00	1.00
0.4	0.5	0.05	0.00
0.4	0.5	0.00	0.84
0.4	0.0	0.05	0.00
0.0	0.5	0.05	0.00
0.4	0.0	0.00	0.92
0.0	0.0	0.05	0.00
0.0	0.5	0.00	0.87

Table 3.1.6. Relative residual oxygen content, extrapolated to time zero, at 55 ^{o}C of pure water and a series of freshly prepared reaction mixtures.

Sensitivity = 10mv, course=4.

3.1.2.6. Oxidation of S(IV) oxospecies.

The rate of the S(IV)-inhibited Maillard reaction is obtained by measuring irreversible S(IV) loss during the constant rate period. If S(IV) is being irreversibly lost by a process not included in the kinetic model for the reaction, such as autoxidation, then the measured rate of loss will be higher than the actual rate of irreversible binding. If this additional process occurs to a significant extent, then measurement of the rate of irreversible binding will be incorrect. It is, therefore, important to determine whether or not S(IV) autoxidation is occurring in the model systems and, if so, its extent must be quantified.

Figures 3.1.41-3.1.43 show S(IV), SO_4^{2-}, and H^+ concentrations as a function of time for mixtures of composition [glucose] = 0.4-2.0 M, [glycine]=0.5 M, [S(IV)] = 0.0438-0.0469 M. It can be seen that the

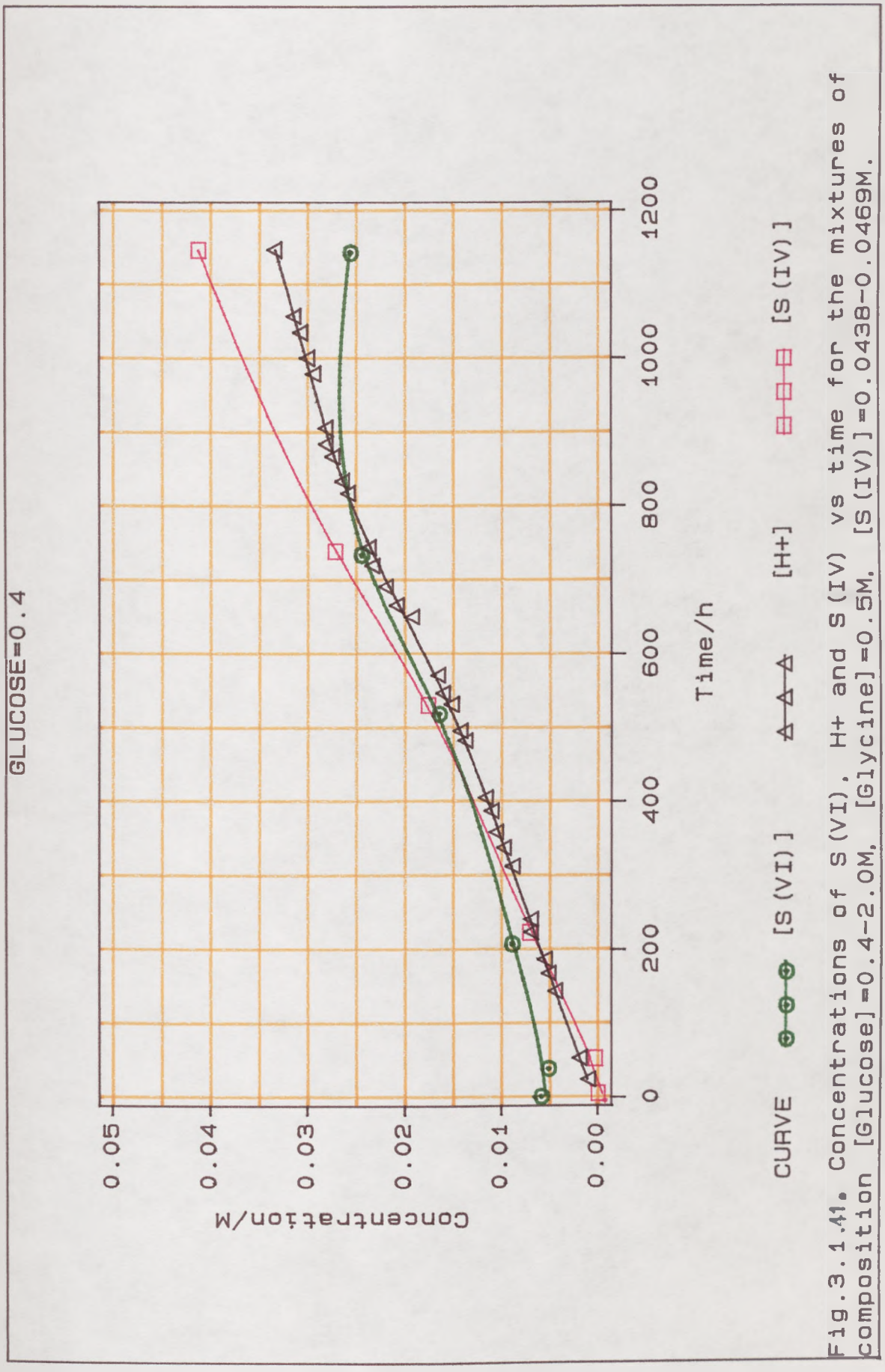


Fig.3.1.41. Concentrations of S(VI), H+ and S(IV) vs time for the mixtures of composition [Glucose]=0.4-2.0M, [Glycine]=0.5M, [S(IV)]=0.0438-0.0469M.

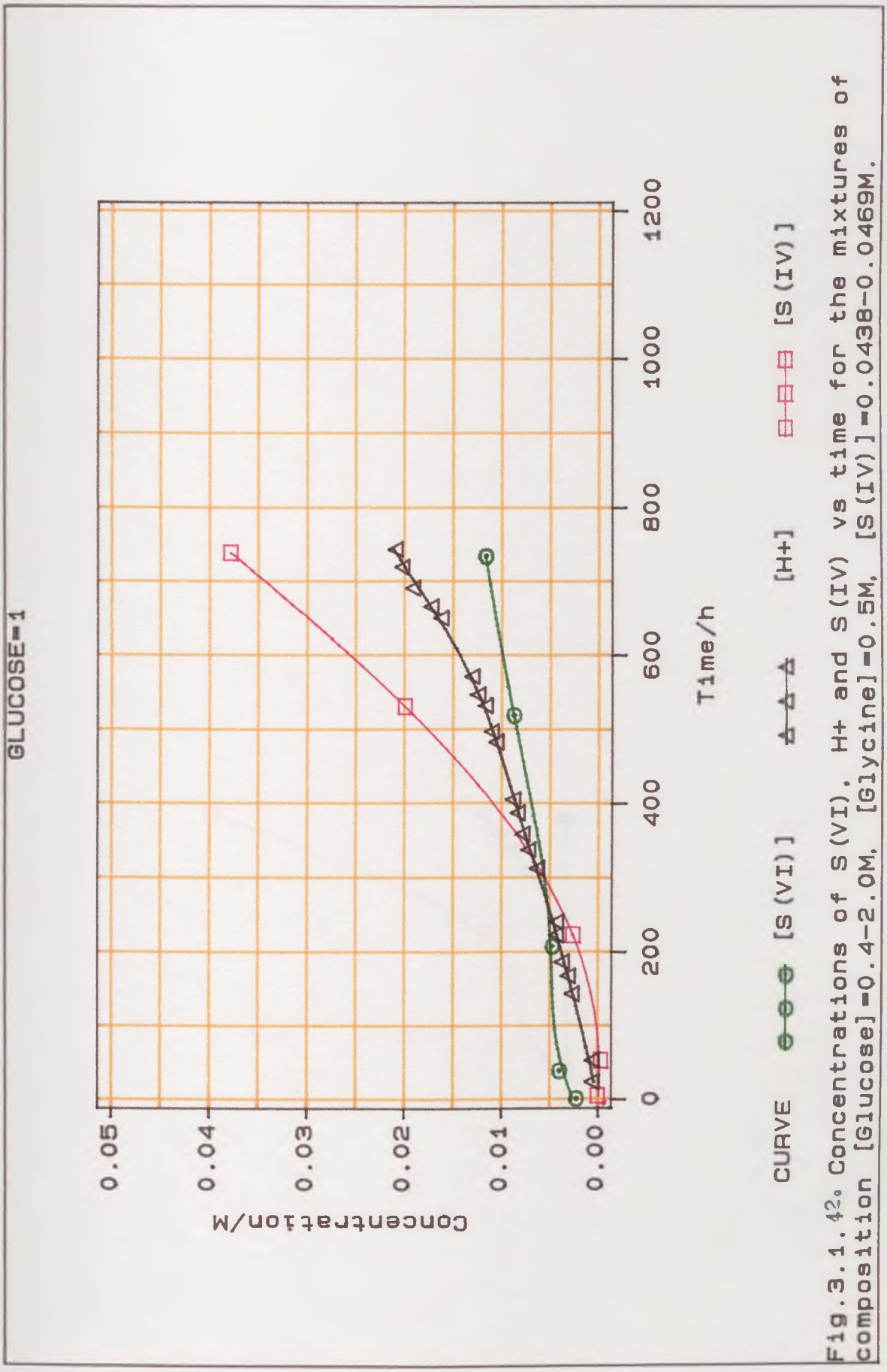


Fig.3.1.42. Concentrations of S(VI), H+ and S(IV) vs time for the mixtures of composition [Glucose]=0.4-2.0M, [Glycine]=0.5M, [S(IV)]=0.0438-0.0469M.

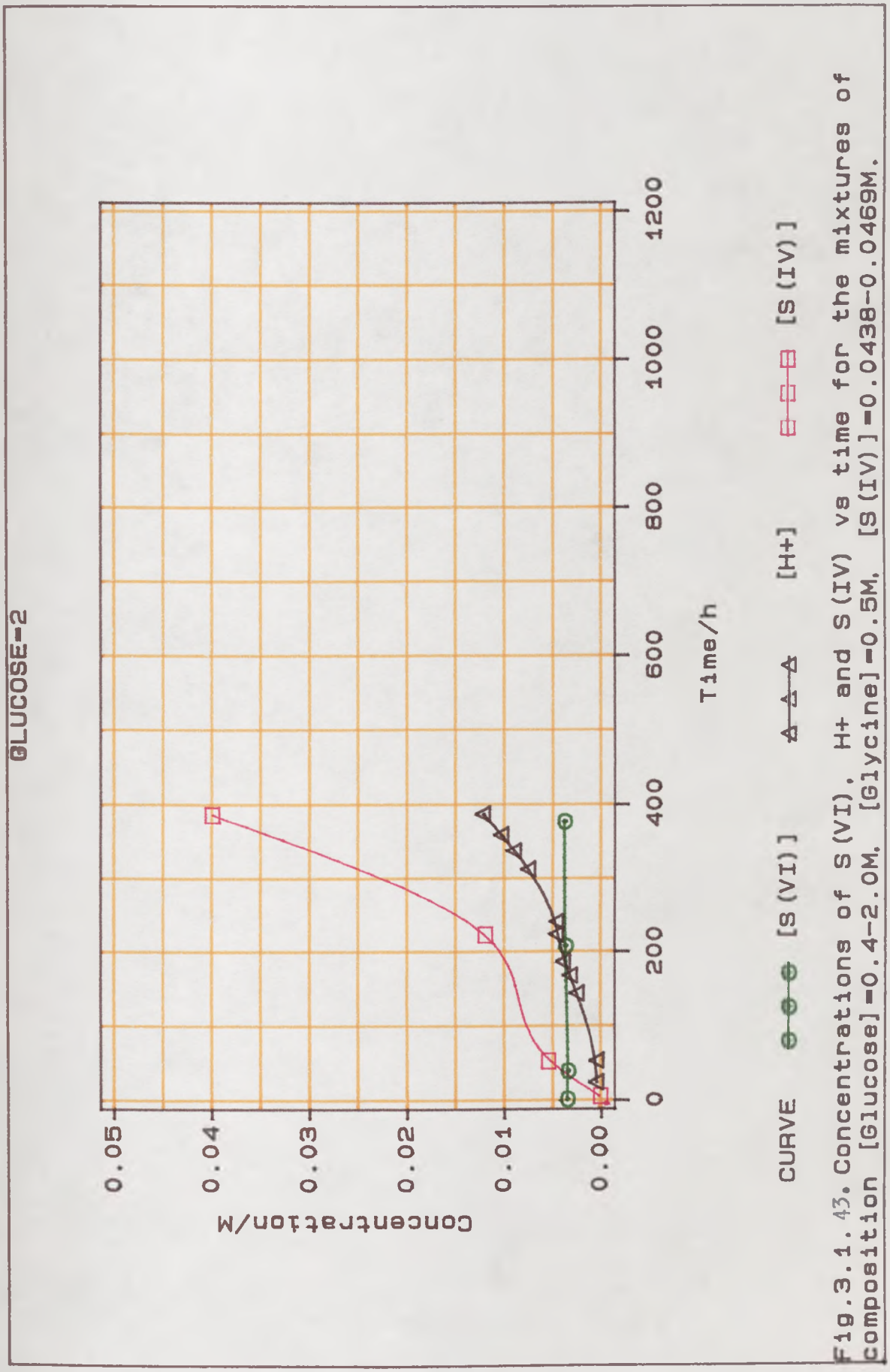


Fig.3.1.43. Concentrations of S(VI), H+ and S(IV) vs time for the mixtures of composition [Glucose]=0.4-2.0M, [Glycine]=0.5M, [S(IV)]=0.0438-0.0469M.

concentrations of S(IV), determined iodimetrically, S(VI), determined by precipitation with excess $BaCl_2$, and H^+, determined by NaOH titration, present in the mixture containing 0.4 M glucose are closely correlated over the first 750 h of the reaction. Sulphur(VI) concentration increases from 5.9 mM to 25.7 mM, i.e. 13.5-58.5% of the total S(IV) initially present. On the other hand, the mixture containing 2.0 M glucose shows no such correlation between the concentrations. In this case S(VI) concentration remains constant at 3.7 mM, or 8% of the total S(IV) initially present. The kinetic link between the measured concentrations at low glucose concentration at $t < 750$ h may be due to autoxidation of S(IV) to S(VI), which is inhibited at high glucose concentration. The analysis of S(VI) involves precipitation of sulphate as $BaSO_4$. Barium ions are known to form complexes with sugar molecules. Formation of a glucose-Ba^{2+} complex at high glucose concentration, could increase the solubility of $BaSO_4$ because of the following equilibria:

$$BaSO_{4\,(s)} \rightleftharpoons Ba^{2+}_{(aq)} + SO_4^{2-}{}_{(aq)}$$

$$Ba^{2+}_{(aq)} + glucose_{(aq)} \rightleftharpoons Complex_{(aq)}$$

This would cause incomplete precipitation of S(VI) and produce an artificially low S(VI) concentration. The effect of glucose concentration on the level of recoverable S(VI) was investigated using mixtures of composition [glucose] = 0.4 M, [glycine] = 0.5 M, [S(VI)] = 0.0437 M, and [glucose] = 2.0 M, [glycine] = 0.5 M, [S(VI)] = 0.0468 M. The concentrations of S(VI) recovered were 0.0440 M and 0.0471 M respectively. Therefore, in the range of concentration studied glucose does not interfere with the determination of S(VI) using barium.

Equation 3.1.8 (section 3.1.2.4) shows dissociation of HSO_3^- to SO_3^{2-} liberating H^+, followed by SO_3^{2-} oxidation to SO_4^{2-}. This explains the connection between S(IV) loss, S(VI) and H^+ liberation at low

glucose concentration; no such connection was observed at high glucose concentration.

3.2. Application of the kinetic model for the S(IV)-Inhibited Maillard reaction to conditions of reduced water activity.

3.2.1. Development of the kinetic model to lead to a description of the action of the preservative in a food.

Using the model system: glucose-glycine-S(IV) in "dilute solution" a kinetic model for the S(IV)-inhibited Maillard reaction has been developed by Wedzicha and Vacalis (1988). This has been critically reviewed above. Having obtained an understanding of such a simplified situation it is possible to progress, in a series of stages, to the more complicated situation which exists in various foods. Knowledge of how the preservative reacts in a food is desired as this may enable the effectiveness of the preservative to be optimised, with perhaps a consequent reduction in the level of use.

Sulphur(IV) oxoanions are added to a variety of foods to inhibit non-enzymic browning, including vegetable products, fruit products, beverages, meat, fish and protein products. The experimental model system used here and in some previous work is relatively dilute compared to some of these foods; as this is a major difference between previous models and many real food situations, it was decided that the effect of water-content on the kinetics of the S(IV)-inhibited Maillard reaction would be studied.

It is now generally accepted that water activity, a_w, is more closely related to the physical, chemical and biological properties of foods and other natural products than is total moisture content (Rockland and Nishi, 1980). Specific changes in colour, aroma,

flavour, texture, stability and acceptability of raw and processed food products have been associated with relatively narrow a_w ranges. For example, empirical studies on the influence of moisture on the stability of walnut kernels (Rockland, 1957), showed that kernels have a primary optimum a_w-moisture range above and below which kernels deteriorate at a more rapid rate. The proliferation of microorganisms occurs only above a_w *c.* 0.75 (Rockland and Nishi, 1980). Kapsalis (1973) reported a sharp maximum when the force for shearing freeze-dried beef was measured as a function of a_w, the maximum being in the region a_w 0.80-0.85. Lea (1951) observed that accelerated non-enzymic browning of a casein-glucose mixture, measured as a loss of free amino nitrogen, occurred during storage at 37 °C at an intermediate a_w. Water activity may have direct, uncomplicated effects upon various chemical reactions (Labuza, 1980), enzymatic reactions (Schwimmer, 1980a; b) and the proliferation of microorganisms (Troller and Christian, 1978; Troller, 1980). Therefore, it was decided that the effect of a_w, and not total moisture content, on the S(IV)-inhibited Maillard reaction would be studied. Sulphur(IV) oxospecies are used in foods of high a_w ($a_w \approx 1$), semi-moist foods (a_w = 0.6-0.9) and dehydrated foods (a_w = 0-0.5) (Labuza *et al*, 1977). It was decided, therefore, that the model would be tested over a wide range of a_w.

Conditions of reduced a_w may be obtained by:

(a) desorption or adsorption. This is achieved by placing the model system, usually a solid, over a saturated salt solution or a specific concentration of sulphuric acid solution., or

(b) direct addition of a humectant to the model system.

Previous workers (Eichner, 1975; Eichner and Karel, 1972; Labuza, 1975; Warmbier *et al*, 1976) have shown that the rate of Maillard

browning is affected by a_w. As the Maillard reaction involves the formation of up to 4 molecules of water per mole of glucose reacted, it is very important that the a_w of the system is constant during the course of the reaction, so that the kinetic effect of a component can be studied in isolation; otherwise the rate may be altered by a change in a_w in addition to any kinetic effect shown by the component under study.

The problems of adjusting the a_w of a solid model system containing glucose-glycine-S(IV) by adsorption or desorption are as follows. Wolf *et al* (1972) showed that when a food is dried down to a given a_w (desorption) or when it is first dried completely and then rehumidified to the same a_w (adsorption), the system subjected to desorption will have a greater moisture content. This effect is called hysteresis. A moisture sorption isotherm for a food product showing sorption hysteresis at a particular a_w, indicated by the dotted line, is shown in Fig.3.2.1.

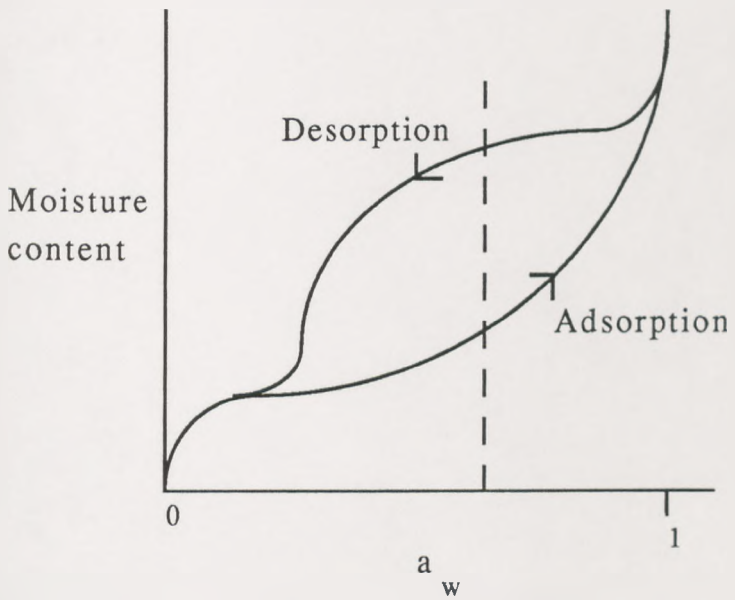


Fig. 3.2.1. Moisture sorption isotherm (moisture content *vs* a_w) for a food product showing sorption hysteresis.

Hysteresis may not represent a true equilibrium condition, but with respect to the normal shelf life of foods it is real and has a significant effect on food stability. It is possible that hysteresis could occur in the model system if the a_w is adjusted in this way. McWeeny (1973) studied the affect of a_w on the Maillard reaction by depositing glucose and glycine on a microcrystaline cellulose ('Avicel') matrix, conditioning this to various a_w values before storage at 37 °C and subsequent measurement of colour. In the present work, mixtures containing glucose-glycine and/or S(IV) were prepared by the method of McWeeny (1973) but using fibrous cellulose, reagent concentrations being given as mol kg^{-1} of cellulose. The total S(IV) concentrations, as a percentage of the total S(IV) originally present, in mixtures of composition [S(IV)] = 0.0516 mol kg^{-1} and [glucose] = 1.0 mol kg^{-1}, [glycine] = 0.5 mol kg^{-1}, [S(IV)] = 0.0853 mol kg^{-1} after 349.5 and 350.4 h were 48.5% and 46.8% respectively. The concentration of S(IV) in the mixture containing S(IV) only, was very similar to that in the Maillard browning mixtures despite the significant differences in composition and it is possible that S(IV) is being lost from both mixtures in the same way. Clearly, in the mixture containing S(IV) only, S(IV) is not being lost as a result of its inhibition of Maillard browning and this may also be the case for the mixture containing glucose, glycine and S(IV). S(IV) was quantitatively extracted by washing a sample of each mixture until the recovery of S(IV) in the extract was constant. The pH of this extract was then measured and the results are shown in table 3.2.1. The pH increases with time in both cases. The equilibrium between SO_2 in the aqueous phase of the sample and SO_2 in the

[S(IV)] = 0.0516 mol kg^{-1}		[S(IV)] = 0.0853 mol kg^{-1}	
Time/h	**pH**	**Time/h**	**pH**
0.0	6.06	0.0	5.44
39.7	6.11	39.0	5.72
110.0	6.08	109.5	5.81
183.6	6.27	184.6	5.91
302.3	6.35	302.1	6.06
349.5	6.69	350.4	6.23

Table 3.2.1. pH vs time for reaction mixtures containing [S(IV)] = 0.0516 mol kg^{-1} only and [glucose] = 1.0 mol kg^{-1}, [glycine] = 0.5 mol kg^{-1}, [S(IV)] = 0.0853 mol kg^{-1} deposited on fibrous cellulose. Reagent concentrations are given as mol kg^{-1} of cellulose.

headspace of the vessel in which the sample is enclosed is as follows:

$$HSO^-_{3(aq)} \underset{}{\overset{H^+}{\rightleftharpoons}} H_2O.SO_{2(aq)} \rightleftharpoons SO_{2(g)} + H_2O_{(aq)}$$

Sulphur dioxide could be lost to the atmosphere on each occasion the lid of the vessel was removed for analysis of the sample. The high solubility of SO_2 in water means that SO_2 could also transfer from the sample to the solution used to control the a_w. Since conversion of HSO_3^- to SO_2 requires a hydrogen ion, the effect will be one of raising the pH in agreement with observation. Loss of gaseous SO_2 would also prevent accurate measurement of the rate of the S(IV)-inhibited Maillard reaction. Since it was decided to study a wide range of a_w in this work, the humidifying of samples from an initial a_w of '0' to an intermediate a_w would require a long time; the S(IV)-inhibited Maillard reaction will proceed whilst this adjustment of a_w is taking

place and its behaviour during this time cannot be properly described. It is also possible that much, if not all, of the S(IV) added would have reacted by the time the desired a_w is obtained; the Maillard reaction would be well advanced and brown products may have begun to form. Hence, only the later stages of the reaction would be studied. In addition, when glucose, glycine and S(IV) are deposited onto cellulose as described, it is envisaged that the cellulose fibre will be coated by the reagents. Adsorption of water onto the surface of the solid and transport to the centre of the sample will occur. The extent of reaction will depend on the degree to which the reactants are solvated. The concentrations of the reagents will be unpredictable and it would be difficult to study the kinetics of such a system. Also, the bulk properties of the sample would affect its reactivity, as the absorption of water into the sample as a whole would be affected by the presence of capillaries and various structural components.

This is in contrast to the situation occurring when a humectant is added in homogeneous solution. The desired a_w is obtained immediately enabling the early stages of the reaction to be studied. Also, as foods contain natural humectants, e.g. sugars and salts, which will effect the a_w of the food; the use of such humectants renders the system a better description of the environment which S(IV) may encounter in a food. Raw red cabbage contains 3.5 g of sugar, 32 mg of Na, 300 mg of K, 53 mg of Ca, and 17 mg of Mg per 100 g of fresh cabbage (Paul and Southgate, 1979). The use of a polyol rather than a salt to alter the a_w seems more appropriate to simulate the situation in dehydrated sulphited cabbage, because sugar would act as the important humectant in the food which has a relatively low salt content. Chirife *et al* (1980) made a systematic study of a_w reduction in single non-electrolyte aqueous solutions. Of

the polyols studied (erythritol, glycerol, mannitol, and sorbitol), glycerol, the only liquid humectant tested, was particuarly effective at reducing the measured a_w. Use of glycerol will enable a lower a_w to be obtained than if a solid humectant were used to adjust the a_w, as glycerol has been found to be miscible with water in all proportions; the a_w reduction by solid humectants is limited to their solubility. This is illustrated by the fact that the minimum a_w's obtained were: 0.90, 0.76, 0.98 and 0.93 for the four polyols respectively (Teng and Lenzi, 1974). It was, therefore, decided that glycerol would be used to adjust the a_w of the reaction mixtures

However, Obanu *et al* (1977) observed that a non-enzymic browning reaction occurred in an eqimolar solution of glycerol and glycine (1 M), at 65 °C and pH 5.5 after an initial induction period of about 4 days. At 35 °C the induction period was approximately 25 days. No absorbance peak was obtained, the absorbance merely increasing with decreasing wavelength as is observed for non-enzymic browning in general. The highest glycine concentration used in the present kinetic experiments at reduced a_w was 0.5 M and the solvents used to control the a_w of the mixtures varied in glycerol content in the range 40.0-81.5% w/w, that is *c.* 4.8-10.8 M glycerol. At 55 °C it is possible, therefore, that brown products could form from the reaction of glycine with glycerol and intermediates or products could subsequently react with S(IV). Seow and Cheah (1985) also found that glycerol actively participated in the formation of brown pigments by reaction with glycine, however, water and glycerol alone at 0.80 a_w did not brown after 32 days, even at 60 °C. In addition Bello and Bello (1976) found that exposure of purified glycerol to air results in the appearance of aldehyde and peroxide impurities. These may participate in browning and could also react with S(IV). If the

glycerol-glycine reaction takes place in the presence of S(IV) and carbonylic intermediates and products arise, then there may be an additional contribution to reversible binding of S(IV). On the other hand inhibition of this reaction by S(IV) could lead to irreversible binding. If glycerol were to react with glycine to produce browning products, it is likely that the reaction would be most significant in the mixtures of highest concentration. Therefore, a reaction mixture of composition [glycine] = 0.5 M, [S(IV)] = 0.0455 M containing 81.5% w/w glycerol at pH 5.5 and 55 °C was tested to see if reversible binding or irreversible loss of S(IV) occurred, the results are shown in table 3.2.2.

Time/h	Free [S(IV)] /M	Total [S(IV)] /M	Reversibly bound [S(IV)]/M
0.6	0.0453	0.0455	0.0001
23.5	0.0464	0.0467	0.0003
96.9	0.0462	0.0465	0.0003
284.1	0.0459	0.0461	0.0003
454.7	0.0460	0.0463	0.0003

Table 3.2.2. Free, total and reversibly bound S(IV) concentration*vs* time for a mixture of composition [glycine] = 0.5 M, [S(IV)] = 0.0455 M and 81.5% w/w glycerol at pH 5.5 and 55 °C.

From table 3.2.2 it can be seen that on the timescale of the S(IV)-inhibited Maillard reaction no significant reversible binding or irreversible loss of S(IV) occurred. It can, therefore, be stated that in the range of concentrations studied, glycerol does not interfere with the system glucose-glycine-S(IV) at pH 5.5 and 55 °C.

Water released during the S(IV)-inhibited Maillard reaction, up

to the formation of DSH, may significantly change the water content of reaction mixtures to which a humectant has been added to adjust the a_w. It is possible to estimate the minimum amount of water formed as a result of the Maillard reaction. Say we consider the composition of a glycerol:water mixture of the lowest a_w studied here, i.e. 81.5:18.5% w/w glycerol:water. The highest S(IV) concentration in these mixtures was 0.0806 M. Therefore, as S(IV) and DDH react on a 1:1 basis to produce a one mole of DSH and two moles of water are released during the sequence of reactions leading to DDH, the amount of water formed after all the S(IV) has been irreversibly bound to form DSH will be 0.1612 M, or 2.9 g l^{-1}. The increase in the water content of such a mixture would, therefore, be *c.* 1.3%. This calculation does not take into account water released as a result of the formation of Schiff's bases or DH which has not been converted to DSH. However, in mixtures containing less glycerol or lower S(IV) concentrations, the percentage increase in water content will be less. Using the reaction mixtures where the amount of glycerol is varied it is not possible to accurately predict the effect on the a_w of a *c.* 1.3% increase in water content, because the a_w is also effected by the concentrations of glycine and S(IV) and these were not always exactly the same for each mixture. Measurement of the a_w of the mixtures at the beginning and end of the reaction is clearly necessary.

3.2.2. Order of reaction at reduced water activity.

If the rate expression obtained at an a_w of *c.* 1.0 is to be used to measure rate constants for the uncatalysed and S(IV)-catalysed Maillard reactions at reduced a_w, then it must first be shown that the order of reaction with respect to each component is unchanged under these conditions. It is assumed here that if this is true at the lowest

a_w studied, then this is also the case at any intermediate a_w.

As the concentration of the reactants will affect the a_w of a reaction mixture and rate is measured over a considerable extent of reaction; it is important to ensure that the a_w of each mixture in a series used to determine kinetic order is the same and constant throughout the duration of the reaction. Otherwise differences in rate produced by differences in a_w may be incorrectly attributed to a kinetic effect of the concentration of a particular component. Chirife *et al* (1980) showed that a solution of glycine (20% w/w, 2.88 M) and a solution of glucose (32.5% w/w, 2.05 M) each gave an a_w of 0.95 at 25 °C. Clearly, glucose is more effective at reducing the a_w than glycine. At pH 5.5 the predominant S(IV) species is HSO_3^-, which is capable of hydrogen bonding to water. However, the concentration of HSO_3^- is low compared to that of glucose; the effect of varying S(IV) concentration on a_w is, therefore, assumed to be insignificant in comparison to the effect of varying glucose concentration.

Water activity was measured by the method of Labuza (1984), as described in section 2.2.6. To illustrate the procedure a series of saturated salt and sulphuric acid solutions of known a_w were chosen, so that the a_w of the sample fell within the range covered by the standards. Samples of a reaction mixture of composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.05 M in 81.5:18.5 w/w glycerol:water solvent were suspended over the standards and the mass change recorded. Table 3.2.3 shows the masses of these samples, per gram of reaction mixture, at intervals of 24 h, each experiment being carried out in triplicate. It can be seen immediately that the a_w of the mixture lies somewhere between 0.30 and 0.50 and that the equilibrium process is complete, that is the change in mass is less than 2% of the original sample mass, after 72 h. Using the data in

Sample	Time /h	Change in mass (/g) per gram of sample			
		Saturated salt solution			
		$MgCl_2$	NaBr	$NaNO_3$	KCl
		a_w=0.30	a_w=0.50	a_w=0.68	a_w = 0.81
A	24	-0.0650	0.0428	0.2211	0.4690
B	24	-0.0647	0.0405	0.2196	0.4650
C	24	-0.0645	0.0405	0.2181	0.4630
A	48	-0.0025	-0.0025	0.0059	0.0646
B	48	-0.0038	-0.0013	0.0075	0.0660
C	48	-0.0035	-0.0012	0.0077	0.0720
A	72	-------	-------	-------	0.0021
B	72	-------	-------	-------	0.0149
C	72	-------	-------	-------	0.0101

Table 3.2.3. Change in mass of the samples, per gram of a sample, held over saturated salt solutions at 55 ^oC, of initial composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.05 M, 81.5% w/w glycerol at intervals of 24 h. Each experiment was carried out in triplicate.

table 3.2.3, the average mass change per gram of sample suspended over each standard was calculated after equilibrium had been reached and is plotted against the a_w of the standards in Fig.3.2.2. This plot is typical of all the results obtained. Table 3.2.4 shows the a_w of mixtures of varying glucose concentration in a solvent of composition 81.5:18.5% w/w glycerol:water at the start and end of the reaction; and the reproducibility of values of a_w at two glycerol contents. In the range of concentrations studied, increasing glucose concentration does not significantly change the a_w of the mixture.

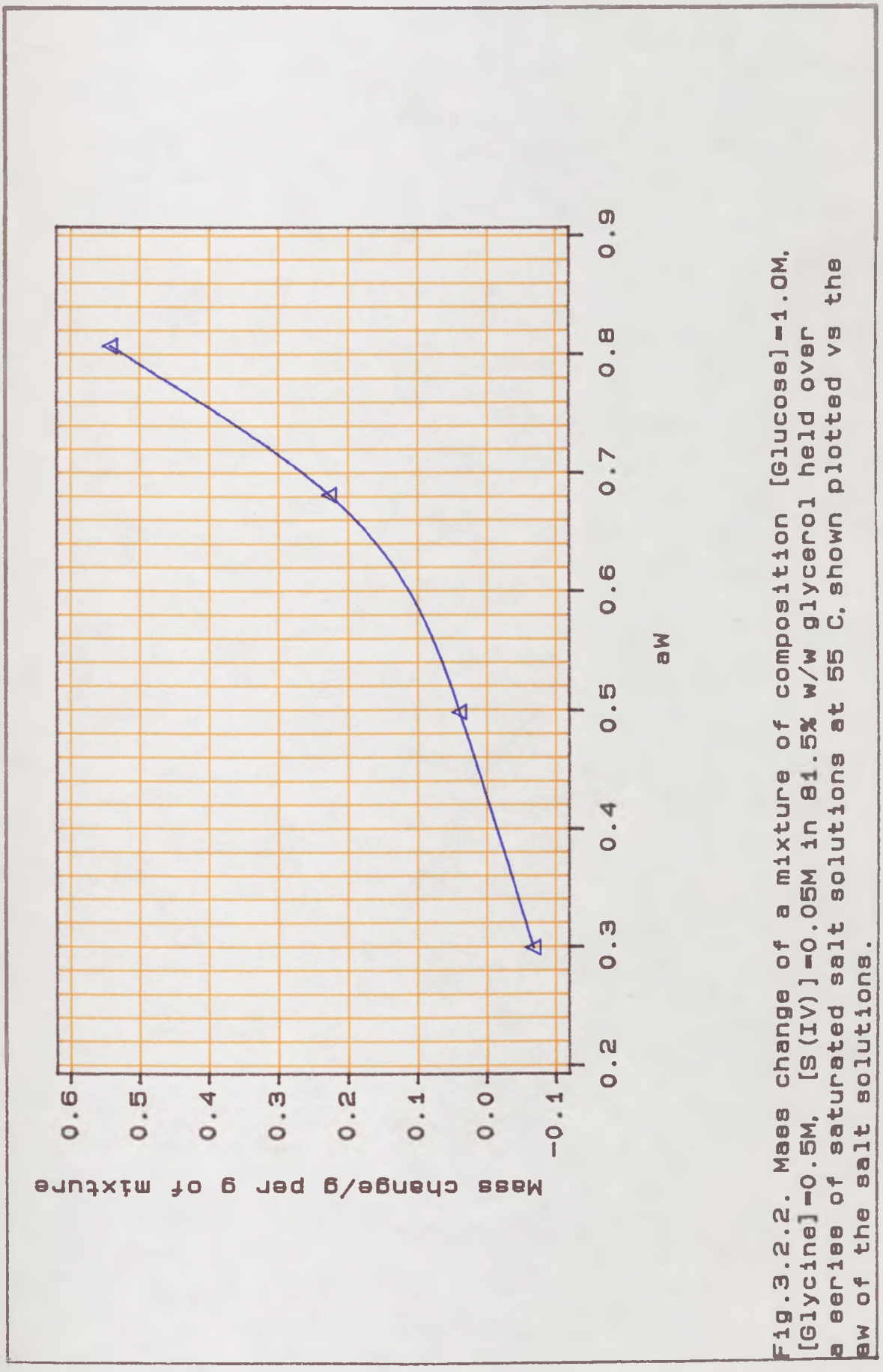


Fig.3.2.2. Mass change of a mixture of composition [Glucose]=1.0M, [Glycine]=0.5M, [S(IV)]=0.05M in 81.5% w/w glycerol held over a series of saturated salt solutions at 55 C, shown plotted vs the aw of the salt solutions.

[glucose] /M	[glycine] /M	[S(IV)] /M	a_w of mixture time=0	time=end	Glycerol /% w/w
0.2	0.5	*c.* 0.05	0.46	----	81.5
0.5	0.5	*c.* 0.05	0.43 (0.43)	----	81.5
1.0	0.5	*c.* 0.05	0.43	0.43	81.5
1.0	0.25	*c.* 0.10	0.65 (0.65)	----	70.3

Table 3.2.4. The a_w of mixtures of varying glucose concentration in a solvent of composition 81.5:18.5% w/w glycerol:water at the start and end of the reaction. The reproducibility of measured a_w at two glycerol contents is shown by the duplicate values in brackets.

Also, the a_w is reproducible and constant throughout the duration of the reaction. It is assumed, therefore, that this is also the case when glycine and S(IV) concentrations are altered. Hence, it is possible to obtain the kinetic order of reaction with respect to glucose, glycine and S(IV) at an a_w of 0.43-0.46, by varying of the concentrations in the ranges 0.2-1.0 M, 0.1-0.5 M, and 0.0116-0.0806 M respectively.

Rate is shown as a function of glucose, glycine, and initial S(IV) concentration in Figs.3.2.3-3.2.5. Correlation coefficients of 0.9918, 0.9944, and 0.9864 respectively were obtained, indicating excellent linear behaviour in each case. An intercept was obtained from the plot of rate *vs* initial S(IV) concentration and confirms that, at this a_w, production of DH occurs by two parallel pathways, one of which is catalysed by S(IV). As expected no such intercept was obtained when rate was plotted *vs* either the glucose or glycine concentration, confirming that glucose and glycine are involved in both pathways in a first order reaction. As the S(IV)-inhibited Maillard reaction is of first order with respect to glucose and glycine and the S(IV)-catalysed

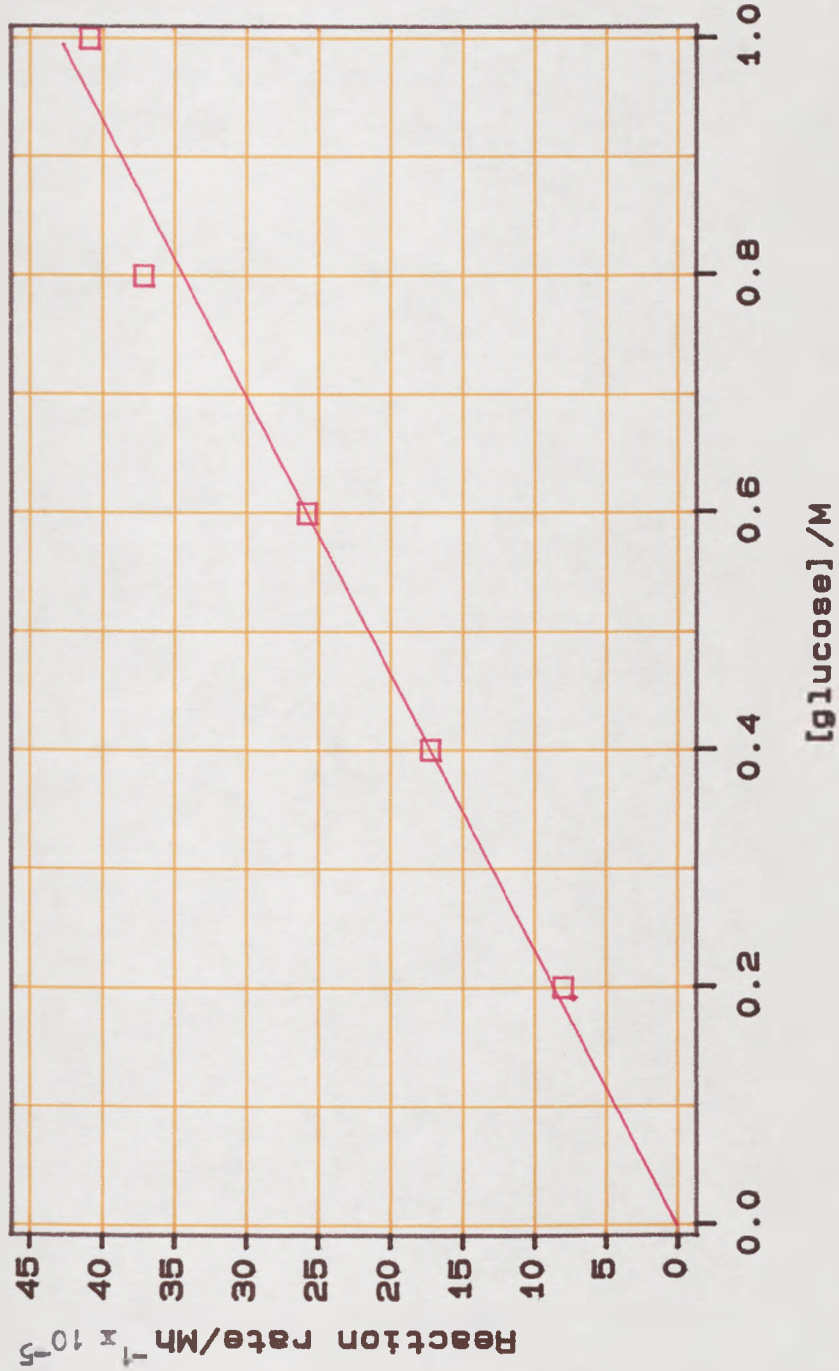


Fig.3.2.3. Reaction rate vs glucose concentration for the mixtures of composition [glucose]=0.2-1.0M, [glycine]=0.5M, [S(IV)]=0.0451-0.0478M at an aw of 0.43-0.46.

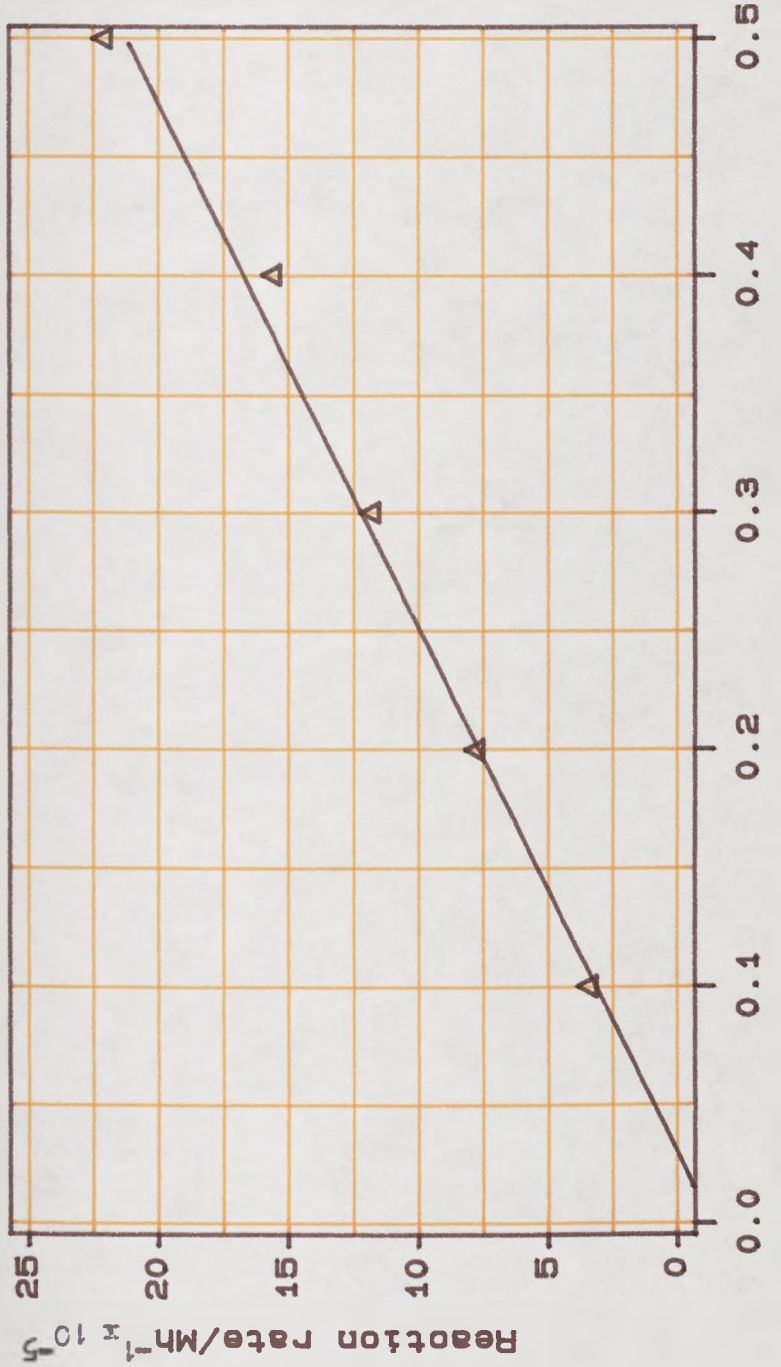


Fig.3.2.4. Reaction rate vs glycine concentration for the mixtures of composition [glycine]=0.1-0.5M, [glucose]=1.0M, [S(IV)]=0.0416-0.0477M at an aw of 0.43-0.46.

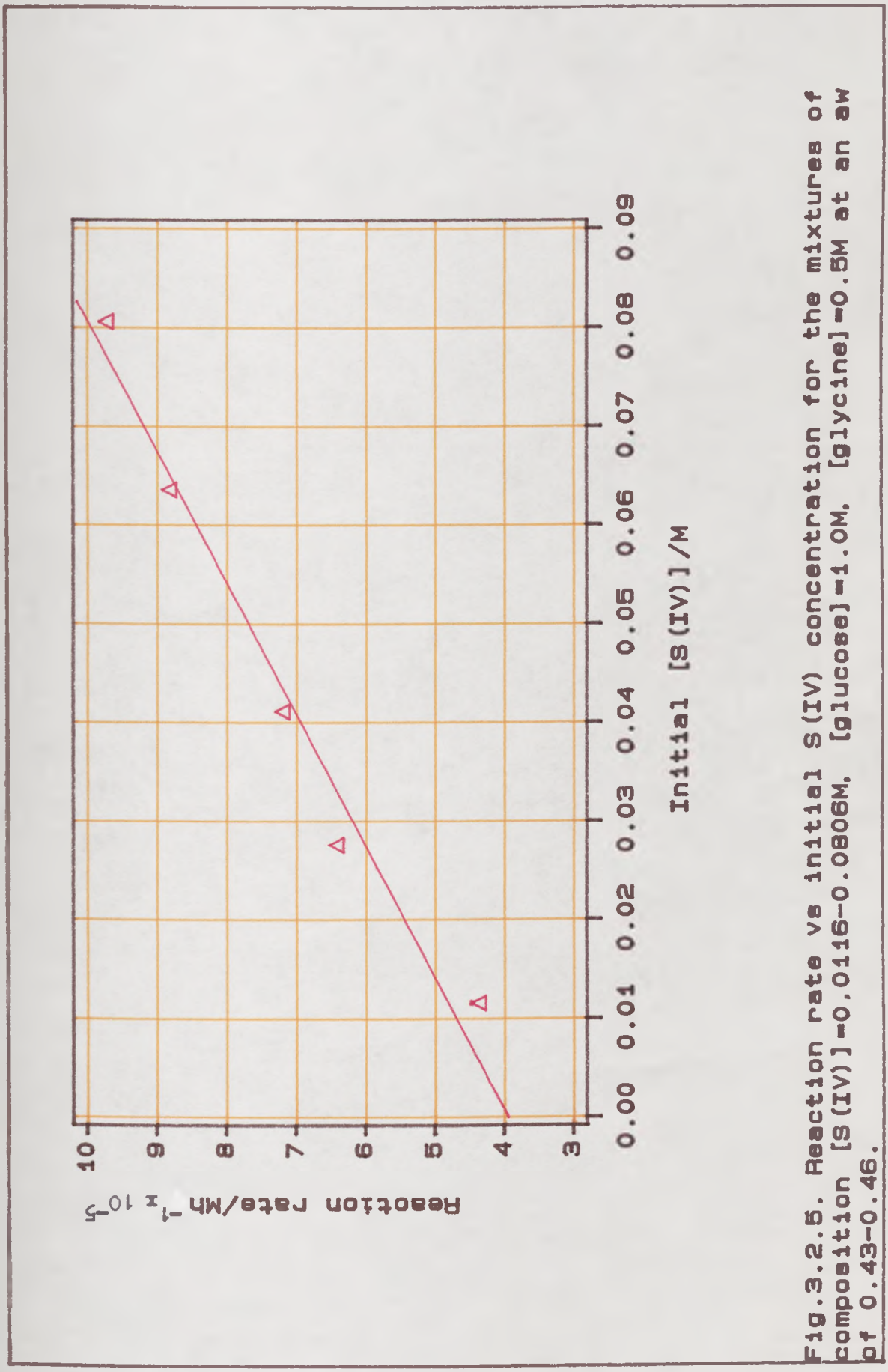


Fig.3.2.5. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0116-0.0806M, [glucose]=1.0M, [glycine]=0.5M at an aw of 0.43-0.46.

reaction is of first order with respect to initial S(IV) concentration at a_w of 0.43-0.46 and *c.* 1.0. It is, therefore, assumed that this is also the case at any intermediate a_w. Hence, it is possible to calculate k_1 and k_2 in the a_w range 0.43-1.0.

3.2.3. Effect of a_w on the rate constants for the S(IV)-inhibited Maillard reaction.

It was shown earlier, section 3.1.2.6, that S(IV) autoxidation is inhibited in a mixture containing 2 M glucose, 0.5 M glycine and 0.0469 M S(IV), but that it can occur to some extent at lower glucose concentration. As the rate constant for an early step in the S(IV)-catalysed Maillard reaction, k_2, is obtained from the gradient of a plot of rate of irreversible S(IV) binding *vs* initial S(IV) concentration; autoxidation of S(IV) will contribute to the rate of irreversible S(IV) loss and, therefore, towards the value of k_2. Therefore, the experimentally derived value of k_2 may contain a contribution from the amount S(IV) autoxidation occuring at that particular a_w. Values of the rate constant for the corresponding early step in the Maillard reaction, k_1, are obtained by extrapolation of reaction rate to zero S(IV) concentration; any S(IV) autoxidation should have little or no effect on the values of k_1. It has been shown that S(IV) autoxidation is first order with respect to the concentration of SO_3^{2-} and independent of the concentration of oxygen at pH 3-7 (Wedzicha, 1984b). Therefore, the rate of autoxidation is first order with respect to S(IV) at constant pH. The rate expression for the constant rate phase of the sulphite inhibited Maillard reaction can be written to include a contribution from autoxidation as follows;

$$\text{Rate} = k_1[\text{glucose}][\text{glycine}] + k_2[\text{glucose}][\text{glycine}][\text{S(IV)}] + k_3[\text{S(IV)}]$$

where k_3 is the rate constant for autoxidation and k_1 and k_2 are the rate constants in Fig.1.4.6. This equation may be simplified to:

$$\text{Rate} = k_1' + k_2'[\text{S(IV)}] + k_3[\text{S(IV)}]$$

where $k_1' = k_1[\text{glucose}][\text{glycine}]$ and $k_2' = k_2[\text{glucose}][\text{glycine}]$

A linear equation in [S(IV)] is obtained as follows:

$$\text{Rate} = k_1' + k''[\text{S(IV)}]$$

where $k'' = k_2' + k_3$.

From a plot of rate *vs* initial S(IV) concentration the intercept gives k_1' and the gradient k''. If k_2' is substituted and the expression rearranged we obtain the following:

$$k_2 = (k'' - k_3) / [\text{glucose}][\text{glycine}]$$

It is not possible to directly obtain a value of k_2 without a value of the rate constant for S(IV) autoxidation, k_3. However, such a value cannot be easily obtained as the extent of S(IV) autoxidation may change due to a variation in the concentration of transition metal ions, complexing agent, reactants etc. However, the glycerol content of the solvent used to control the a_w varied from 40-81.5% w/w, which represents a range of concentrations of approximately 4.8-10.8 M. Air oxidation of sulphite is effectively prevented by glycerol (Schroeter; 1966). It is likely that S(IV) autoxidation will be suppressed by the combined antioxidant effects of glucose, glycerol and glycine. Therefore, in the mixtures containing glycerol, autoxidation of S(IV) is unlikely to contribute towards values of k_2.

To obtain the rate constants k_1 and k_2 as a function of a_w, reaction mixtures of varying initial S(IV) concentration were studied in solvents of differing glycerol content. Table 3.2.5 shows the effect of glycerol content on the a_w of mixtures of composition [S(IV)] = 0.0819-0.0914 M, [glucose] = 1.0 M, [glycine] = 0.5 M at the begining and end of the reaction:

[S(IV)] /M	Water activity t=zero	t=end	glycerol content of solvent/%w/w
0.0914	0.85	0.85	40.0
0.0819	0.79	0.78	46.9
0.0882	0.70	0.70	60.6
0.0911	0.65	0.62	70.3

Table 3.2.5. Water activity of reaction mixtures of composition [S(IV)] = 0.0819-0.0914 M, [glucose] = 1.0 M, [glycine] = 0.5 M in solvents of varying glycerol content.

It can be seen that the a_w is effectively constant throughout the duration of the reaction and that increasing glycerol concentration reduces the reaction mixture a_w. The a_w of mixtures containing 81.5% w/w glycerol can be obtained from table 3.2.4, where the effect of glucose concentration on the a_w of a mixture was tested.

The constant rate of the S(IV)-inhibited Maillard reaction is plotted as a function of initial S(IV) concentration in Figs.3.2.5-3.2.9. The extent of any linear correlation between reaction rate and initial S(IV) concentration is given by the correlation coefficient at each a_w in table 3.2.6:

a_w of reaction mixture	Correlation coefficient
0.85	0.9939
0.78-0.79	0.9909
0.70	0.9953
0.62-0.65	0.9889
0.43-0.46	0.9864

Table 3.2.6. The extent of correlation between reaction rate, for the sulphite inhibited Maillard reaction, and initial S(IV) concentration as a function of a_w.

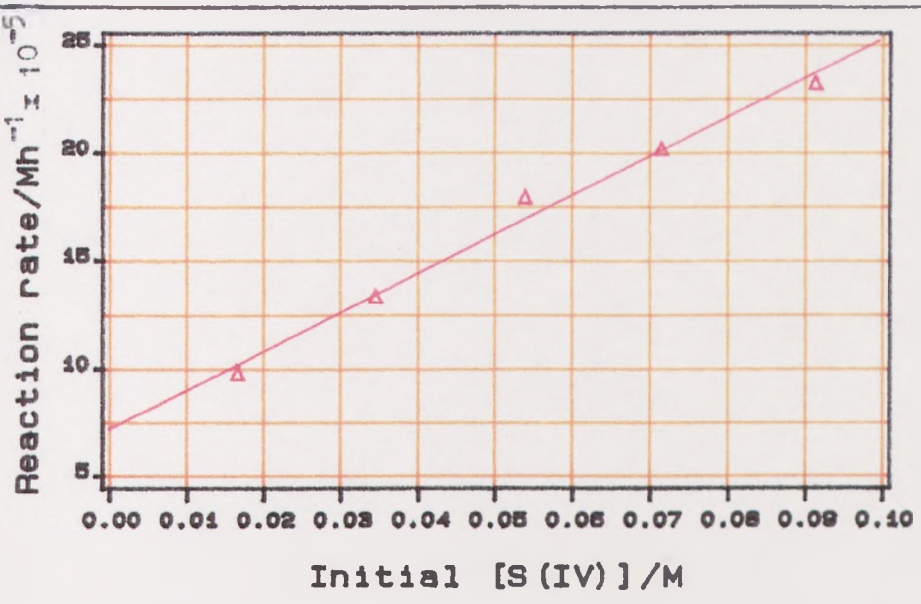


Fig.3.2.6. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0167-0.0914M, [Glucose]=1.0M, [Glycine]=0.5M at an aw of 0.85.

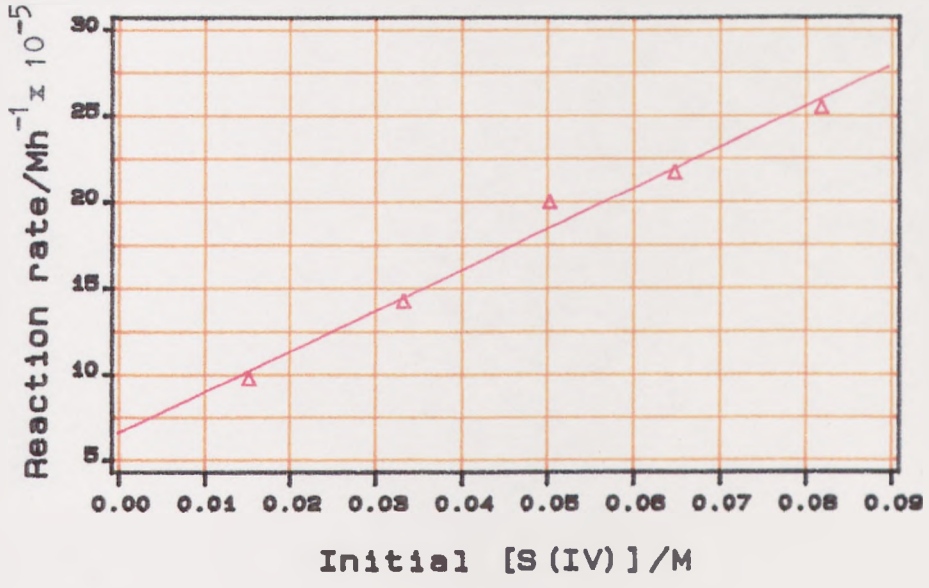


Fig.3.2.7. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0153-0.0819M, [Glucose]=1.0M, [Glycine]=0.5M at an aw of 0.78-0.79.

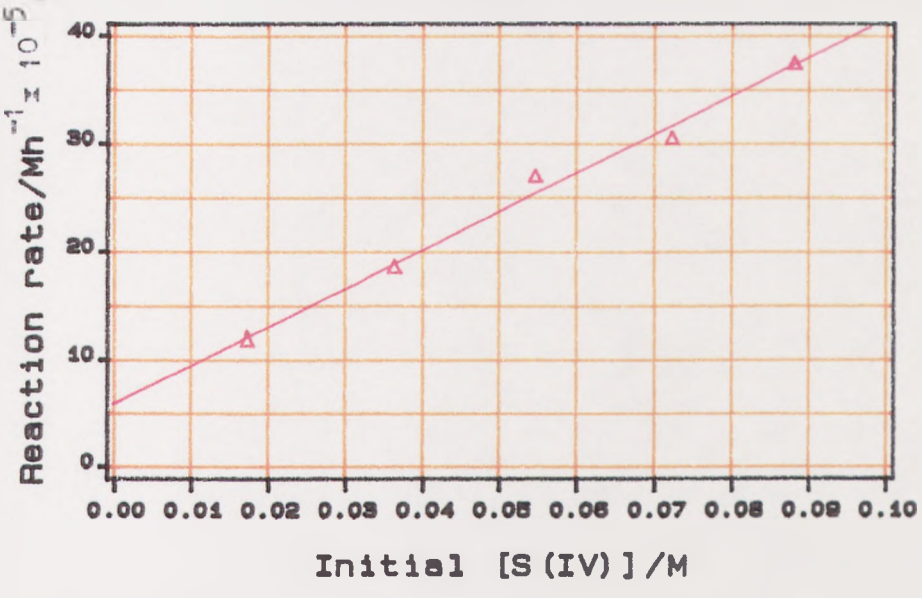


Fig.3.2.8. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0174-0.0882M, [Glucose]=1.0M, [Glycine]=0.5M at an aw of 0.70.

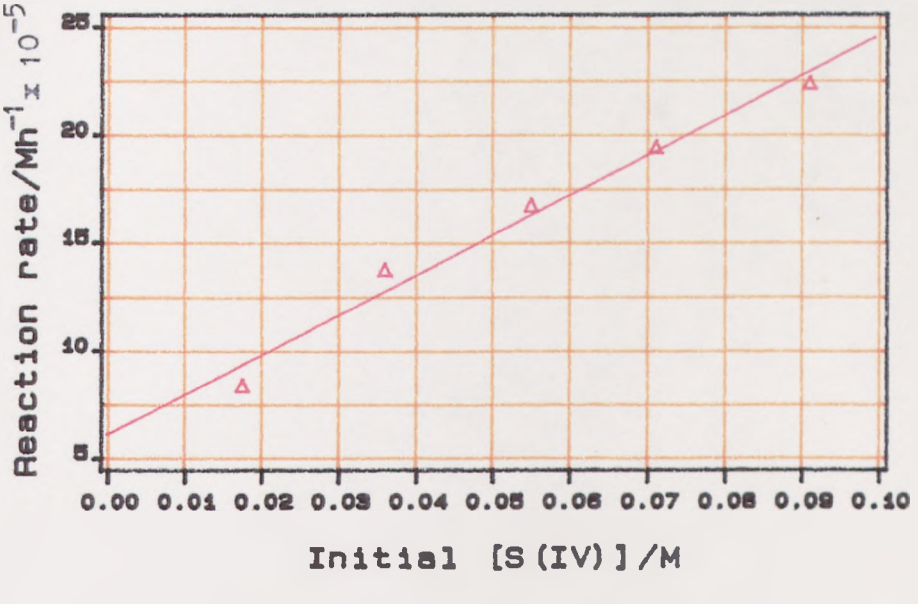


Fig.3.2.9. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0176-0.0911M, [Glucose]=1.0M, [Glycine]=0.25M at an aw of 0.62-0.65.

It can be seen that there is a high degree of correlation between the variables.

A linear regression analysis has been carried out on the 5 rate-initial S(IV) concentration data points at each a_w. The computed standard error is an estimate of the standard deviation (SD) for the rate constant. Values of k_1 and k_2 are shown plus or minus one SD from the mean and as a function of the measured a_w at the begining and end of the reaction in Figs.3.2.10 and 3.2.11. There is, therefore, a probability of 0.66 that a value of the rate constant will fall within this range. This is a relatively narrow specification for allowed values of the rate constants, which we see as being markedly affected by a_w at $a_w < 0.7$ for k_1 and $a_w > 0.7$ for k_2.

The a_w of the mixture used to obtain k_1 and k_2 which contained no glycerol was not measured. However, a solution containing 2 M glucose has an a_w of *c.* 0.95 (Chirife *et al*, 1980). As the reaction mixtures of varying S(IV) concentration contained 1 M glucose, the a_w of the mixture that contained no glycerol is likely to be higher than 0.95 and has , therefore, been rounded to 1.0.

It can be seen that the shape of the plots of k_1 and k_2 *vs* a_w are notably different, confirming that the early stages of the S(IV)-catalysed and the uncatalysed Maillard reactions proceed by differing mechanisms.

The rate constant k_1 increases with reduced a_w and in the range studied, a_w 0.43-1.0, does not reach a maxima. The standard deviation also increases with decreasing a_w, but there is no overlap of the SD ranges. Therefore, the increase is probably not due to error in the values of k_1 and must be due to the effect of a_w on the Maillard reaction. Eichner and Karel (1972) found that the rate of browning,

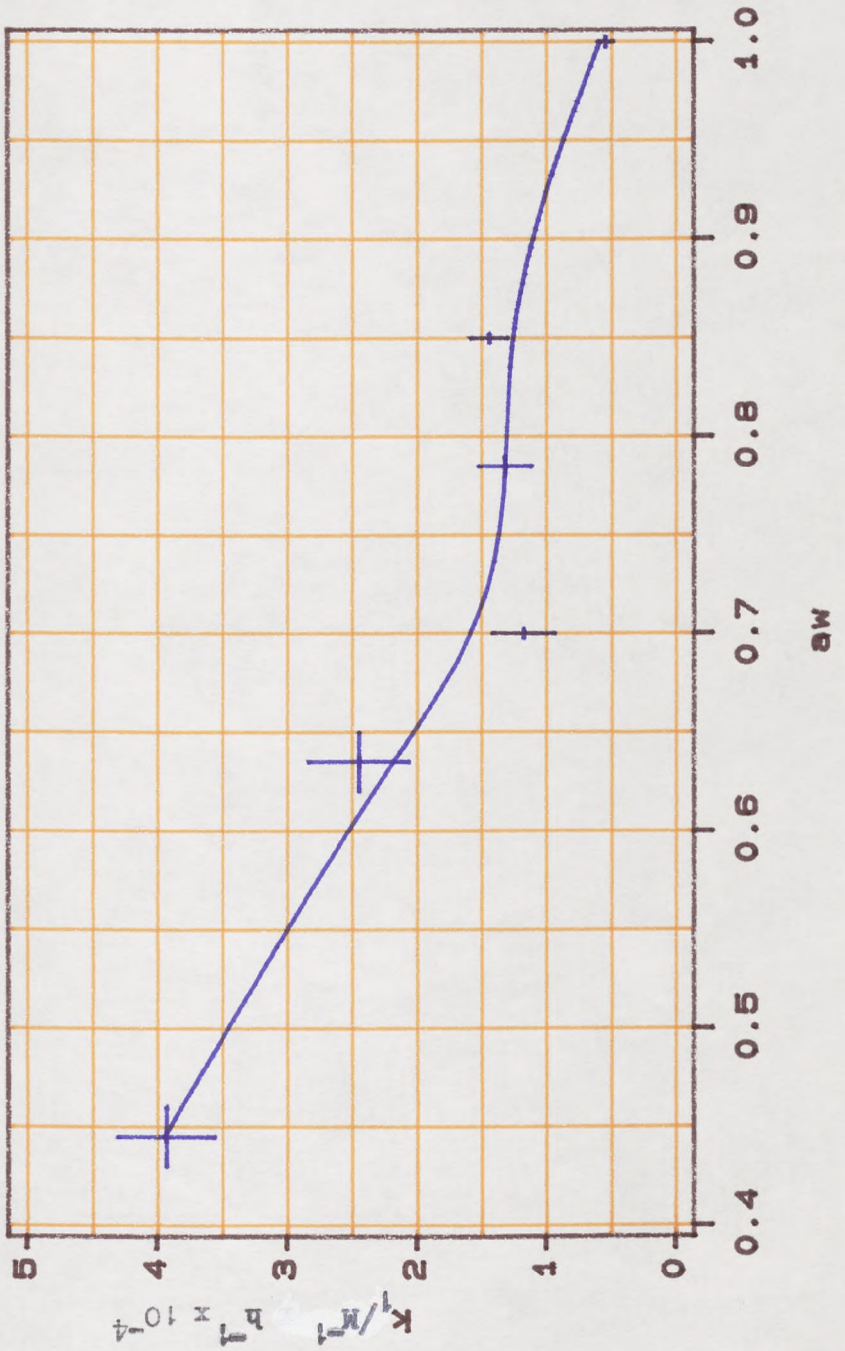


Fig.3.2.10. Graph of rate constant k_1, for an early step of the Maillard reaction, vs aw.

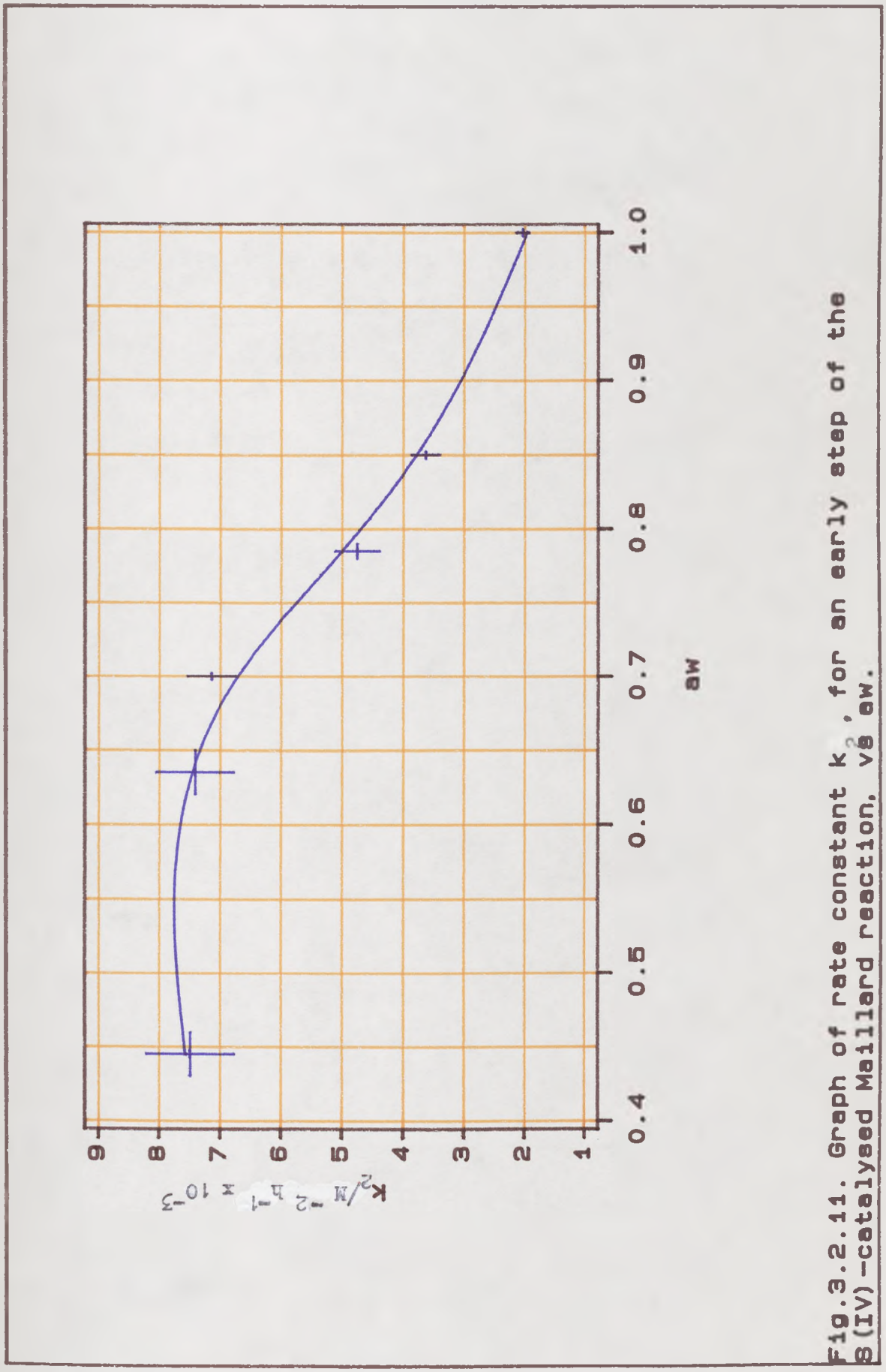


Fig.3.2.11. Graph of rate constant k_2, for an early step of the S(IV)-catalysed Maillard reaction, vs aw.

obtained by absorbance measurements at 420 nm, increased down to an a_w of 0.4 in a model system containing glucose, glycine and glycerol. No such maximum has been obtained in this work but it is expected to lie outside the range of a_w studied, as one imagines that no chemical reaction would be possible at $a_w = 0$ because no transport of the reactants would be possible.

The concentration of water is reduced by the addition of glycerol to a point where it is no longer in large excess. According to Labuza *et al* (1977), the occurrence of a rate of browning maximum as a_w increases from zero is probably due to four factors. (1) Up to 3.5 mol water per mol sugar are formed during browning (Eichner and Karel, 1972). Thus, water can act through product inhibition by retarding the initial glycosylamine reaction. (2) Water may enhance deamination reactions later on in the sequence as shown by Reynolds (1963). (3) Probably the most important contribution of water is the dilution of reactant components (Eichner, 1975; and Labuza, 1971). (4) The aqueous phase becomes less viscous with increase in water content allowing greater mobility of reactants. Thus, as a_w is increased from zero factors 2 and 4 serve to increase the rate which reaches a maximum and then begins to fall as factors 1 and 3 dominate.

In addition to the rate increasing with reduced a_w, previous workers (Eichner, 1975; Eichner and Karel, 1972; Warmbier *et al*, 1976) have found that the position of the rate maximum for the Maillard reaction is affected by the presence of glycerol. Eichner (1975) stated that on increasing the amount of glycerol, reaction rate was increased at low water activities. This was attributed to a decreasing diffusion resistance of the system caused by the presence of a liquid medium. At higher water activities, on the other hand, the

dilution and inhibitory effects of water prevail decreasing the reaction rate. This seems reasonable as the total amount of liquid phase, water + glycerol, is greater when glycerol is used compared to the amount of liquid phase, water only, present when the a_w is adjusted by equilibration over a saturated salt solution. It is likely that glycerol also exerts a specific solvent effect in the present investigation.

At any a_w, values of k_2 are significantly larger than those of k_1, demonstrating that the predominant pathway for DH production in the model system glucose-glycine-S(IV) is by the S(IV)-catalysed Maillard reaction. Thus, in food dehydration, S(IV) could exert a substantial effect on the rate of reaction of glucose with amino acids.

It can be seen that k_2 increases with reduced a_w and reaches a constant value at $a_w < 0.7$. The standard deviation of the data increases with decreasing a_w, but the behaviour is clear cut. The a_w at which the constant value of k_2 is obtained may have been shifted to a lower a_w as a result of the solvent effect of glycerol.

Water activity may affect the condensation reactions occurring in the S(IV)-catalysed reaction in the same way as it affects the Maillard reaction. However, the predominant S(IV) species in solution may be affected by a change in a_w, thus changing the extent of catalysis and reaction rate. It is expected that as a_w is reduced, this will promote the formation of $S_2O_5^{2-}$ as a result of the effect of water on the law of mass action expression for the reaction,

$$2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O$$

In situations where the concentration of water is limiting the formation constant for $S_2O_5^{2-}$ is given by:

$$K = [S_2O_5^{2-}][H_2O] / [HSO_3^-]^2$$

Rearrangement of this equation yields:

$$[S_2O_5^{2-}] = [HSO_3^-]^2 K / [H_2O]$$

If significant $S_2O_5^{2-}$ formation occurs and $S_2O_5^{2-}$ is a catalyst of the S(IV)-inhibited Maillard reaction, then the rate equation (equation 3.1.1) for the S(IV)-inhibited Maillard reaction may be written as:

$$\text{Rate} = k_1[\text{glucose}][\text{glycine}] + k_2[\text{glucose}][\text{glycine}][S_2O_5^{2-}]$$

and substitution for $S_2O_5^{2-}$ would yield:

$$\text{Rate} = k_1[\text{glucose}][\text{glycine}] + k_2[\text{glucose}][\text{glycine}][HSO_3^-]^2 K / [H_2O]$$

Therefore, the rate of the S(IV)-catalysed reaction would be of second order with respect to initial S(IV) concentration. The good linear behaviour of rate*vs* [S(IV)] plots indicates that either significant $S_2O_5^{2-}$ formation has not occurred, or that it is not kinetically significant. The effect of a_w on k_2 could be the result of a change in the extent of base catalysis, due to variation in the proportions of HSO_3^- and SO_3^{2-} in solution. It was decided, therefore, to investigate whether or not $S_2O_5^{2-}$ is formed at low a_w in the presence of glycerol.

3.2.4. Effect of glycerol on the predominant S(IV) oxospecies in solution and the implications for the S(IV)-inhibited Maillard reaction.

There is interest in the species $S_2O_5^{2-}$ because it is a poor nucleophile (Wedzicha and Goddard, 1991). The ability of sulphite species to inhibit non-enzymic browning arises from the nucleophilicity of the sulphite ion, and the possible conversion of HSO_3^- to $S_2O_5^{2-}$ in concentrated foods (e.g. dehydrated and partially dehydrated fruits and vegetables) has prompted speculation (Wedzicha, 1987) that the high demand for the additive to control browning in such foods is the result of it being converted to a relatively unreactive form. Additionally, catalysis of the early stages of the Maillard reaction by S(IV) (Wedzicha and Vacalis, 1988) has

been ascribed to general acid-base catalysis. Whereas SO_3^{2-} and HSO_3^- are good acid base catalysts, it is possible that $S_2O_5^{2-}$ may be considerably more effective (Slae and Shapiro, 1978). If this speculation is correct, the formation of such a catalyst in concentrated sulphited foods could be another reason for the need of high levels of use of the additive in such products.

Herrera-Viloria (1984) showed that the yield of C-sulphonate (not including hydroxysulphonate) formed during the dehydration of potato strips at 80 °C, is of first order with respect to the amount of S(IV) added to inhibit browning. This suggests that C-sulphonate production is not catalysed by $S_2O_5^{2-}$ and that catalysis must be due to another S(IV) species. There is, however, no unequivocal evidence for the formation of $S_2O_5^{2-}$ in model systems or dehydrated foods and the lack of second order behaviour indicative of catalysis by $S_2O_5^{2-}$ could simply be due to the species not being formed. Measurement of the relative proportions of the individual S(IV) oxospecies may help to explain the behaviour of the rate constant for an early step in the S(IV)-catalysed Maillard reaction, k_2, as the a_w is reduced by addition of glycerol.

Reference I.R. spectra of S(IV) oxospecies were obtained from 0.1 M solutions at pH 1 (Fig.3.2.12), pH 5.5 (Fig.3.2.13) and pH 9 (Fig.3.2.14). Thus, $SO_2 \cdot H_2O$ absorbs at 1151 cm^{-1} (peak B, symmetric stretch) and 1331 cm^{-1} (peak A, non-symmetric stretch) (Davis and Chatterjee, 1975), the weak feature at 1052 cm^{-1} (peak C) possibly being due to a trace of HSO_3^-, in equilibrium with $SO_2 \cdot H_2O$. Solutions in which the predominating species is HSO_3^- give peaks at 1210 cm^{-1} (peak D, $S_2O_5^{2-}$, non-symmetric S-O stretch), 1054-1065 cm^{-1} (peak E, $S_2O_5^{2-}$) and 1023 cm^{-1} (peak F, HSO_3^-) (Herlinger and Long, 1969; Davis and Chatterjee, 1975) whilst SO_3^{2-} gives a weak broad peak at

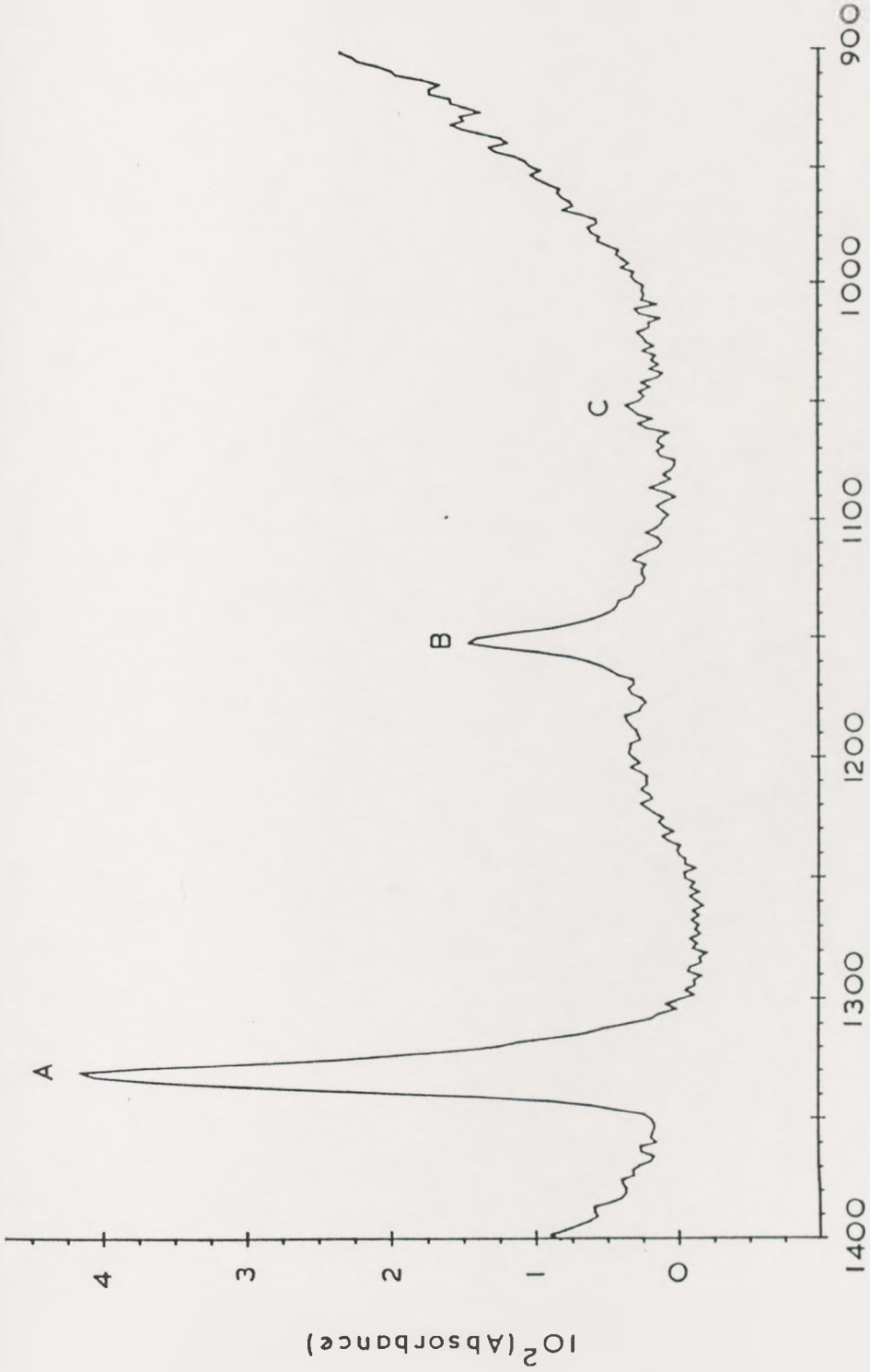


Fig.3.2.12. FTIR spectra of a 0.1 M solution of S(IV) at pH 1.

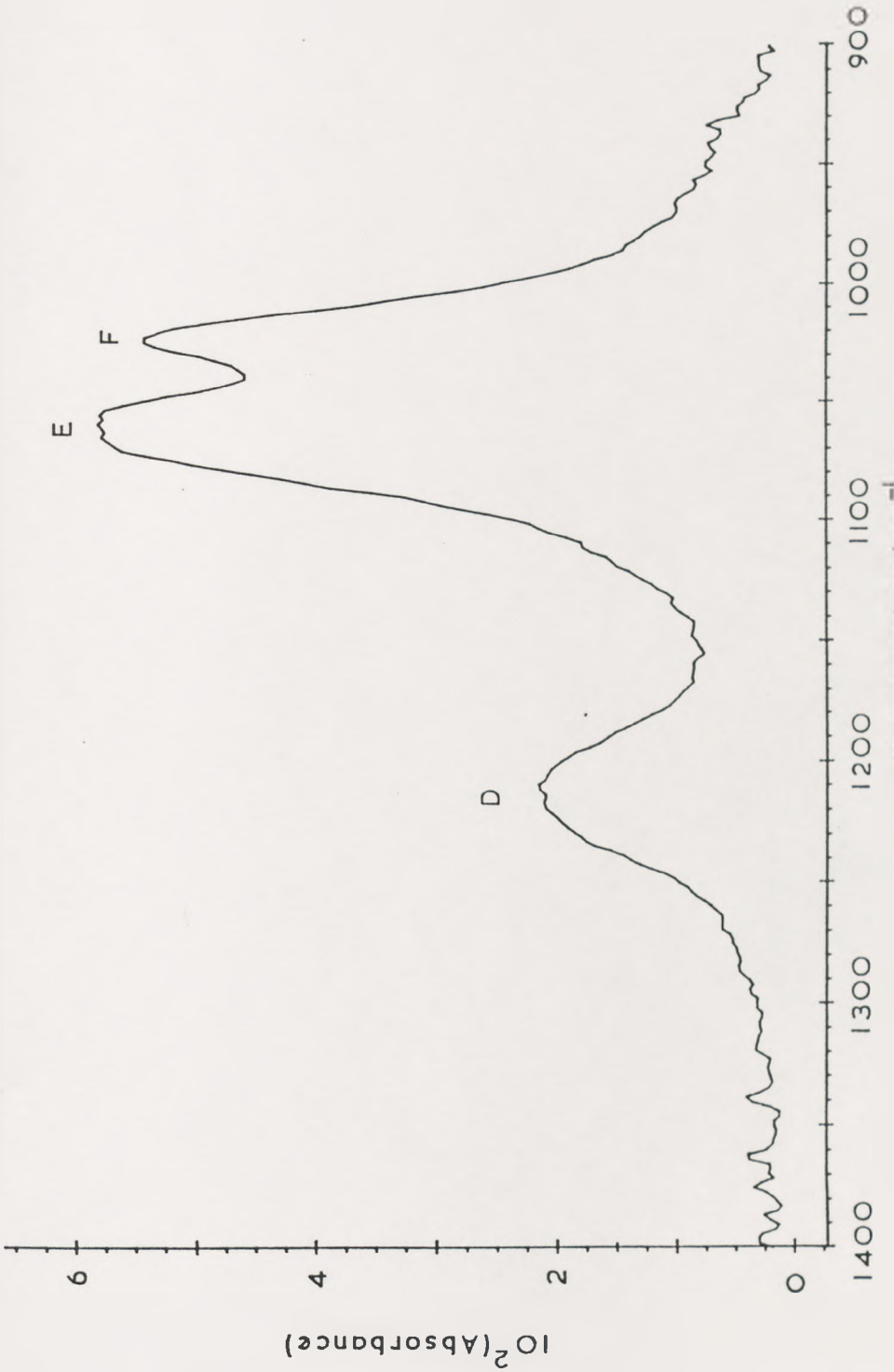


Fig.3.2.13. FTIR spectra of a 0.1 M solution of S(IV) at pH 5.5.

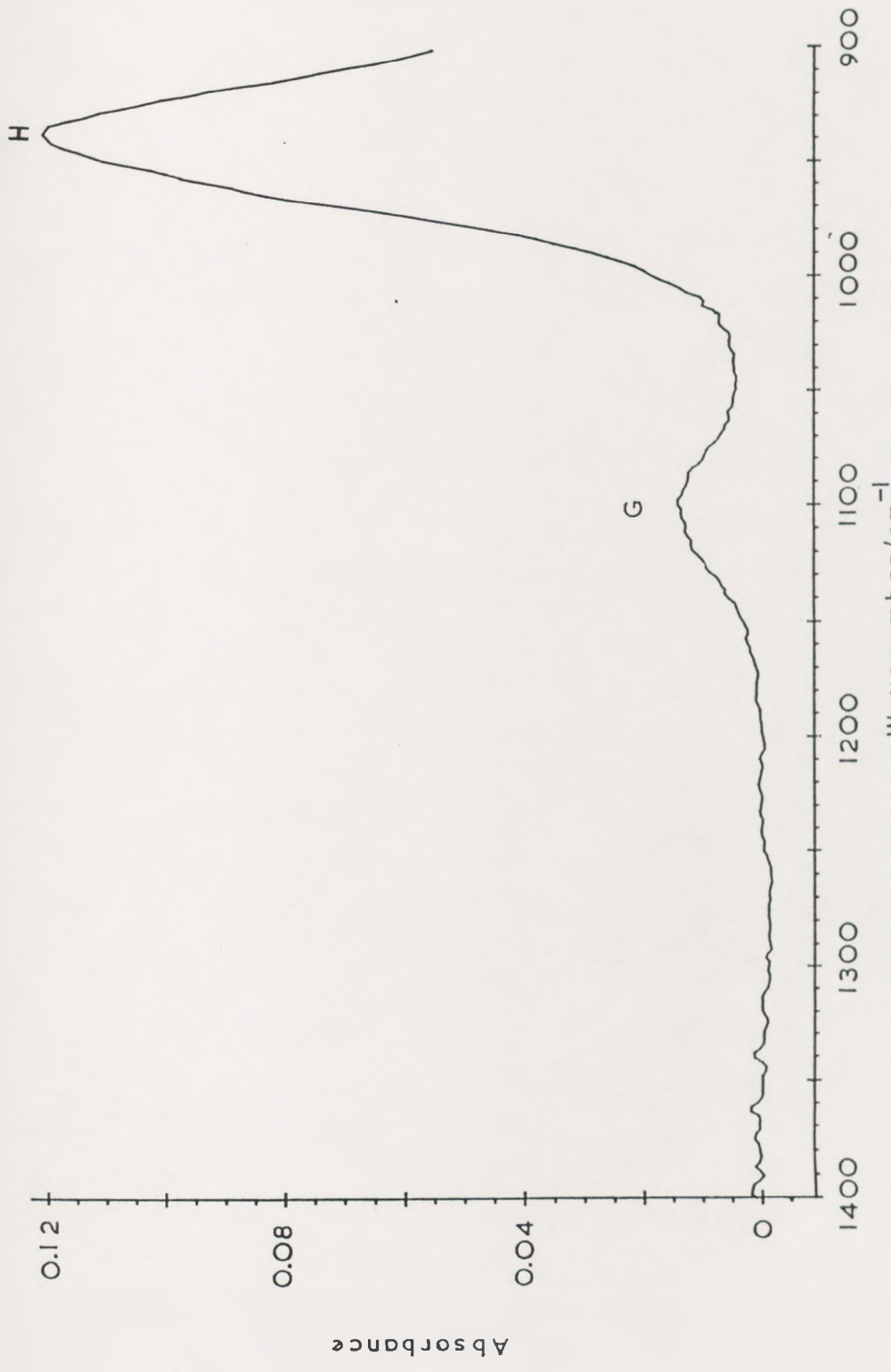


Fig.3.2.14. FTIR spectra of a 0.1 M solution of S(IV) at pH 9.

1098 cm^{-1} (peak G, symmetric stretch) and an intense absorbance at 938 cm^{-1} (peak H, non-symmetric stretch). Sulphate ion gives a single peak at 1100 cm^{-1} (Fig.3.2.15, non-symmetric stretch), the symmetric mode being only Raman-active. We see that the solution of S(IV) at pH 5.5 shows no evidence of containing $SO_2 \cdot H_2O$ or SO_3^{2-} ions. It is probably relatively free from SO_4^{2-} ion because this absorbs at least twice as strongly as the S(IV) and there is no evidence of even a shoulder on the high frequency side of the peak at 1054-1065 cm^{-1} in Fig.3.2.13.

When spectra were recorded from a 1 M solution of S(IV) at pH 5.5 an additional weak feature was seen at 965 cm^{-1} and the relative heights of the peaks at 1054-1065 and 1023 cm^{-1} were slightly altered. A new broad and weak absorbance was also seen at *c.* 1160 cm^{-1}. Both new peaks are due to increased concentration of $S_2O_5^{2-}$, deduced from previously reported assignments (Davis and Chatterjee, 1975; Herlinger and Long, 1969).

It was decided to investigate the effect of glycerol in the concentration range 0-30% (w/w), because this gives the greatest change in U.V. absorbance at 255 nm as described in section 2.2.7. The I.R. spectra of aqueous glycerol solutions are shown in Fig.3.2.16 and illustrate that, with increasing glycerol concentration, one simply obtains scaled traces with no evidence of new features arising from changes in solute-solvent interactions. However, at the highest glycerol concentration, the absorbance is in the region of 1.0 and, therefore, the glycerol-water reference has a high absorbance compared with that of S(IV) species; the main problem is that the absorbance maxima shown by the reference are in the region of interest. This was found to limit the concentration of glycerol used to 30% (w/w). The appropriate reference was subtracted (1:1) from

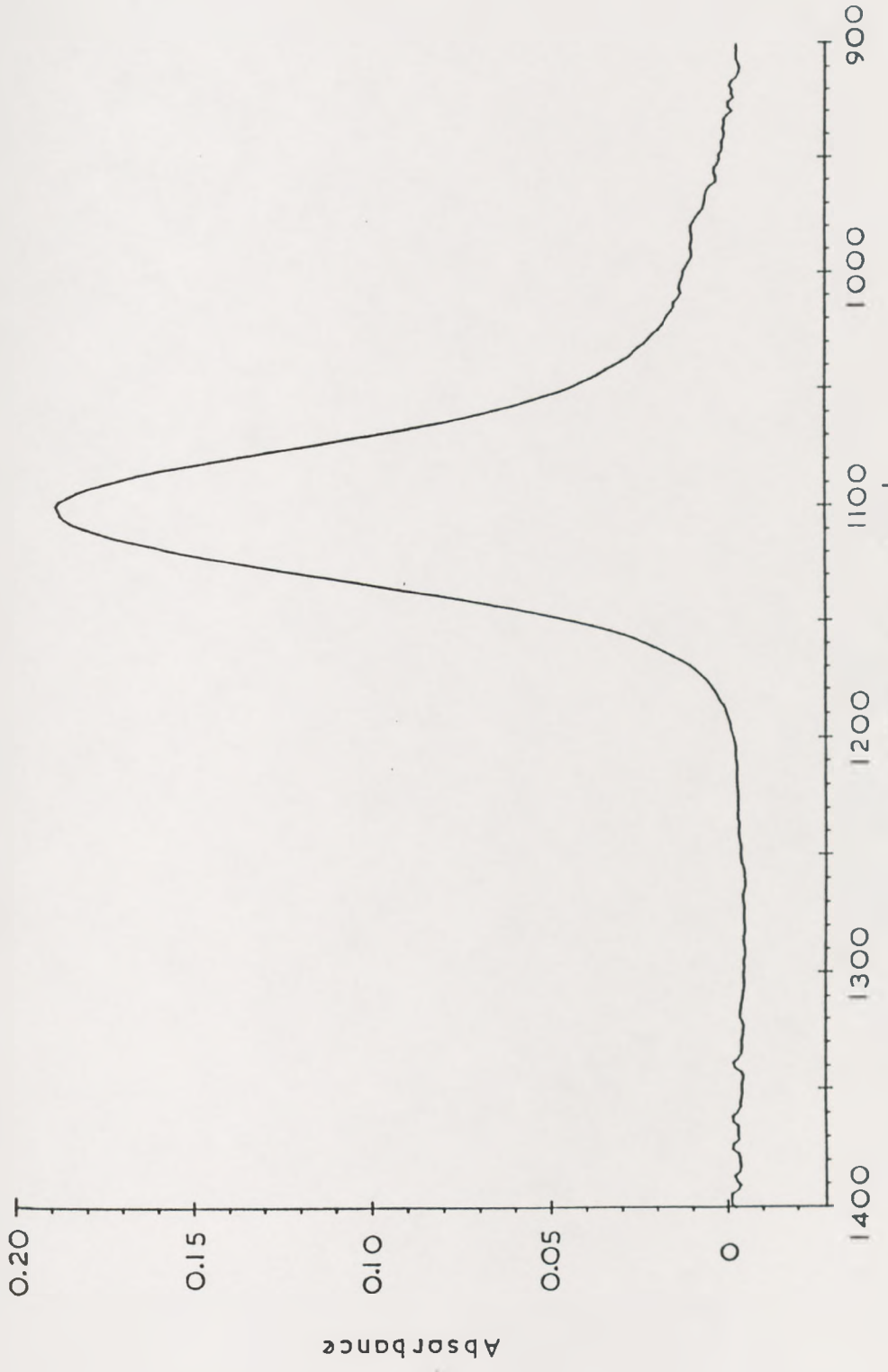


Fig.3.2.15. FTIR spectra of a 0.1 M solution of Na_2SO_4.

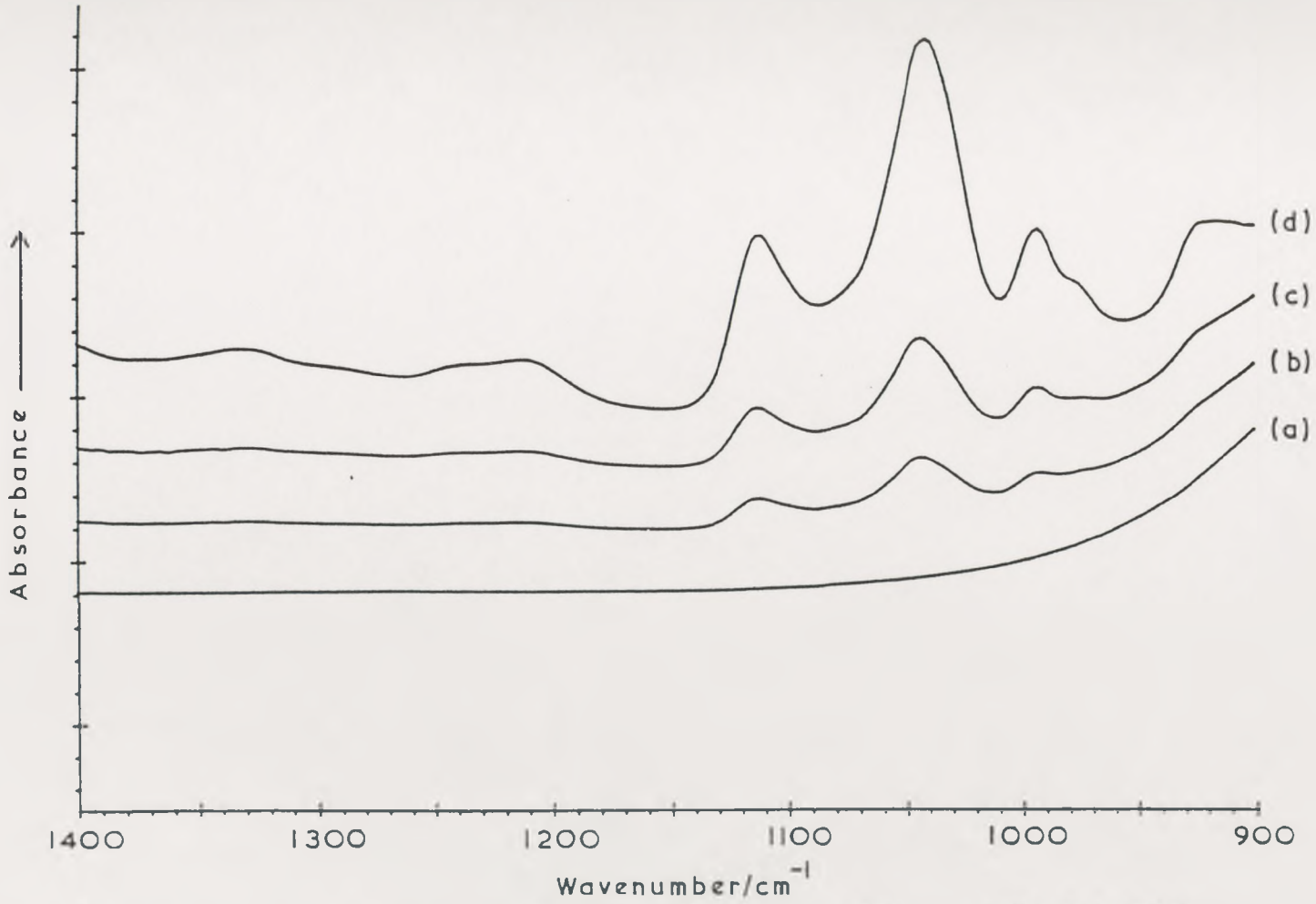


Fig.3.2.16. FTIR spectra of water and water-glycerol mixtures. (a) water; (b) 5 % (w/w) glycerol; (c) 10 % (w/w) glycerol; (d) 30 % (w/w) glycerol. Each division on the absorbance axis represents 0.01 absorbance units. Spectra have been displaced by arbitrary amounts along the absorbance axis for clarity.

samples containing S(IV) oxospecies to obtain spectra of the desired solutes and the success of removing this background was judged by the fact that a peak due to water at 1650 cm^{-1} had disappeared into the noise. There is no way of checking that the peaks due to glycerol had been fully subtracted. However, changing the ratio of reference to sample by ±10% did not introduce any new features into the spectra and only affected the relative heights of the resulting peaks in that region to a small extent.

Difference spectra for a constant concentration of S(IV) but a variable amount of glycerol are plotted in Fig.3.2.17. Here, the comparison, in each case, is made with the sample dissolved in water and a positive peak denotes a higher absorbance in water than in water-glycerol mixtures. Thus, we see that increase in glycerol-content causes a marked increase in absorbance of the samples at 1000-1010 and 1095 cm^{-1} (peaks I and J, respectively) and a decrease at 1052 and 1210 cm^{-1} (peaks K and L, respectively). The changing intensity of peaks at 1052 and 1210 cm^{-1} is a clear indication of a reduction in $S_2O_5^{2-}$-content with increase in glycerol-content; the use of the peak at 1210 cm^{-1} is particularly reliable as it is well removed from any prominent features of the water-glycerol spectra. The absorbance difference at 1098 cm^{-1}, SO_3^{2-}, is seen to decrease with increasing glycerol content, but this peak is too close to that at 1100 cm^{-1} to rule out interference from SO_4^{2-}. Similarly, the absorbance difference at 938 cm^{-1} due to SO_3^{2-} which is not present in the absence of glycerol becomes more negative with glycerol-content. This suggests that SO_3^{2-} concentration increases with increasing glycerol content.

The position of the HSO_3^- peak at 1022 cm^{-1} in water is displaced slightly to 1018 cm^{-1} as the glycerol content is increased to

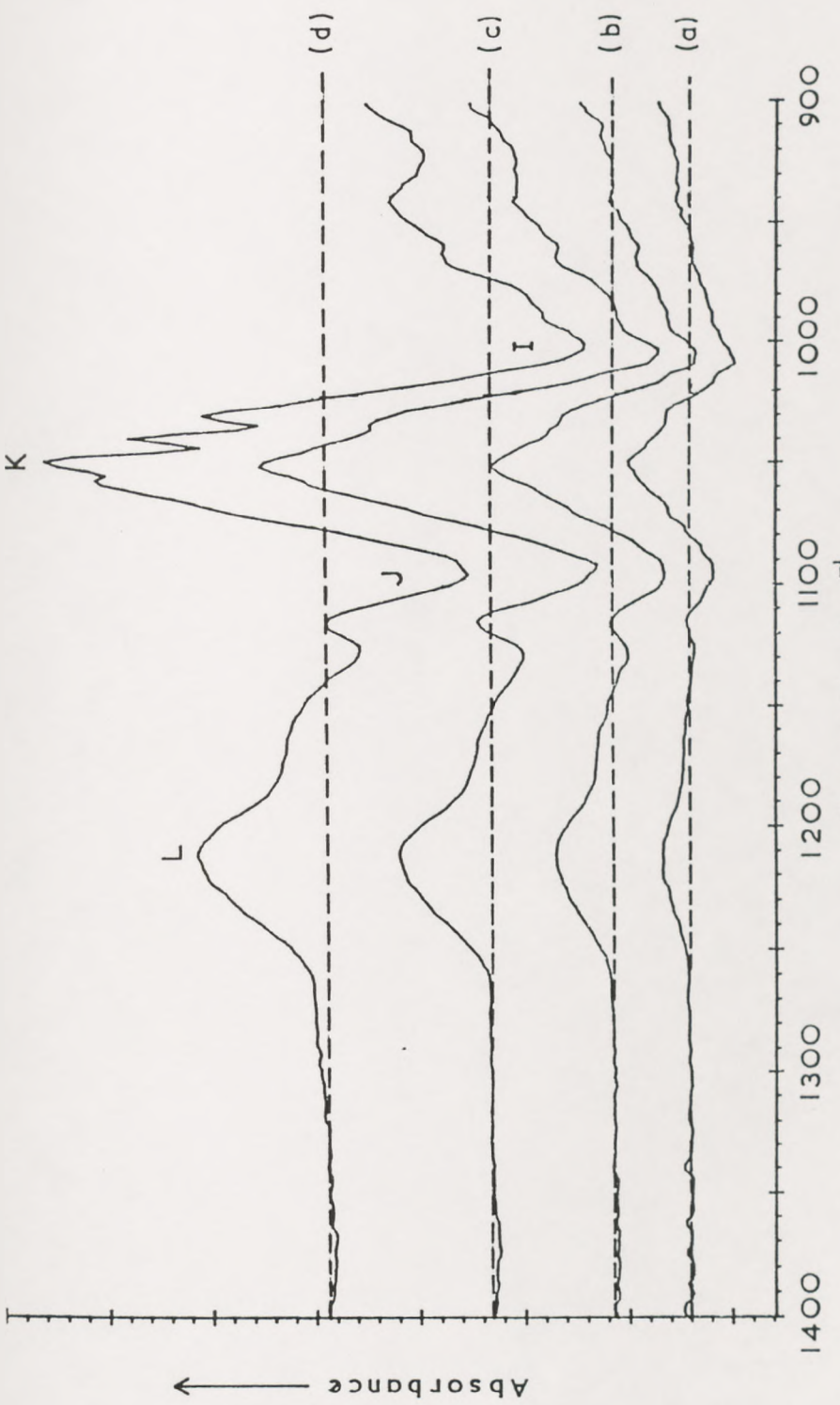


Fig.3.2.17. Difference FTIR spectra of 1 M S(IV) in water and water-glycerol mixtures. A positive peak implies that the substance has a greater absorbance at a given wavelength in water. Solvents: (a) 5 % (w/w) glycerol; (b) 10 % (w/w) glycerol; (c) 20 % (w/w) glycerol; (d) 30 % (w/w) glycerol. Each division on the absorbance axis represents 0.01 absorbance units. The broken line shows the baseline for each subtraction and baselines are shown displaced by arbitrary amounts along the absorbance axis.

30% (w/w), but the absorbance due to HSO_3^- remains constant (0.48 and 0.49 in water and 30% (w/w) glycerol, respectively). The lack of effect of changing glycerol-content on the HSO_3^- peak is illustrated well in Fig.3.2.17 where the spectrum crosses the base line at this location for all solvent mixtures. The increasing absorbance due to peak I, with glycerol content, is therefore not due to an increasing concentration of HSO_3^-. Figure 3.2.18 shows that the absorbances due to peaks I, J and K are well correlated to those of peak L although the lines do not pass through the origin. Perhaps, peaks I and J both represent new species formed from $S_2O_5^{2-}$ with increasing glycerol-content. A weak feature at 1128 cm^{-1} could, likewise be related to $S_2O_5^{2-}$. The difference peak at 1095 cm^{-1} (peak J) is too close to that for SO_4^{2-} (1100 cm^{-1}) and errors in interpretation could arise if samples were to contain small, but variable, amounts of SO_4^{2-}.

The success in interpreting the difference spectra given in Fig.3.2.17 rests with the validity of subtracting a glycerol-water reference from a glycerol-water-S(IV) sample, i.e. assuming that subtraction is perfect in all regions of the spectrum and not just where there is an isolated peak due to water at 1650 cm^{-1}. This must remain an assumption on which the results are conditional, though the correlation between absorbance of the peaks of interest add credence to the interpretation.

From the evidence presented here, it appears that addition of a non-electrolyte decreases the amount of $S_2O_5^{2-}$ present but does not change the concentration of HSO_3^-. Disulphite ion may be converted to another S(IV) species. A possible product is SO_3^{2-} because of absorbance changes at 938 cm^{-1}. In order to ascertain whether or not water activity is important in changing the U.V. absorbance of $S_2O_5^{2-}$, consider first the concentration of non-electrolyte required to reduce

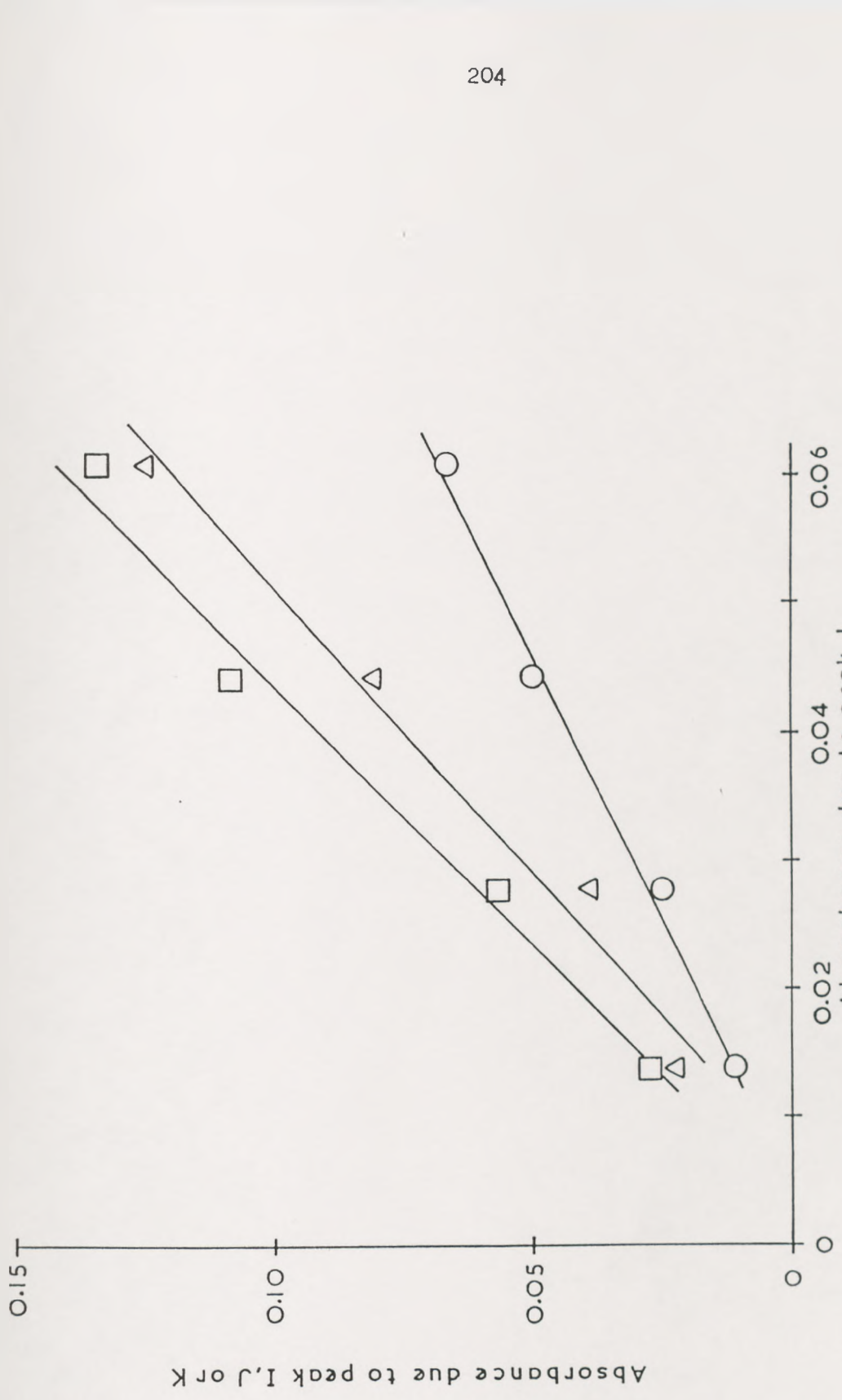


Fig.3.2.18. Graphs of absorbance due to peaks I,J and K vs. absorbance due to peak L in the difference spectra shown in Fig.3.2.17. For each peak the absorbance is measured from the baseline and absolute values are plotted. Peak I vs. peak L △ ; peak J vs. peak L ○ ; peak K vs. peak L □ .

the U.V. absorbance due to $S_2O_5^{2-}$ by, say, 20%, i.e. 5% glycerol, 33% sucrose and 68% PEG-400 (see Fig.2.2.3). The corresponding a_w = 0.99, 0.98 and 0.73 (Norrish, 1966; Sloan and Labuza, 1976; Chirife and Fontan, 1980). Clearly, PEG-400 behaves differently from glycerol and sucrose. Secondly, consider the reduction in absorbance at a_w = 0.92 (that corresponding to the most concentrated sucrose solution used). The absorbances of solutions containing the 3 non-electrolytes are reduced by 62, 41 and 10% respectively at this value of a_w. There exists, therefore, no correlation between water activity and observed changes in the U.V. absorbance of $S_2O_5^{2-}$, such changes are likely to be due to specific solute-solute or solute-solvent interactions.

Preliminary consideration should be given to other possible reasons for the observed changes in I.R., and U.V., spectra. The formation of $NaS_2O_5^-$ ion pairs is likely. It is found that ion-pair formation leads to a reduction in U.V. absorbance due to $S_2O_3^{2-}$ (Thomas and Monk, 1956) and SO_3^{2-} (Wedzicha and Goddard, 1991). Whereas ion-pairs between 1:1 electrolytes are highly dissociated in aqueous solution, those involving 2:1 electrolytes show significant stability which is increased on addition of water-miscible organic solvents. Thus, the formation constants of $NaS_2O_3^-$ and $KS_2O_3^-$ are increased by a factor of of *c.* 30 on making the solvents 50% (v/v) ethanol (Thomas and Monk, 1956). The formation constant of $NaS_2O_3^-$ in this solvent is 143 M^{-1}.

If one incorporates ion-pair formation in the HSO_3^--$S_2O_5^{2-}$ equilibrium, the overall behaviour will be given by at least 3 equations, i.e.,

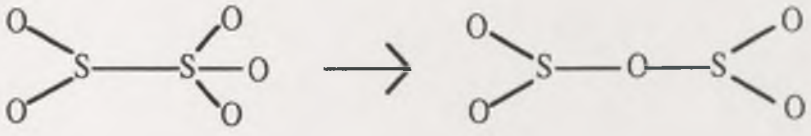
$$2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O \quad (3.2.1)$$

$$Na^+ + S_2O_5^{2-} \rightleftharpoons NaS_2O_5^- \quad (3.2.2)$$

$$Na^+ + HSO_3^- \rightleftharpoons NaHSO_3 \qquad (3.2.3)$$

The formation of $NaS_2O_5^-$ would tend to reduce the activity of $S_2O_5^{2-}$ in solution and promote the "condensation" of HSO_3^-. This does not occur. The possibilities which would give rise to the observed effects include the fortuitous compensation for the formation of $NaS_2O_5^-$ by a combination of solvent effects on the reactions shown in equations 3.2.1 and 3.2.3. The species which give rise to the new peaks observed in Fig.3.2.17 could be speculated as one or more of the ion-pairs because their concentrations would be related to the change in $S_2O_5^{2-}$ concentration.

An alternative speculation is that $S_2O_5^{2-}$ could undergo isomerisation, on addition of non-electrolyte, as follows:



The unsymmetrical structure is the accepted form in aqueous solution. Goodall (1984) consider that the symmetrical S-O-S bonded ion could exist in non-aqueous media to account for the facile autoxidation of S(IV) in such systems. However, this isomerisation has only been considered in anhydrous solvents and the formation of the symmetrical $S_2O_5^{2-}$ species in solvents with a high water-content is a less likely possibility.

3.2.5. Variation of the solvent and the effect on the rate of the S(IV)-inhibited Maillard reaction.

Production of DH from the reaction of glucose, glycine and S(IV) is catalysed by S(IV) (Wedzicha and Vacalis, 1988). The apparent pK

of HSO_3^- is increased by the addition of ethanol, glycerol and polyethylene glycol, but only ethanol and polyethylene glycol give rise to a substantial increase in the pK of sulphurous acid (Wedzicha and Goddard, 1991). It is possible that the change in relative concentration of SO_3^{2-}, HSO_3^- and $SO_2 \cdot H_2O$ may contribute to the observed effect of a_w on the S(IV)-catalysed reaction. Table 3.2.7 shows the effect of S(IV) and humectant concentration on the a_w of mixtures of composition [S(IV)] = 0.0160-0.0902 M, [glucose] = 1.0 M, [glycine] = 0.5 M in solvents containing 40% w/w humectant at the begining and end of the reaction.

Humectant	**[S(IV)] /M**	**a_w t=zero**	**a_w t=end**
PEG-400	0.0160	0.90	----
PEG-400	0.0902	0.90	0.90
Ethanol	0.0176	0.88	----
Ethanol	0.0901	0.90	0.92

Table 3.2.7. a_w of reaction mixtures of composition [S(IV)] = 0.0160-0.0902 M, [glucose] = 1.0 M, [glycine] = 0.5, 0.75 M in solvents containing 40% w/w humectant.

It can be seen that S(IV) concentration has little effect on the a_w of a reaction mixture in the range of concentration studied and that this a_w is effectively constant throughout the duration of the reaction. From tables 3.2.7. and 3.2.5 it can be seen that the measured a_w of mixtures containing 40% w/w ethanol, PEG-400 and glycerol were 0.88-0.92, 0.90 and 0.85 respectively.

Plots of reaction rate, for the S(IV)-inhibited Maillard reaction, *vs* initial S(IV) concentration gave correlation coefficients of 0.9939,

0.9944 and 0.9952 for the reaction mixtures containing 40% w/w glycerol, ethanol (Fig.3.2.19) and PEG-400 (Fig.3.2.20) respectively. From Figs.3.2.11 and 3.2.10 it can be seen that the value of k_2 is *c.* 20% larger at an a_w of 0.85 than 0.90, but the effect of this a_w difference on k_1 is negligible. Therefore, the value of k_2 obtained in the presence of glycerol, at $a_w = 0.85$, could, to a first approximation, be decreased by *c.* 20% so as to make it comparable with the values of k_2 obtained in the presence of ethanol and PEG-400 which were at an a_w of 0.90. The values of k_1 obtained in reaction mixtures of composition [S(IV)] = 0.0160-0.0902 M, [glucose] = 1.0 M, [glycine] = 0.5, 0.75 M in solvents containing 40% w/w humectant are shown in table 3.2.8.

Solvent	**k_1 /M^{-1} h^{-1} x 10^{-4}**	**Standard deviation /M^{-1} h^{-1} x 10^{-5}**
Glycerol	1.44	1.39
PEG-400	1.62	2.63
Ethanol	2.16	3.29

Table 3.2.8. Rate constants, k_1, for an early step of the Maillard reaction in reaction mixtures of composition [S(IV)] = 0.0160-0.0902 M, [glucose] = 1.0 M, [glycine] = 0.5, 0.75 M in solvents containing 40% w/w humectant.

There is a 1.5 fold increase in k_1 as the solvent is changed from glycerol to ethanol which cannot be due to the experimental spread of values, as the value of k_1 in glycerol lies outside one standard deviation of the value of k_1 in ethanol and vice versa. The values of k_1 have been obtained by extrapolation of plots of reaction rate, for the S(IV)-inhibited Maillard reaction, *vs* initial S(IV) concentration to zero S(IV) concentration. Therefore, the increase in k_1 as the solvent is changed cannot be caused by variation in the proportions of S(IV)

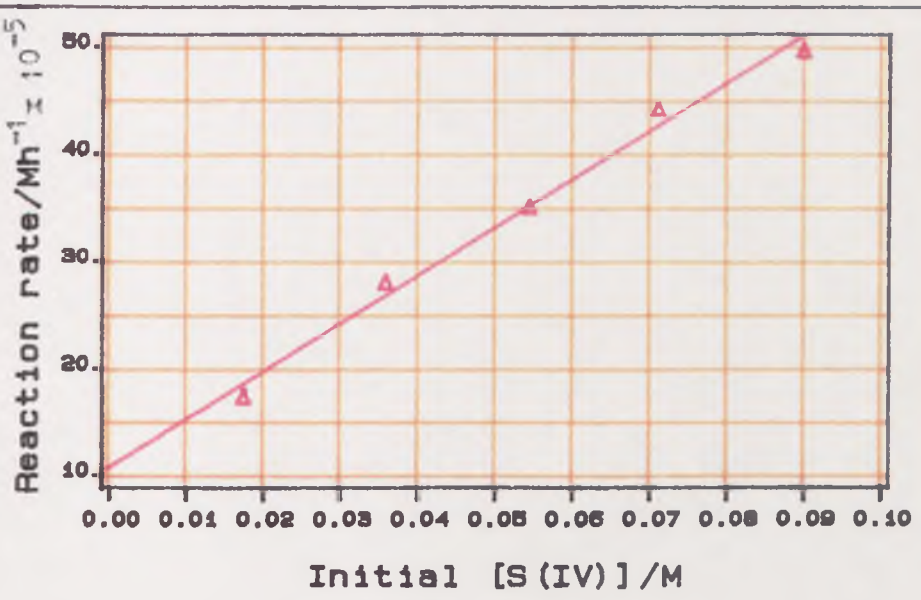


Fig.3.2.19. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0176-0.0901M, [Glucose]=1.0M, [Glycine]=0.5M at an aw of 0.88-0.92.

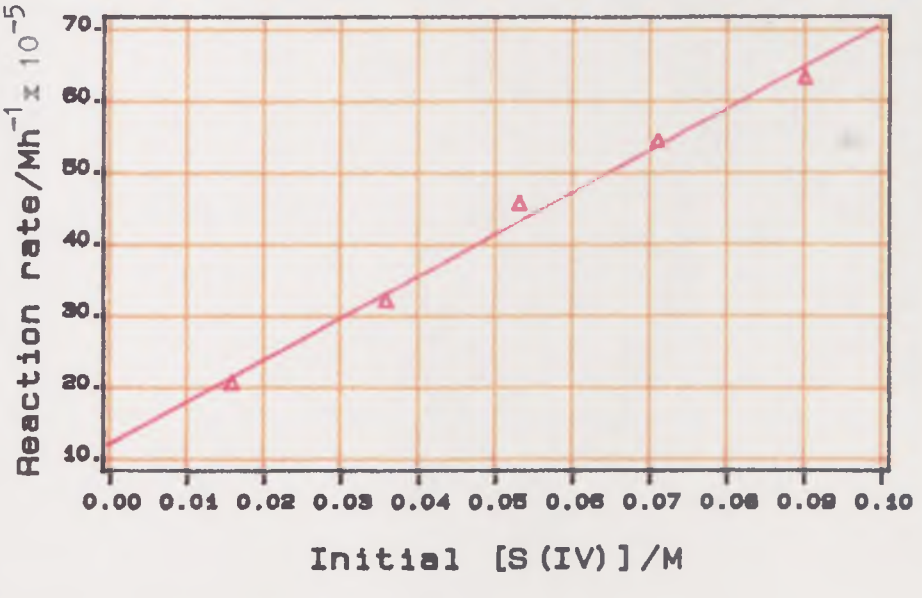


Fig.3.2.20. Reaction rate vs initial S(IV) concentration for the mixtures of composition [S(IV)]=0.0160-0.0902M, [Glucose]=1.0M, [Glycine]=0.75M at an aw of 0.90.

species, but is likely to be due to the differing solvent properties of the two systems. Consideration of the transition-state or activated complex theory may help to explain the solvent effect on k_1. Suppose that the potential energy of the interacting molecules at the time of collision is known as a function of the relative positions of the various nuclei. There will be a configuration of nuclei of maximum potential energy, related to the activation energy, through which, or near which, the system would be expected to pass in going from reactants to products. This region of configuration space is called the *transition state*. A system in the transition state is called an *activated complex*. It is assumed that the rate of reaction is given by the rate of passage through the transition state (passage over the potential energy barrier), the number of activated complexes at any instant being determined by an equilibrium with the reactant molecules (Frost and Pearson, 1961). It may be that the solvent causes an effect in one of the following ways.

(1) If a reaction proceeds by two pathways, one catalysed and one uncatalysed, then from transition state theory, the activated state in the catalysed reaction will have a lower potential energy than that of the uncatalysed reaction. The amount of energy required to enable the reactants to pass over this lower energy barrier is less, a higher proportion of the reactants possesses this energy and, therefore, the reaction rate of the catalysed reaction will be greater. The S(IV)-inhibited Maillard reaction is catalysed by S(IV) oxospecies and it may be that the Maillard reaction can also be catalysed by the different humectants used to adjust the proportions of the S(IV) species. However, it is unlikely that the solvents will act as base catalysts because ethanol, the solvent for which the highest value of k_1 was obtained, is protonated only by

strong acids. It is also unlikely that ethanol will act as an acid catalyst, as it is only a very weak acid.

(2) Elevation of the potential energy of the reactants to that of the activated state may be described as involving absorption of energy by the reactants and promotion of electrons to higher energy levels. If this process causes a net production of unpaired electrons, then the activated state will be more polar than the ground state. However, if the environment in which the reaction occurs is made more polar, say, by changing the solvent, then the polar excited state may be formed more easily, and the activation energy reduced. Hence, a greater proportion of the reactants have sufficient energy to attain the activated state and react.

An indication of the extent of the polar nature of a molecule can be obtained from its dipole moment, which is defined as the product of one of the charges of a dipole unit by the distance separating the two dipolar charges. The dipole moment is obtained from measurement of the dielectric constant; an increased value of the dielectric constant should be indicative of an increase in polar nature. A small increase in the value of k_1 was seen when the humectant was changed from glycerol to ethanol. It may be that this increase is due to an increase in polarity as the solvent is changed. However, dielectric constants for glycerol and ethanol are 42.5 and 24.3 respectively (C.R.C., 1981-82), indicating that glycerol is more polar than ethanol. Therefore, it seems unlikely that the increase in rate constant is produced simply by an increase in the polarity of the solvent.

(3) As the solvent is changed this may affect the extent to which the reactants and activation complex are solvated and hence reaction rate.

(4) Kaanane and Labuza (1989) stated that the solvent in which a sugar is dissolved has a significant effect on the kinetics of its mutarotation. Burton and McWeeny (1963) reported that the higher the level of acyclic sugar form in solution the faster the rate of browning. As mutarotation of α to β-glucose proceeds through an open chain form of glucose, the solvent could effect the rate at which the acyclic form of the sugar is formed and hence the value of k_1.

The different solvents may cause a change in the extent to which the Maillard reaction is catalysed by S(IV), due to variation in the equilibrium constants of the S(IV) species. The fraction of an acid, HA, ionised, A^-, is related to the equilibrium constant, K, by,

$$[A^-] / c = K / (K + [H^+])$$

where $c = [HA] + [A^-]$; similarly the fraction protonated is,

$$[HA] / c = [H^+] / (K + [H^+])$$

If we assume that pH is constant, then if $[H^+] << K$ (as in the case of $SO_2 \cdot H_2O$) then,

$$[SO_2 \cdot H_2O] \propto 1 / K \quad \text{and} \quad [HSO_3^-] / c \approx 1$$

On the other hand if $[H^+] >> K$ (as in the case of HSO_3^-) then,

$$[SO_3^{2-}] \propto K \quad \text{and} \quad [HSO_3^-] / c \approx 1$$

The rate constant k_2 is shown $\pm$ one standard deviation from the mean and as a function of the dissociation constants, obtained from Wedzicha and Goddard (1991), of $SO_2 \cdot H_2O$ and HSO_3^- in Figs.3.2.21 and 3.2.22. A correlation coefficient of -0.9988 was obtained from the plot of k_2 *vs* the dissociation constant of HSO_3^- and this increases to -0.9998 when the value of k_2 obtained in the presence of glycerol is reduced by 20% to allow for the effect of the water activity described above. These graphs are equivalent to plots of k_2 *vs* $[SO_2 \cdot H_2O]$ and $[SO_3^{2-}]$. Variation of the solvent produces a significant change in the

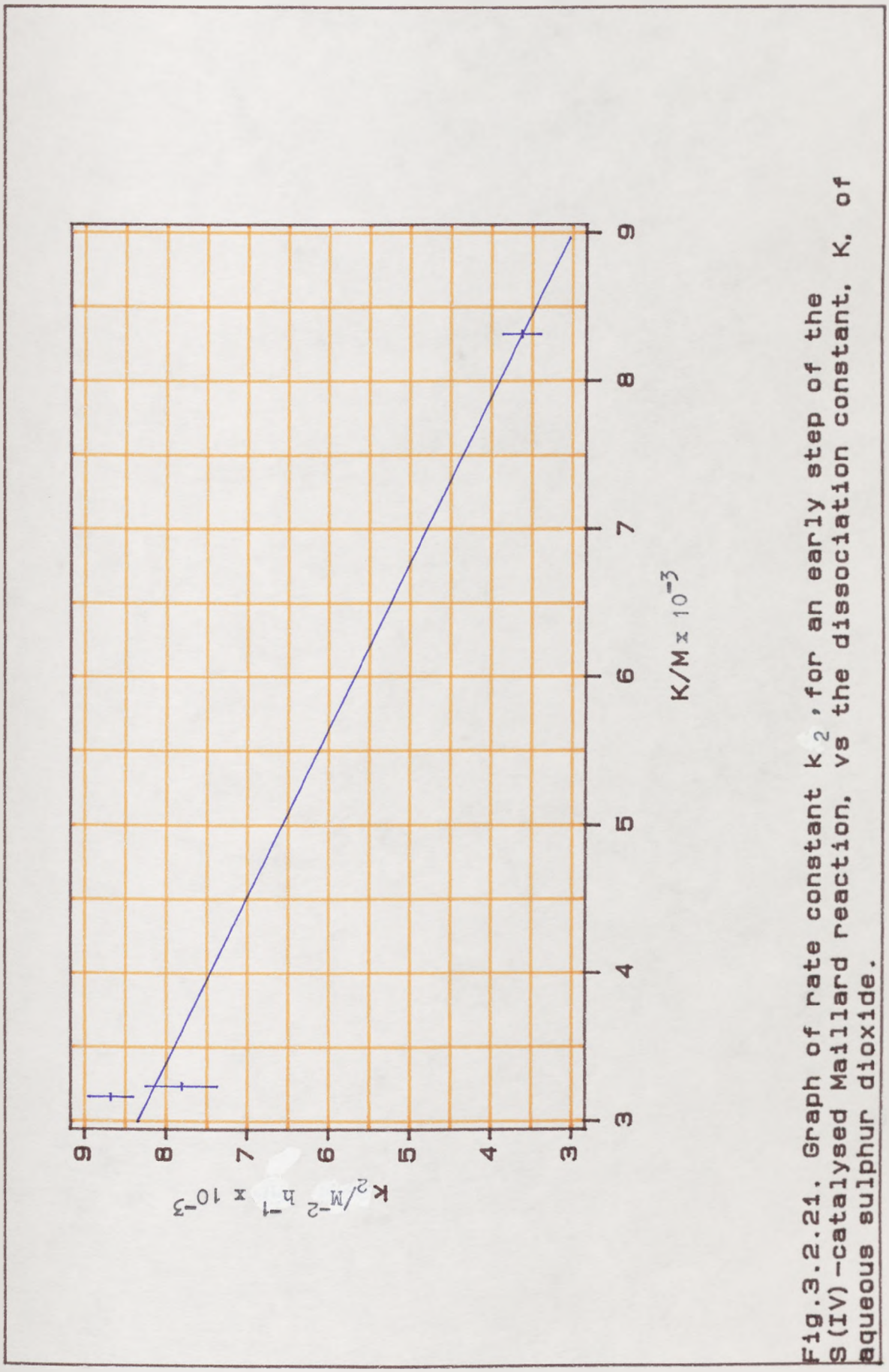


Fig.3.2.21. Graph of rate constant k_2, for an early step of the S(IV)-catalysed Maillard reaction, vs the dissociation constant, K, of aqueous sulphur dioxide.

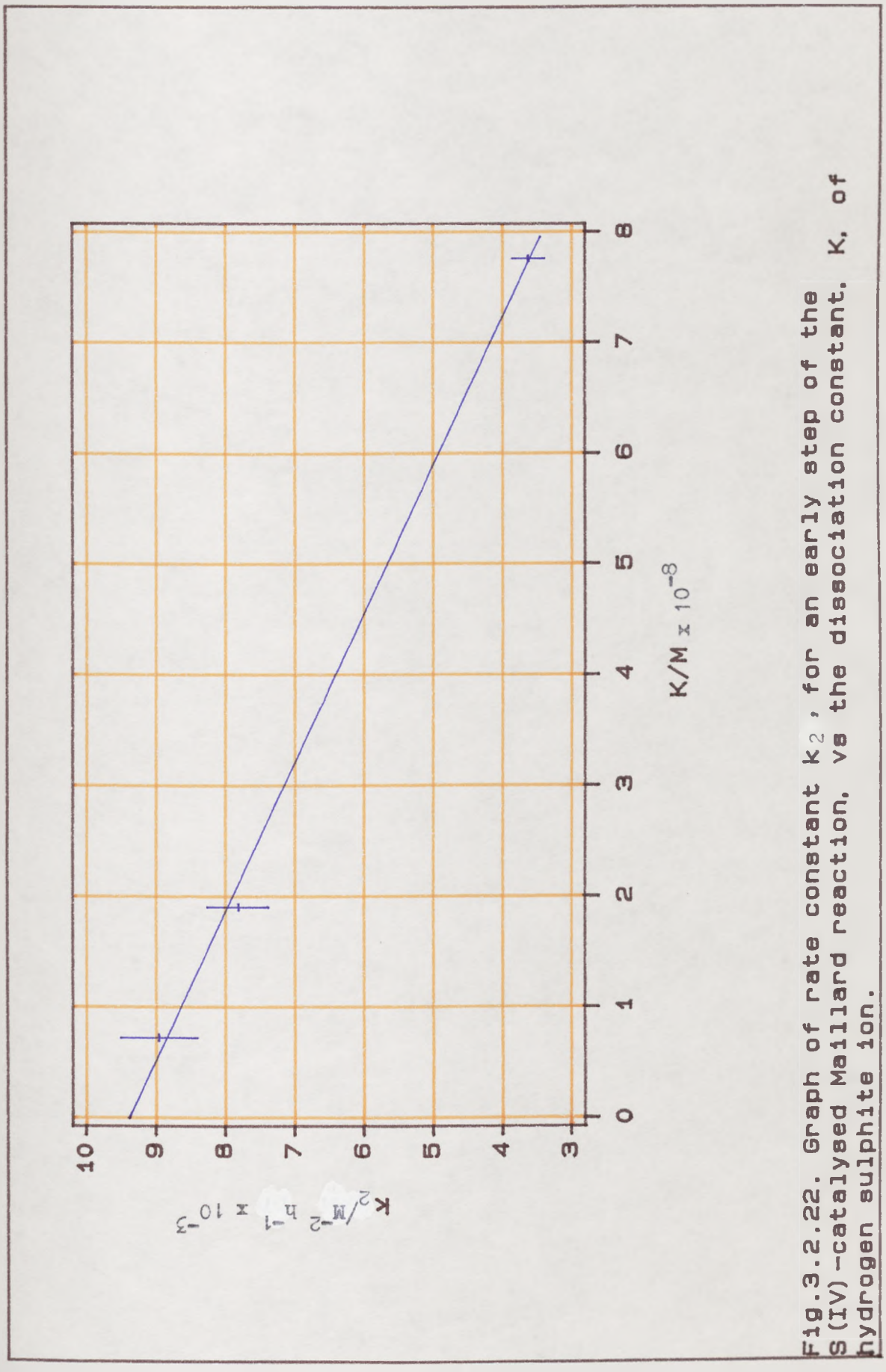


Fig.3.2.22. Graph of rate constant k_2, for an early step of the S(IV)-catalysed Maillard reaction, vs the dissociation constant, K, of hydrogen sulphite ion.

value of k_2 which cannot be due to the experimental spread of values, as there is no overlap of the respective standard deviation ranges. There could be three or more factors contributing to this change in k_2. Firstly, the solvent could affect the rate of production of the acyclic sugar as described for k_1 above. Secondly, a change in the extent to which the reaction species are solvated as the solvent changes. Thirdly, a change in the extent of catalysis by S(IV) as the concentrations of SO_3^{2-} and HSO_3^- vary. It is thought that SO_3^{2-} rather than HSO_3^- is more likely to catalyse the Maillard reaction, as SO_3^{2-} is a more effective catalyst for the deamination of 1-methyl-5,6-dihydrocytosine than HSO_3^- (Slae and Shapiro, 1978). Thus, base catalysis of the Maillard reaction by SO_3^{2-} could contribute significantly to the effect of a_w on k_2. It was shown in the previous section (see Fig.3.2.17) that, using FTIR, the absorbance difference at 938 cm^{-1} between a solution of S(IV) in water and a solution containing the same concentration of S(IV) but in a water-glycerol solvent, due to SO_3^{2-}, becomes more negative with glycerol-content, suggesting that SO_3^{2-} concentration increases with increasing glycerol-content; the concentration of $S_2O_5^{2-}$ which has been thought of as a catalyst for the Maillard reaction falls and that of HSO_3^- remains constant. This would be in agreement with Wedzicha and Vacalis (1988), who proposed that the production of the ketoseamine in the Maillard reaction could be catalysed by SO_3^{2-} as shown in Fig.3.2.23. This would also be in keeping with the findings of Potman and Van Wijk (1989), who state that the dihydrogen phosphate ion base catalyses the conversion of the glycosylamine into Amadori rearrangement products at pH 5.6 and 100 °C. The proposal of Wedzicha and Vacalis (1988) is further strengthened by the findings in section 3.1.2.3, where several possible rate-determining steps were

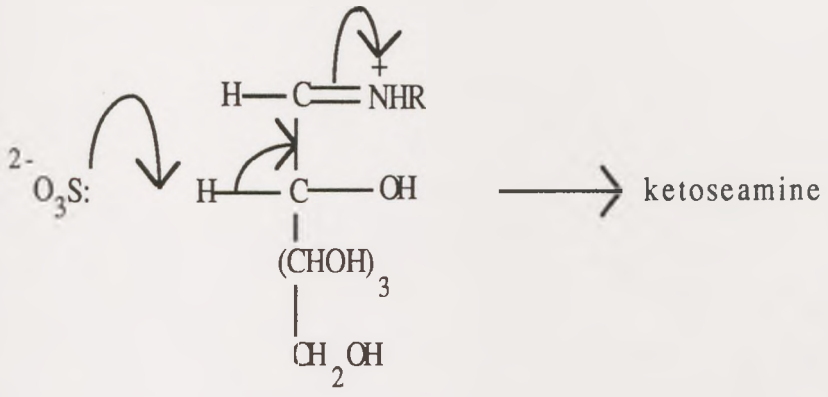


Fig.3.2.23. Proposed base catalysis of ketoseamine production by SO_3^{2-} (Wedzicha and Vacalis, 1988).

considered and the resulting theoretical kinetics compared to the observed kinetics. It was suggested that production of MFG from the first Schiff's base formed during the glucose-glycine reaction is most likely to be the rate-determining step.

On the other hand, the increase in k_2 may be due to the formation of $SO_2 \cdot H_2O$ as its dissociation constant is reduced from 8.32×10^{-3} to 3.16×10^{-3} M. If the Maillard reaction is base catalysed, then $SO_2 \cdot H_2O$ cannot be the catalytic species. This is because the sulphur atom in $SO_2 \cdot H_2O$ cannot accept a proton, as its four 3p electrons are all involved in covalent bonds to oxygen. However, $SO_2 \cdot H_2O$ could act as an acid catalyst as could HSO_3^-, but SO_3^{2-} and $S_2O_5^{2-}$ could not. The Maillard reaction may be acid catalysed, as is imine production (Sykes, 1986; McMurry, 1984), but the case for base catalysis of the Maillard reaction by SO_3^{2-} is at present stronger. The increase in the rate of the S(IV)-catalysed Maillard reaction with reduced a_w, could also be due to the effect of a_w on the transition state energy. If the reaction is as shown in Fig.3.2.24 we see that at the transition state there is a reduction in the

number of charged species and some of the charge is delocalised.

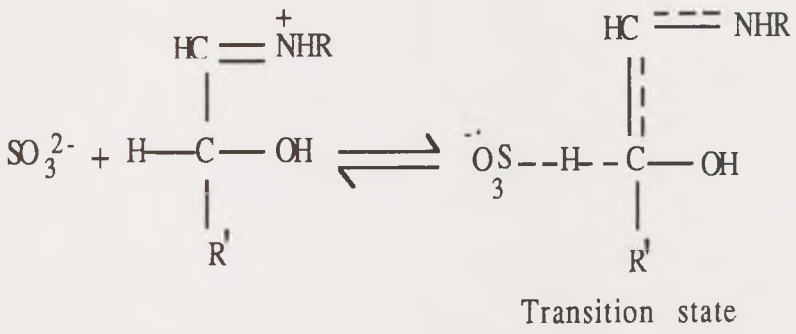


Fig.3.2.24. Postulated formation of a transition state complex during the S(IV)-catalysed reaction of glucose and glycine.

Less solvation of the transition state than reactants is required and hence at low a_w when the amount of water available for solvation is reduced, the position of equilibrium will favour the transition state.

CHAPTER 4: DISCUSSION

4.1. Prediction of the concentrations of total, free and reversibly bound S(IV) using the kinetic model of the S(IV)-inhibited Maillard reaction.

4.1.1. Introduction.

The kinetics of the S(IV)-inhibited Maillard reaction are investigated in this thesis. The study is a continuation of the work started by McWeeny *et al* (1969), Wedzicha (1984a), Wedzicha *et al* (1985), Wedzicha and Kaban (1986), Vacalis (1986) and Wedzicha and Vacalis (1988). Vacalis (1986) found that a hydroxysulphonate adduct of DH need not be included in the kinetic model of the S(IV)-inhibited Maillard reaction, shown in Fig.4.1.1, to obtain satisfactory agreement between theoretically generated data and experimental results. This is despite Wedzicha and Kaban (1986) showing that pure DH forms such an adduct with S(IV) in the presence of glycine. Vacalis (1986) did not control the pH of model systems. However, it was shown in section 3.1.2.2, that the rate of irreversible S(IV) loss during the constant rate phase of the glucose-glycine-S(IV) reaction was greater when the pH was maintained than when it falls. The incorporation of DH hydroxysulphonate into the kinetic model may now be required in order to obtain a satisfactory fit of theoretical data to experimental results.

4.1.2. Construction of a computer program to describe the kinetic model of the S(IV)-inhibited Maillard reaction.

It was shown earlier, section 3.1.2.6, that S(IV) autoxidation can occur in reaction mixtures containing glucose, glycine and S(IV).

The kinetic model of the S(IV)-inhibited Maillard reaction proposed by Wedzicha and Vacalis (1988) has, therefore, been altered to take account of this, the improved model being shown in Fig.4.1.1.

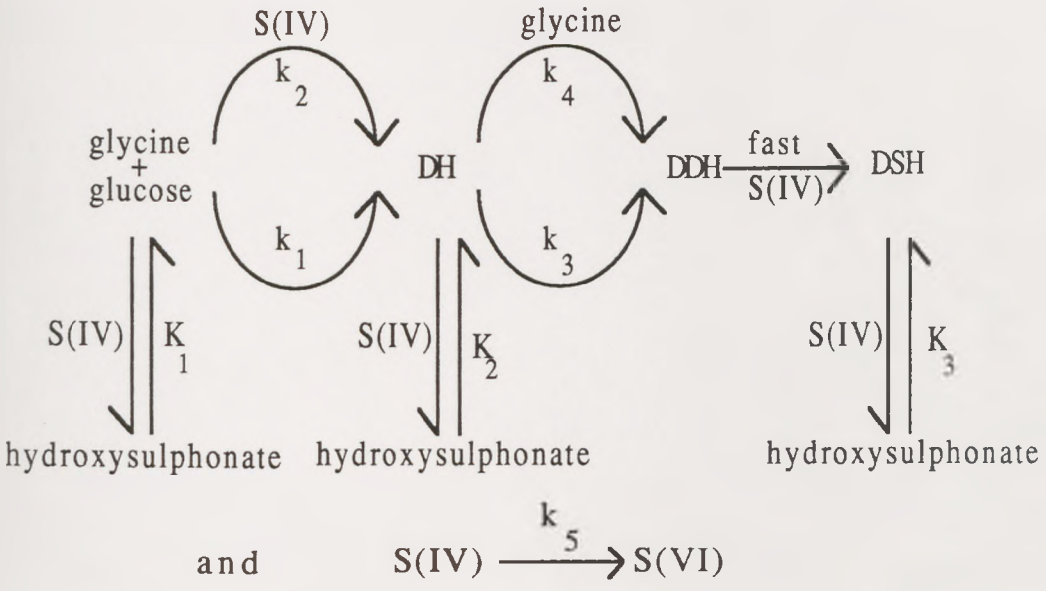


Fig 4.1.1. Kinetic model for the S(IV)-inhibited Maillard reaction.

As there is no analytical solution of the rate equations describing the model, a numerical integration of the rate equations using the finite step method was performed. To illustrate the procedure, consider the general reaction of reactant R giving product P;

$$R \xrightarrow{k} P$$

where k is the rate constant. The rate of reaction is given by,

$$\text{Rate} = -d[R] / dt = d[P] / dt$$

If the reaction is of first order with respect to R, at zero time (t=0) the initial concentration of reactant R is $[R]_0$ and the reaction rate is given by,

$$\text{Rate} = k\,[R]_0$$

After a small time interval, Δt, the amount of R that has reacted is,

$$\Delta R = k\,[R]_0\,\Delta t$$

this being equal to the amount of P produced. If the time interval Δt is very small (c 0.01% of the total reaction time), then the reaction rate over this period can be considered constant. The concentration of reactant R at the end of the first time period T_1 ($T_1 = \Delta t$) is,

$$[R]_1 = [R]_0 - \Delta R = [R]_0 - k\,[R]_0\Delta t$$

The procedure is then repeated. During the second successive time interval, the amount of R that has reacted will be:

$$\Delta R = k\,[R]_1\Delta t$$

The concentration of R at the end of the second time period ($T_2 = 2\Delta t$) will be:

$$[R]_2 = [R]_1 - \Delta R = [R]_1 - k\,[R]_1\Delta t$$

In this way a predicted concentration/time curve, of the type shown in Fig.4.1.2, can be obtained.

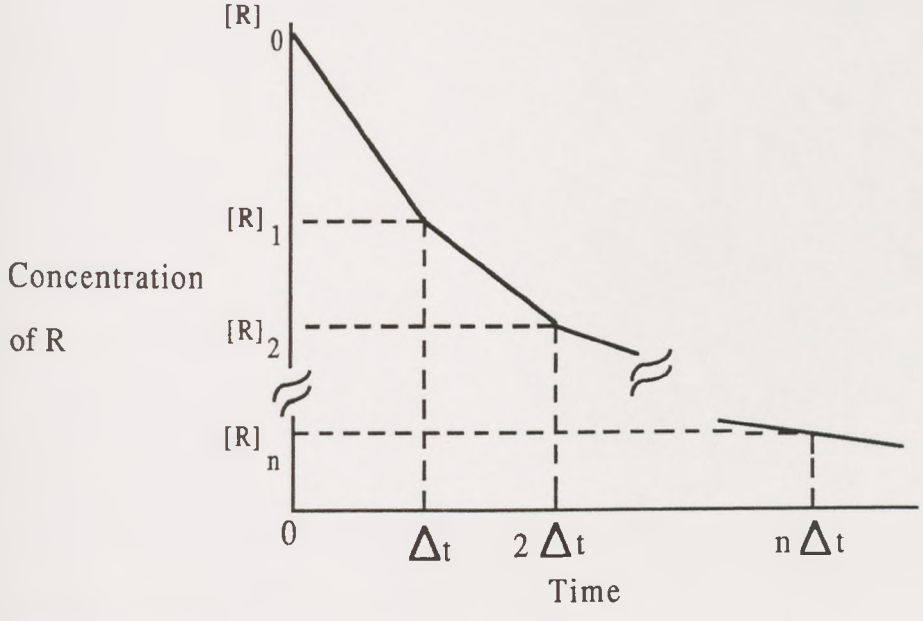


Fig.4.1.2. Use of finite step method for the numerical intergration of the rate equation: Rate = -d[R]/dt = k[R].

The proposed model of the S(IV)-inhibited Maillard reaction

includes three simultaneous equilibria (glucose with S(IV), DH with S(IV), DSH and S(IV)) which can change the concentrations of glucose, DH and DSH at every increment. If, at any moment, the initial concentrations of glucose, DH, DSH and S(IV) are a, b, c and s respectively and the concentrations of the hydroxysulphonates of glucose, DH and DSH (S-glucose, S-DH and S-DSH respectively) are x, y and z respectively; then the three simultaneous equilibria involving these species are:

Glucose	+	S(IV)	$\rightleftharpoons$	S-glucose
(a-x)		(s-x-y-z)	K_1	x
DH	+	S(IV)	$\rightleftharpoons$	S-DH
(b-y)		(s-x-y-z)	K_2	y
DSH	+	S(IV)	$\rightleftharpoons$	S-DSH
(c-z)		(s-x-y-z)	K_3	z

Law of mass action expressions for the respective dissociation constants are:

$$K_1 = \{(a-x)(s-x-y-z)\} / x \qquad (4.1.1)$$

$$K_2 = \{(b-y)(s-x-y-z)\} / y \qquad (4.1.2)$$

$$K_3 = \{(c-z)(s-x-y-z)\} / z \qquad (4.1.3)$$

Expressions (4.1.1)-(4.1.3) can be rearranged into the form of the quadratic equations (4.1.4)-(4.1.6) respectively,

$$x^2 - x(K_1+a+s-y-z) + a(s-y-z) = 0 \qquad (4.1.4)$$

$$y^2 - y(K_2+b+s-x-z) + b(s-x-z) = 0 \qquad (4.1.5)$$

$$z^2 - z(K_3+c+s-x-y) + c(s-y-x) = 0 \qquad (4.1.6)$$

It is not possible to obtain an analytical solution of equations (4.1.4),

(4.1.5) and (4.1.6) to give x, y and z directly, but this can be done easily using a numerical technique. The method introduced by Wedzicha and Chishya (1983) involves setting x, y and z initially to zero and values for these variables are then successively evaluated from equations (4.1.4), (4.1.5) and (4.1.6) (iteration 1). These values are substituted back into these equations and improved values of x, y and z obtained (iteration 2). Iterations can be repeated until the procedure is halted when no significant difference exists between two successive sets of x, y, z values. For example, sensible values for concentrations and equilibrium constants are as follows:

$a = 1.0$ M, $b = 0.05$ M, $c = 0.02$ M, $s = 0.02$ M,

$K_1 = 1.33$ M, $K_2 = 0.0044$ M, $K_3 = 0.0040$ M

The values of x, y and z obtained by successive iteration appear in table 4.1.1. When shown initially to 4 significant figures the values of x, y and z are constant after 22 iterations.

Iteration	x	y	z
1	0.06668	-0.05054	0.003118
2	0.02846	-0.01080	0.001913
etc			
19	0.00107	0.01225	0.005257
20	0.00107	0.01225	0.005257
21	0.00107	0.01224	0.005258
22	0.00107	0.01224	0.005258
23	0.00107	0.01224	0.005258

Table 4.1.1. Successive evaluation of the concentrations of the hydroxysulphonates of glucose (x), DH (y) and DSH (z), obtained by continuous iteration of the quadratic equations (4.1.4), (4.1.5) and (4.1.6).

If these derived values are substituted into equations (4.1.1), (4.1.2) and (4.1.3), the apparent equilibrium constants which control x, y and z are found to be:

$K_1 = 1.3347$ M

$K_2 = 0.004415$ M

$K_3 = 0.004012$ M

These compare very favourably with the values of 1.33, 0.0044 and 0.0040 M used initially.

A computer program (appendix 1) was developed to describe the model of the S(IV)-inhibited Maillard reaction shown in Fig.4.1.1. The constants in the model are adjusted until the fit between theoretically generated data and experimental results is maximised. Essentially the profile of the plot of the experimental values of total, free or reversibly bound S(IV) concentration *vs* time is described using of a large number of small concentration/time segments. The length of the time segment or step size, Δt, is selected and calculation of the component concentrations performed using this time increment. The theoretical reaction rate is assumed constant during each time increment and it is, therefore, important that the increment is small enough for this to be the case. The rates of chemical reactions are not necessarily constant during the course of a kinetic experiment, this being illustrated for the reaction profile shown in Fig.4.1.3. If the rate changes substantially, e.g. if the reaction is of non-zero order, the time integral must be sufficiently small to ensure the fastest part of the reaction (period b) conforms to the constant rate assumption and is sampled at appropriate frequency. This means that at some stage of the reaction the interval will be unnecessarily short (period a) and this has two consequences. First the computing of the concentration-time

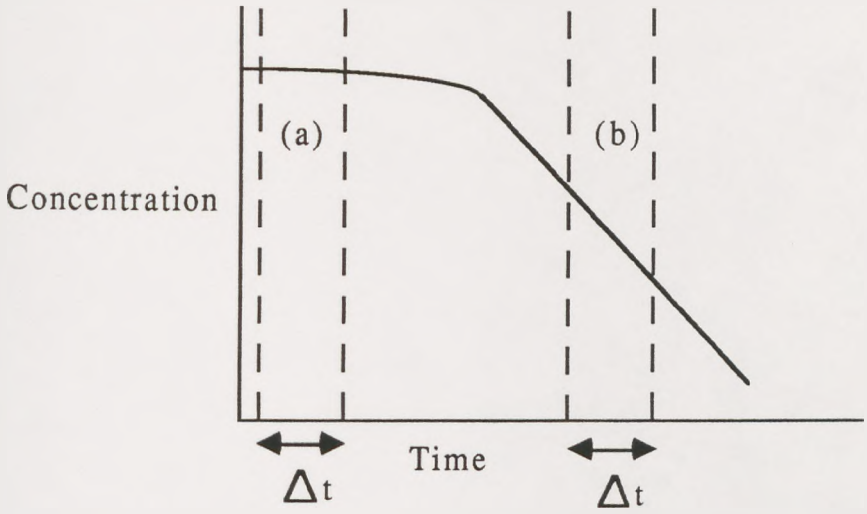


Fig.4.1.3. A typical reaction profile showing an induction period (a) where the reaction rate is slow and a constant rate period (b) where the reaction rate is faster.

profile will be inefficient at some stage of the reaction; the calculation requires a larger than necessary number of steps. Secondly rounding errors in the calculations may accumulate and give rise to an overall error in concentration. Fortunately it is a simple matter to check whether or not a chosen time interval is too large. This is done by varying the step size and comparing concentration-time data at various step size values. The best step size is the value at which the concentration-time data become independent of step size; this is, of course, linked to the resolution of the data and it may be necessary to reduce the step size should the number of significant figures used in reporting the data be increased. This is illustrated in table 4.1.2, by the fact that the data become independent of the size of the time increment as it is reduced beyond 0.05 h.

A mixture containing 2.0 M glucose, 0.5 M glycine and 0.0434 M S(IV) gave the fastest reaction rate during the linear portion of S(IV) concentration-time graphs. The rate and equilibrium

	Predicted % reversibly bound S(IV)				
	Step size/h				
Time/h	5.0	0.5	0.05	0.005	0.0005
0.0	66.344	66.344	66.344	66.344	66.344
50.0	65.661	65.731	65.738	65.739	65.739
100.0	63.807	63.944	63.962	63.959	63.959
150.0	60.990	61.189	61.214	61.212	61.211
200.0	57.349	57.609	57.641	57.638	57.638
250.0	52.824	53.164	53.202	53.201	53.201
300.0	46.710	47.219	47.270	47.273	47.274
350.0	37.730	38.467	38.540	38.547	38.548
400.0	26.696	27.547	27.632	27.640	27.641
450.0	14.943	15.843	15.935	15.942	15.943

Table 4.1.2. Effect of step size on values of reversibly bound S(IV), calculated using the kinetic model of the S(IV)-inhibited Maillard reaction shown in Fig.4.1.1.

constants in the kinetic model were systematically varied, until the best agreement between theoretical data and experimental results was obtained using an initial step size of 0.05 h. The values of the constants that gave this agreement were as follows:
$k_1 = 1.091 \times 10^{-4}\ M^{-1}h^{-1}$, $k_2 = 4.053 \times 10^{-3}\ M^{-2}\ h^{-1}$,
$k_3 = 14.45 \times 10^{-4}\ h^{-1}$, $k_4 = 5.95 \times 10^{-3}\ M^{-1}\ h^{-1}$, $k_5 = 1.5 \times 10^{-4}\ M^2\ h^{-1}$,
$K_1 = 1.0$ M, $K_2 = 0.28$ M, $K_3 = 0.00066$ M. From this reaction mixture it was observed that the rate of loss of reversibly bound S(IV), was greater than that of free S(IV). It was decided to use this quantity because its value depends not only on the integration of the rate equations, but is sensitive to the simultaneous solution of the equilibria for the formation of hydroxysulphonates. Thus, the

behaviour of the data in table 4.1.2 includes all parameters used in modelling. From table 4.1.2 it can be seen that if Δt is smaller than 0.05 h, the concentrations are independent of Δt.

4.1.3. Fitting theoretically generated data to experimental results.

As shown in section 3.1.2.2, maintaining reaction mixture pH significantly increases the rate of irreversible S(IV) loss during the constant rate phase of the glucose-glycine-S(IV) reaction. Therefore, rate constants used by previous workers (Wedzicha and Vacalis, 1988) to obtain a fit of theoretical data to experimental results in reaction mixtures where no pH control was exerted, are clearly of no use here, unless the reaction steps in question are independent of pH. This could be the case if, for example, the decrease in rate brought about by allowing the reaction mixture pH to fall was due to a reduction in the extent of acid base catalysis, caused by a change in the relative proportions of SO_3^{2-} to HSO_3^-. If at the same time the change in pH decreased the rate of DH formation, then the rate of formation of DDH from DH could be independent of pH. New constants to describe the model of Wedzicha and Vacalis (1988) under conditions of "constant" pH have been obtained here.

First approximations to rate constants that correspond to early steps in the uncatalysed, k_1, and S(IV)-catalysed, k_2, Maillard reactions were obtained from the plot of rate, for the S(IV)-inhibited Maillard reaction, *vs* initial S(IV) concentration. These constants were used with those derived by Wedzicha and Kaban (1986), for the formation of DSH from DH, and the reported values of dissociation constants for glucose (Wedzicha, 1984a), DH and DSH hydroxysulphonates (Wedzicha and Vacalis, 1988) to calculate

theoretical values of total, free and reversibly bound S(IV) during the course of the reaction. These values are shown with those obtained experimentally as a function of time in Figs.4.1.4-4.1.8. It can be seen that there are large differences between predicted and observed values. Clearly these constants require adjustment and can only be used as starting values for the fitting procedure which is now described.

The experimental and predicted S(IV) concentrations are still significantly different from each other. Given that a suitable value of Δt had been chosen, the process of fitting theoretically calculated values to experimental results involves adjusting the constants in the model until the maximum agreement between the two concentration/time profiles is seen. It was decided to obtain the best overall fit between observed and predicted values of total and free S(IV) concentration, rather than attempting to maximise the fit of either one or the other. In the latter case it may be possible to obtain a high degree of correlation between predicted and observed values of, say, free S(IV) concentration, whilst causing the quality of fit between the values of total S(IV) concentration to be reduced. This may imply that the behaviour of the free species was well understood, which could be misleading, because it may be possible to adjust the constants so that for the same set of experimental data, predicted and observed values of total rather than free S(IV) showed the higher correlation. It was shown in section 3.1.2.6, that S(IV) autoxidation is inhibited in a reaction mixture containing [glucose] = 2.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0469 M. Therefore, the constants in the model were adjusted to obtain the best fit of theoretical data to experimental results at this glucose concentration. However, as the experimental rate of irreversible

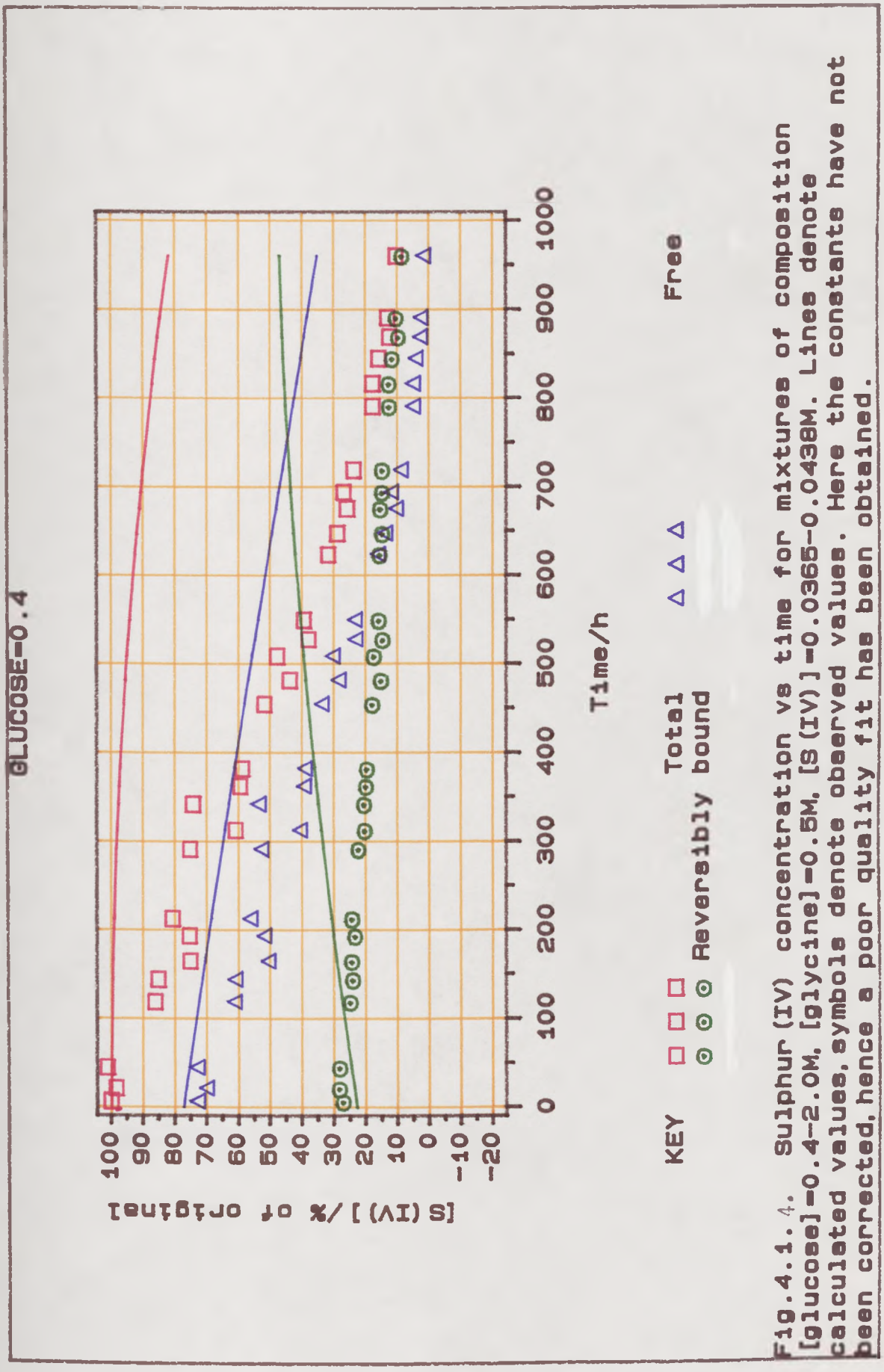


Fig.4.1.4. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values. Here the constants have not been corrected, hence a poor quality fit has been obtained.

Fig.4.1.5. Sulphur(IV) concentration ve time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values. Here the constants have not been corrected, hence a poor quality fit has been obtained.

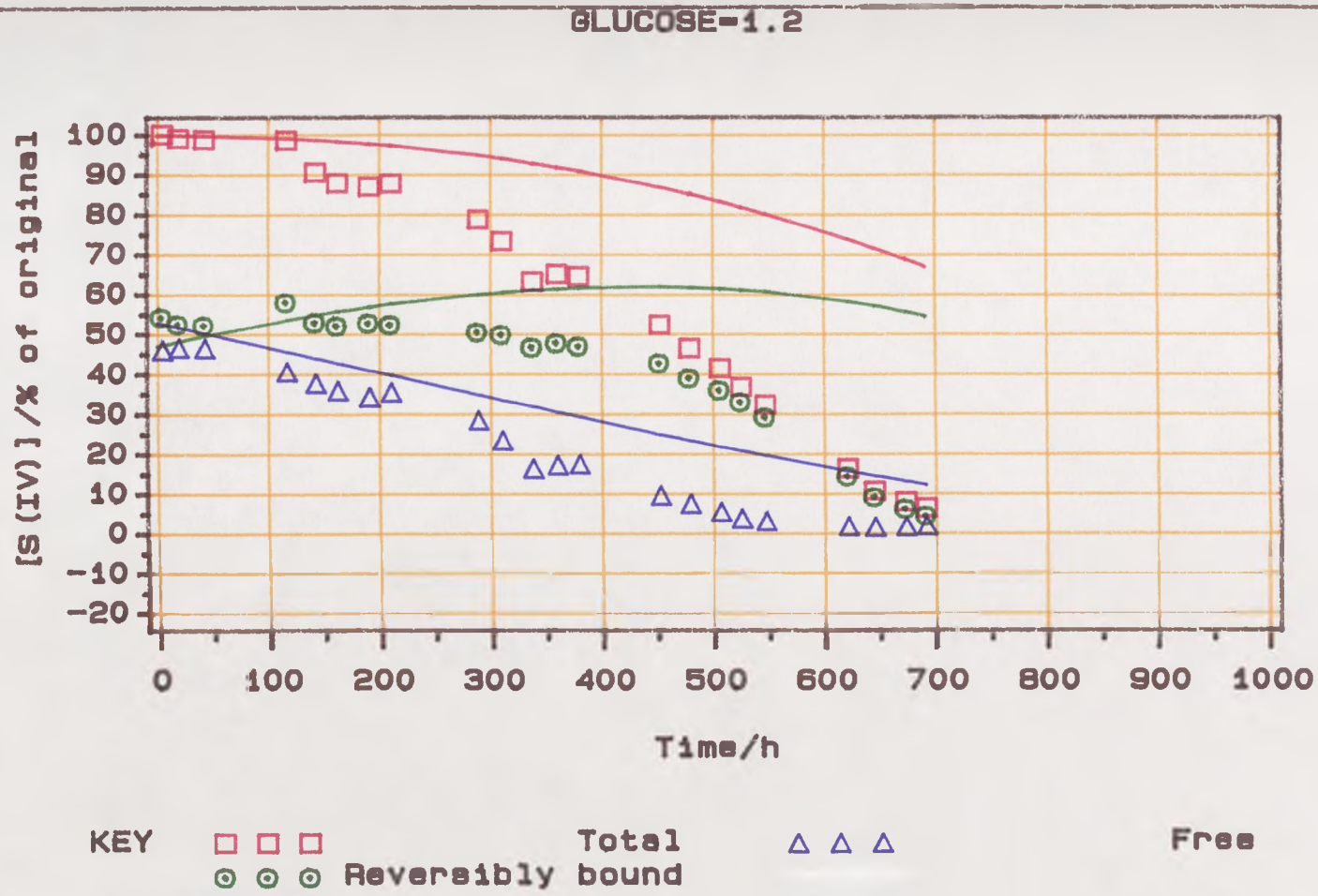


Fig.4.1.6. Sulphur(IV) concentration vs time for mixtures of compoeition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Linee denote calculeted velues, eymbole denote observed velues. Here the conetente heve not been corrected, hence e poor quelity fit hee been obtained.

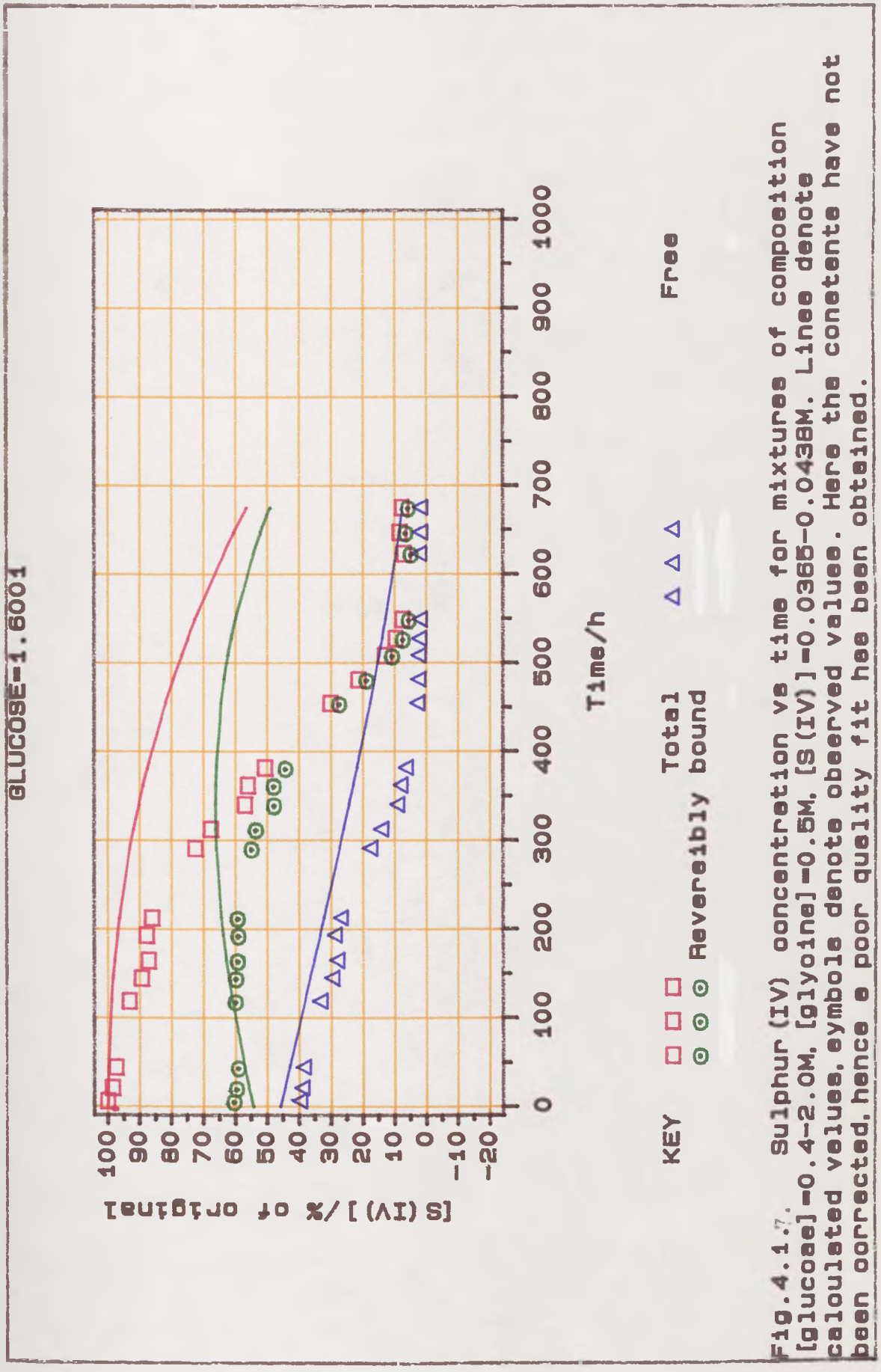


Fig.4.1.7. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values. Here the constants have not been corrected, hence a poor quality fit has been obtained.

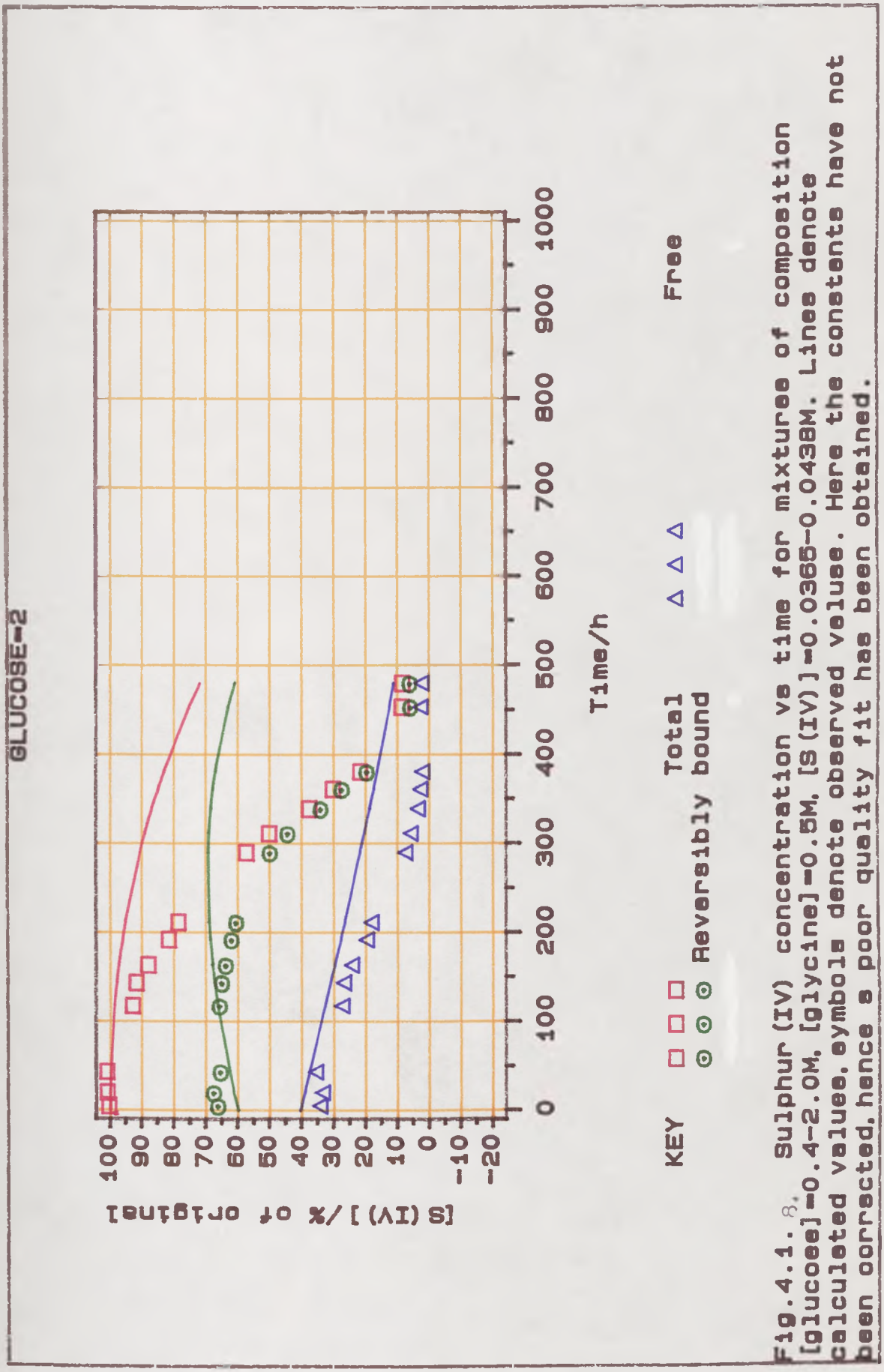


Fig.4.1.8. Sulphur (IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values. Here the contents have not been corrected, hence a poor quality fit has been obtained.

S(IV) loss significantly exceeded that of the theoretical rate at low glucose concentration, an additional constant was added to account for possible S(IV) autoxidation. In the case of inhibition of S(IV) autoxidation by mannitol (Fuller and Crist, 1941) and by N,N-dimethylformamide and N,N-dimethylacetamide (Schroeter, 1963), the rate law for the inhibited reaction is given by,

$$- d[S(IV)] / dt = k_5 [S(IV)] \{A / (A + [I])\} \quad (4.1.7)$$

where A is a constant describing the inhibition, I represents the inhibitor and k_5 is the rate constant for the uninhibited reaction. This rate law is reported to be in agreement with experimental data over a change in inhibitor concentration of two orders of magnitude, with a value of A of 10^{-5} M in all cases. Each of glucose and glycine will contribute towards the inhibition of S(IV) autoxidation and each must be accounted for. It is not possible to simply add glucose and glycine concentrations to give a single value of I, because this would assume that glucose and glycine would inhibit S(IV) autoxidation to the same extent. It is likely that these reagents will differ in their relative antioxidant abilities and hence separate equations should be used for each contribution towards inhibition. The amount by which the S(IV) concentration changes, ΔR, due to S(IV) autoxidation during the interval Δt is taken as:

$$\Delta R = \Delta t \, k_5 [S(IV)] \{A / (A + [I])\}$$

where I is either glucose or glycine.

The quality of the fit was assessed as the sum of squared deviations between corresponding theoretical and experimental values at each measurement time, i.e., for free S(IV),

$$SSD = \sum_{i=1}^{n} (free_{th} [S(IV)] - free_{ex} [S(IV)])^2$$

where the subscripts th and ex denote calculated and experimental values respectively and n is the number of data points. However, as reaction mixtures with different compositions react at different rates, the number of pairs of theoretical and experimental values defining the respective concentration/time profiles will vary accordingly, as will the sum of the squares of the deviations. Therefore, to assess the effect of reaction mixture composition on the quality of fit, the average squared deviation per pair of data values was obtained by dividing the sum of the squares of the deviations by the number of measurements, i.e.,

$$ASSD = \sum_{i=1}^{n} \{(free_{th}\,[S(IV)] - free_{ex}\,[S(IV)])^2\} / n$$

The same procedure was followed for total and reversibly bound S(IV). These three average sums were added to produce the total average squared deviation per pair of theoretical and experimental values for the reaction mixture. This procedure is then repeated for each reaction mixture in a kinetic run. This gives a measure of the agreement between theoretical data and experimental results relative to any other reaction mixture, a perfect fit having a value of zero, and enables the effect of reagent concentration on the quality of fit to be assessed.

In this procedure the deviations are a percentage of the measured initial S(IV) concentration. Hence, if the difference between theoretical and experimental S(IV) concentrations was, say, 0.005 M at every instant, this would represent a consistent deviation of 10% throughout the duration of the reaction, if the initial S(IV) concentration had been 0.05 M; the deviations are weighted uniformly. Alternatively, the deviation occurring at any particular

time could be expressed as a percentage of the measured S(IV) concentration at that time. For example, if at the end of the reaction the S(IV) concentration was 0.005 M and the deviation throughout the reaction between theoretical and experimental values was as before 0.005 M, then this would represent a deviation of 100%. This approach would lead to the values towards the end of the reaction being weighted more heavily, and to keep the total error to a minimum it would be more important to fit these later data points accurately. However, this procedure is undesirable as it implies that the prediction of S(IV) concentration becomes worse as the reaction proceeds, when actually the deviation from the measured value may remain constant. The increase in perceived error is not due to a worsening of the prediction process, but rather to the unavoidable loss of S(IV) due to irreversible binding to components of the mixture.

If one were to fit data by minimising the deviation expressed in terms of the percentage deviation at a given concentration, the result shown in Fig.4.1.9 could well arise.

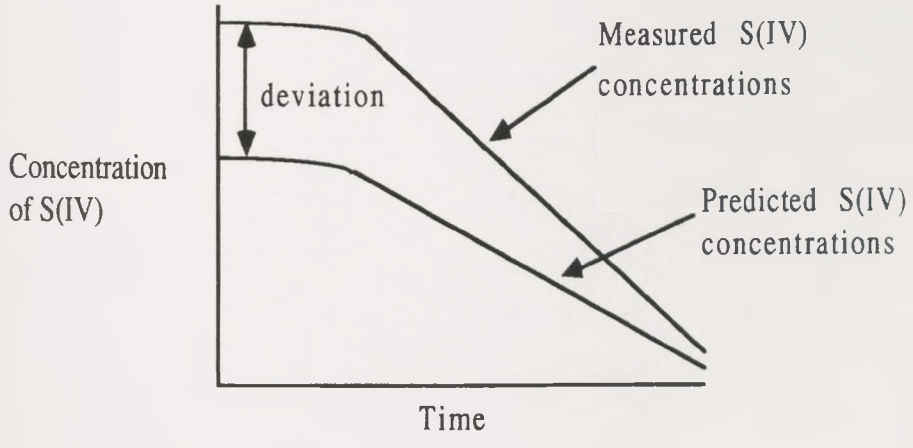


Fig.4.1.9. Predicted and measured S(IV) concentrations *vs* time, produced by minimising the deviation expressed in terms of the percentage deviation at a given concentration.

It can be clearly seen that the predicted reaction rate, obtained from the linear constant rate phase, could be markedly different from the measured rate. Hence, an apparent good fit could give rise to a very poor estimate of the measured reaction rate. To avoid this, actual deviations were used rather than fractional or % deviations.

The combinations of constants that gave the best correspondence between theoretically calculated data and experimental results for mixtures of varying glucose, glycine and S(IV) concentration are shown in table 4.1.3, their respective measures of fit being given in tables 4.1.4-4.1.6.

Constant	**Original value**	**Value obtained when the concentration of the following were varied:**		
		glucose	**glycine**	**S(IV)**
$k_1/M^{-1}h^{-1}10^{-5}$	5.46	10.91	8.73	19.10
$k_2/M^{-2}h^{-1}10^{-3}$	2.0263	4.053	3.242	7.092
$k_3/h^{-1}10^{-4}$	17.000	14.450	10.200	5.100
$k_4/M^{-1}h^{-1}10^{-3}$	7.0000	5.9500	4.2000	2.1000
$k_5/M\,h^{-1}$	0.0	15.0	25.0	0.0
K_1/M	1.33	1.00	1.00	1.00
$K_2/M\,10^{-1}$	0.0400	2.8	2.8	2.8
$K_3/M\,10^{-4}$	44.0	6.6	6.6	6.6

Table 4.1.3 Values of rate and equilibrium constants required to give the best agreement between experimental and theoretical values of total, free and reversibly bound S(IV), using the model of the S(IV)-inhibited Maillard reaction shown in Fig.4.1.1, .

Theoretically generated, using adjusted rate and equilibrium

Glucose /M	Average squared deviation per pair of calculated and measured values			total average
	Total S(IV)	Free S(IV)	Reversibly bound S(IV)	
0.4	97.6	36.9	185.3	319.9
0.8	194.9	86.6	60.2	341.8
1.2	84.8	30.1	23.0	138.0
1.6	94.9	10.6	59.4	164.8
2.0	27.0	2.7	24.3	53.9
MEAN	99.8	33.4	70.4	

Table 4.1.4.

Glycine /M	Average squared deviation per pair of calculated and measured values			total average
	Total S(IV)	Free S(IV)	Reversibly bound S(IV)	
0.2	31.8	19.8	12.9	64.5
0.4	26.2	8.9	22.1	57.1
0.6	36.2	14.0	20.1	70.3
0.8	57.1	12.1	44.3	113.4
1.0	45.2	13.8	38.9	97.9
MEAN	39.3	13.7	27.7	

Table 4.1.5.

constants, and experimentally obtained values of total, free and reversibly bound S(IV) are shown as a function of time in Figs.4.1.10-4.1.29. From Figs.4.1.10-4.1.29 it can be seen that in the early stages of the reaction, 0-100 h, experimental and theoretical

S(IV) /M	Average squared deviation per pair of calculated and measured values			total average
	Total S(IV)	Free S(IV)	Reversibly bound S(IV)	
0.0078	632.9	20.5	481.3	1134.6
0.0159	37.9	13.7	18.2	69.9
0.0243	126.2	29.8	69.6	225.6
0.0324	5.4	14.8	9.0	29.3
0.0423	6.5	15.6	13.7	35.8
0.0480	40.5	24.2	15.2	80.0
0.0602	19.0	11.4	11.2	41.7
0.0677	24.7	9.3	24.2	58.2
0.0773	8.3	6.2	9.5	24.0
0.0830	8.7	10.1	8.1	27.0
MEAN	91.0	15.6	66.0	

Table 4.1.6. Tables 4.1.4-4.1.6 give the average squared deviations of theoretical from experimental values of total, free and reversibly bound S(IV); total average squared deviations and mean of the average squared deviations for reaction mixtures of composition [glucose] = 0.4-2.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0365-0.0438 M, table 4.1.4: [glucose] = 1.0 M, [glycine] = 0.2-1.0 M, [S(IV)] = 0.0406-0.0423 M, table 4.1.5: [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0078-0.0830 M, table 4.1.6.

values show a high degree of agreement in each mixture. This indicates that after adjustment of the constants, the model of the glucose-glycine-S(IV) reaction gives a good description of the concentration/time profile during this period. After this time significant deviation between values occurs in some mixtures, presumably because the model does not accurately describe all the

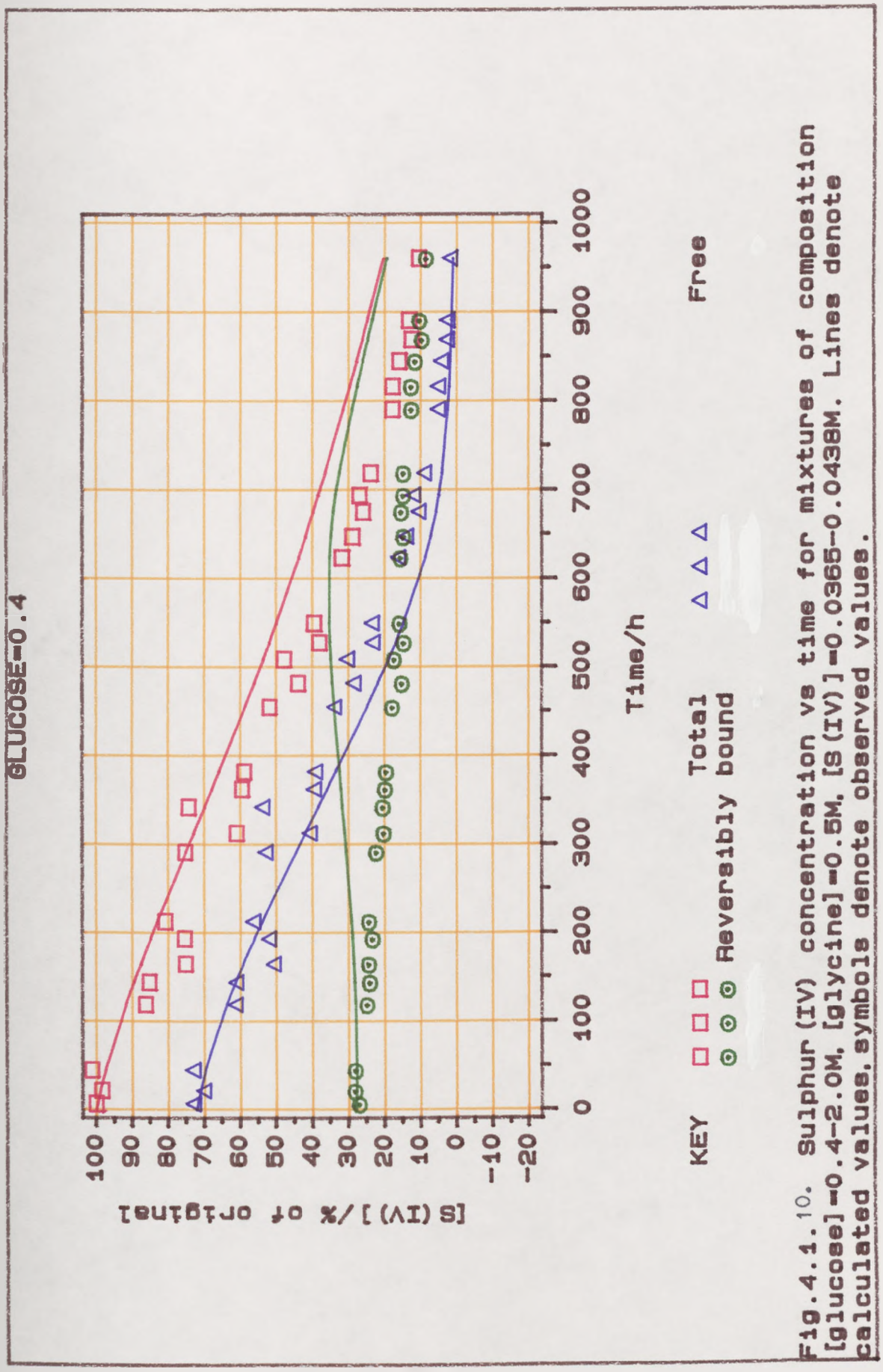


Fig.4.4.1.10. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values.

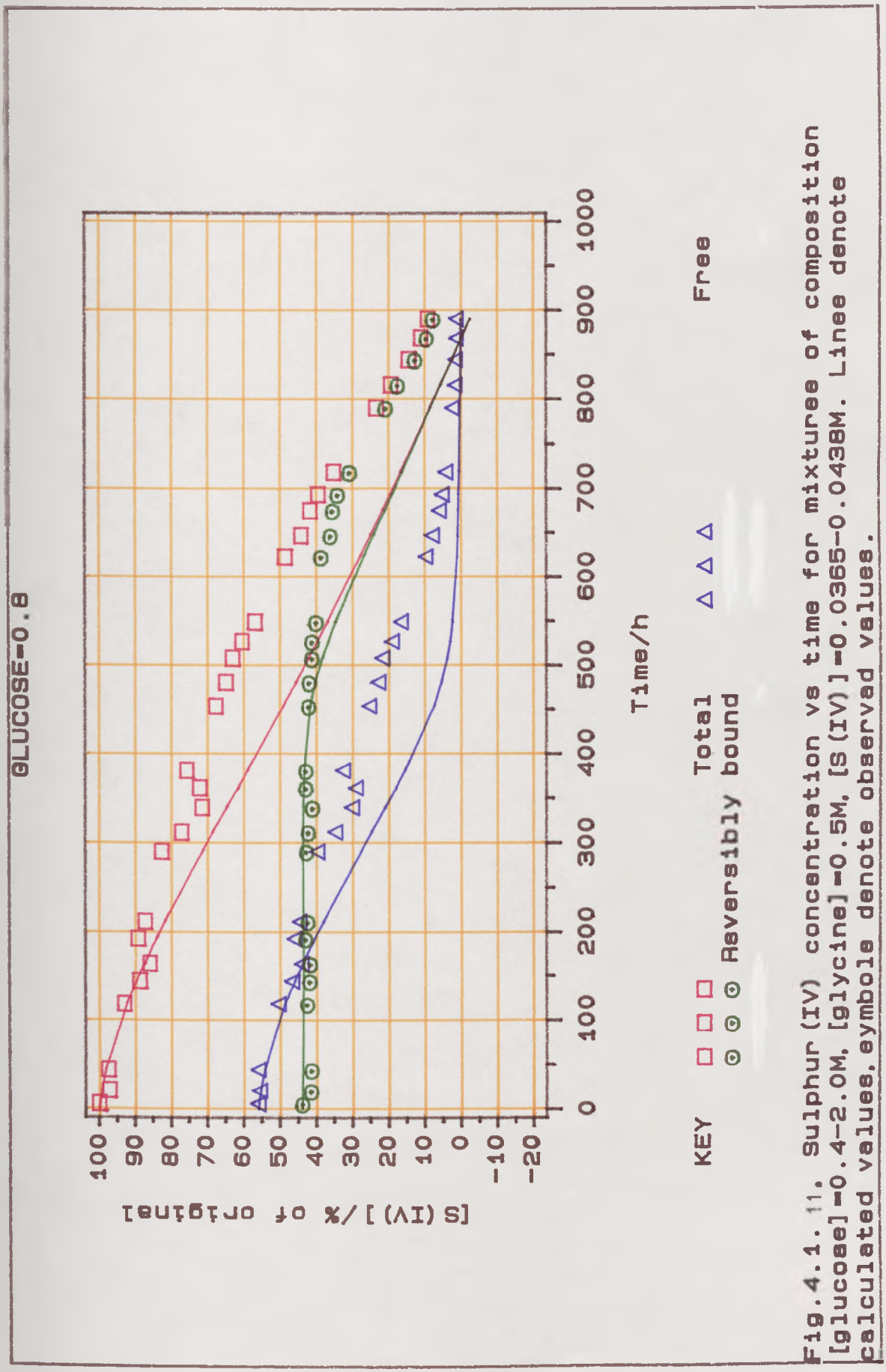


Fig.4.1.11. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values.

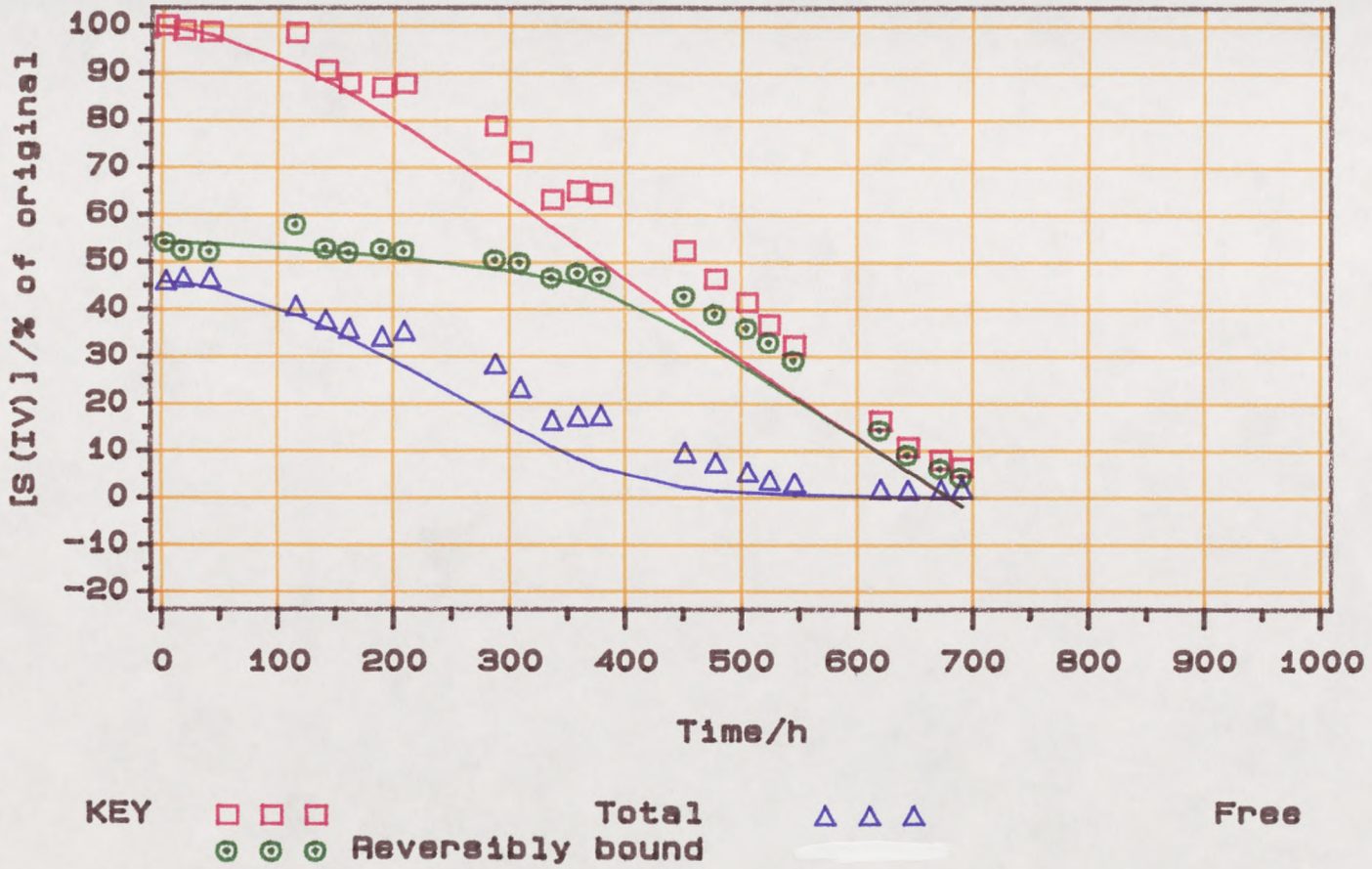


Fig.4.1.12. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values.

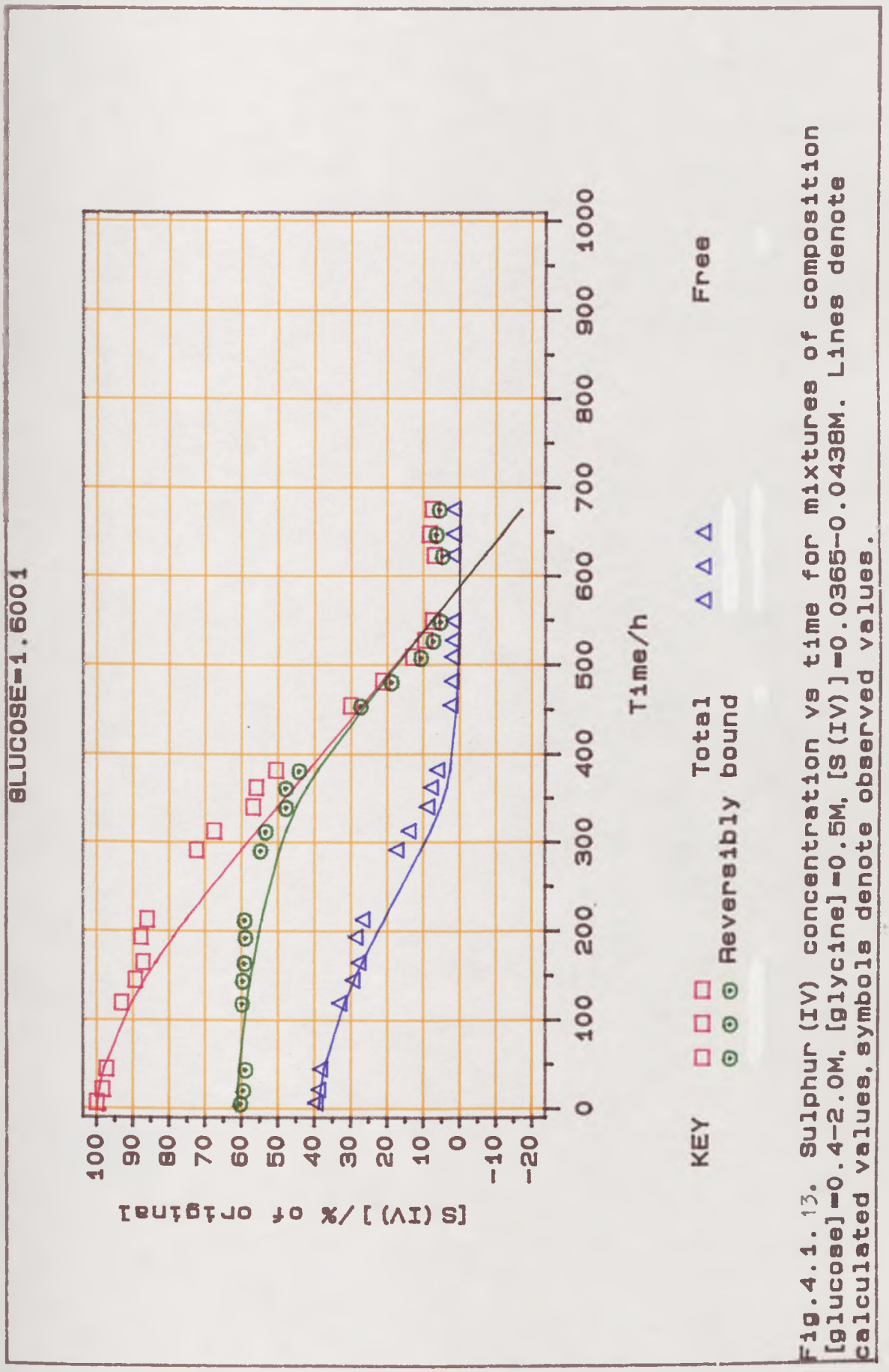


Fig.4.1.13. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values.

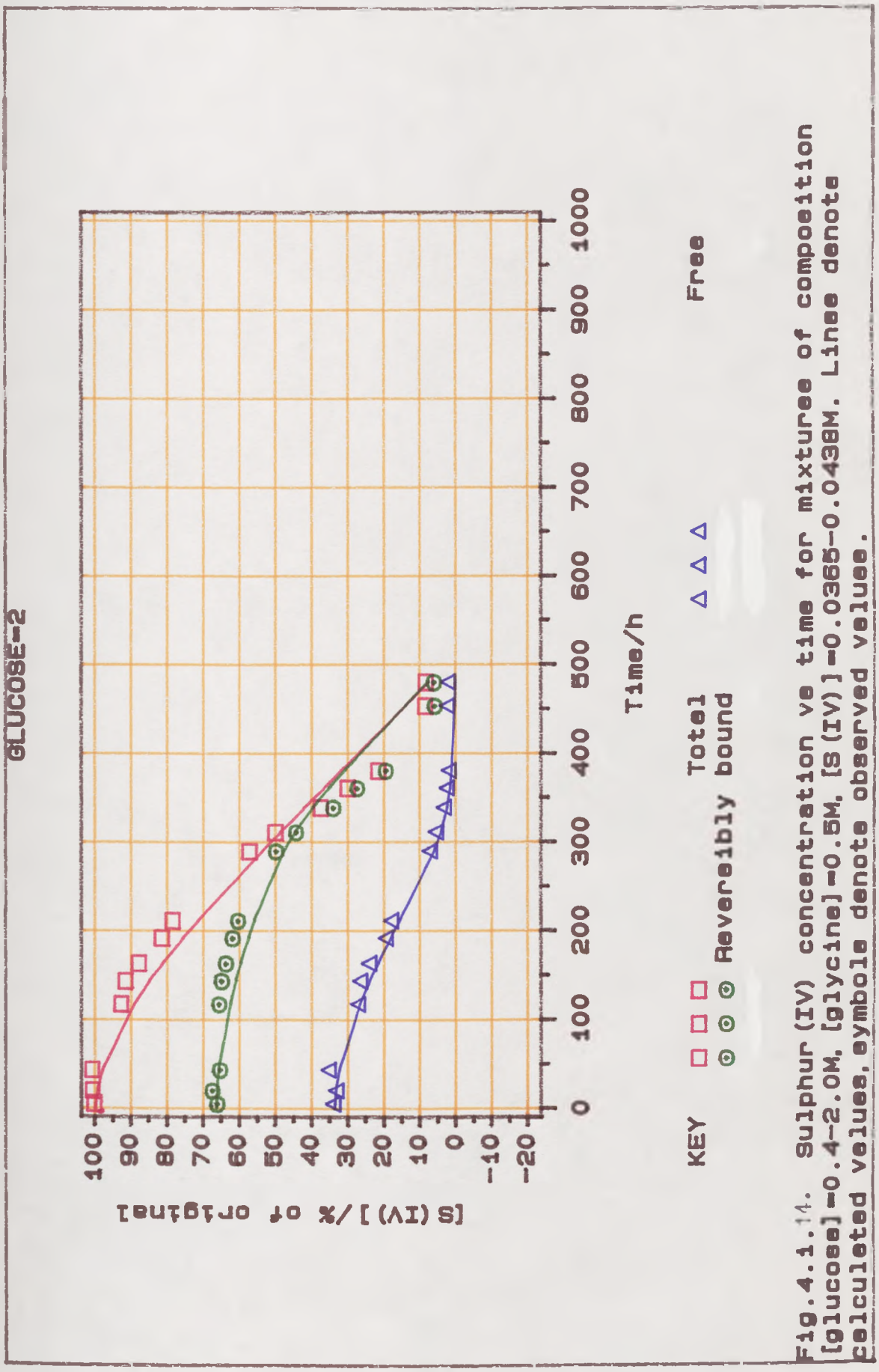


Fig.4.1.14. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=0.4-2.0M, [glycine]=0.5M, [S(IV)]=0.0365-0.0438M. Lines denote calculated values, symbols denote observed values.

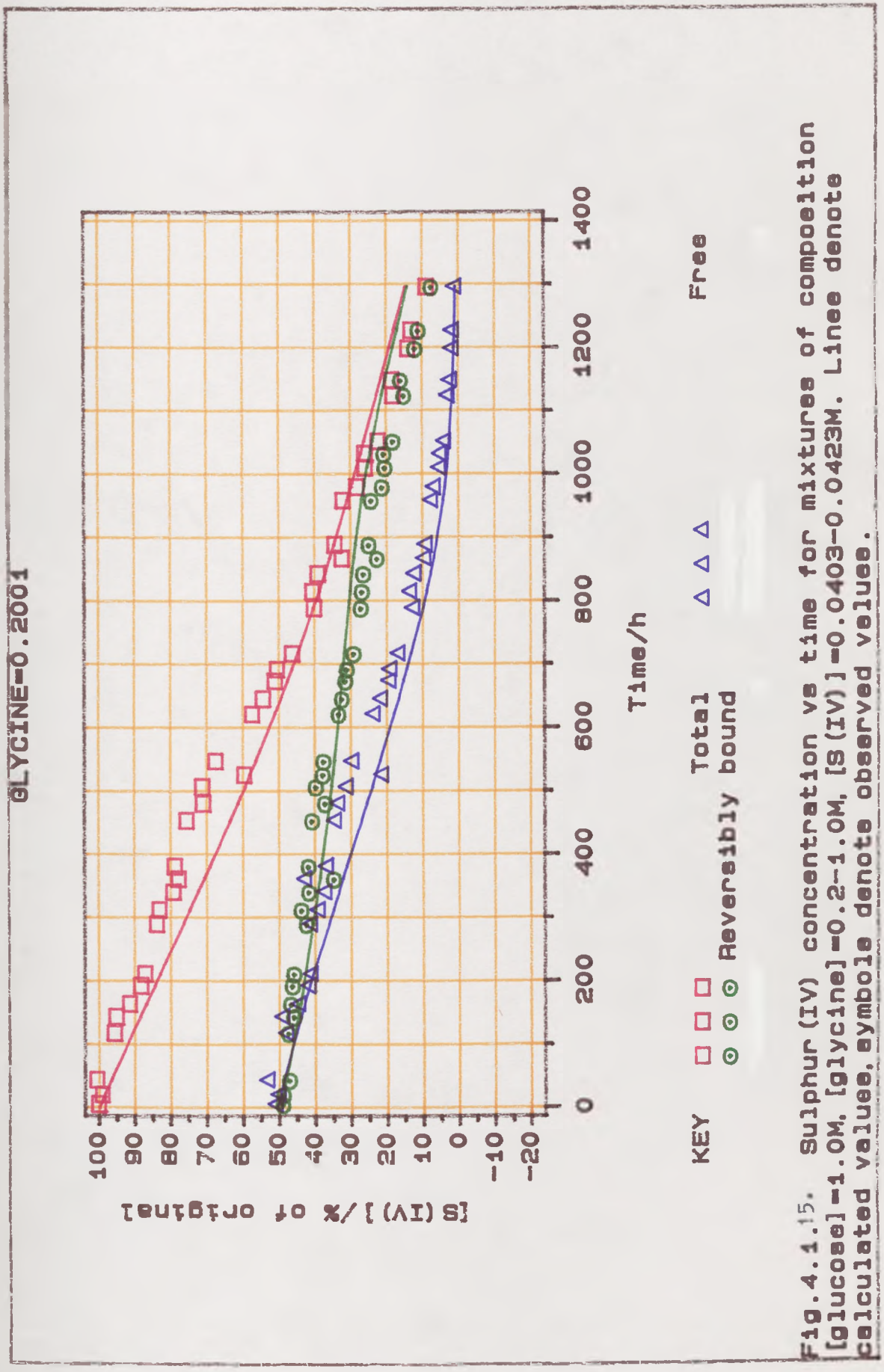


Fig.4.4.15. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M. Lines denote calculated values, symbols denote observed values.

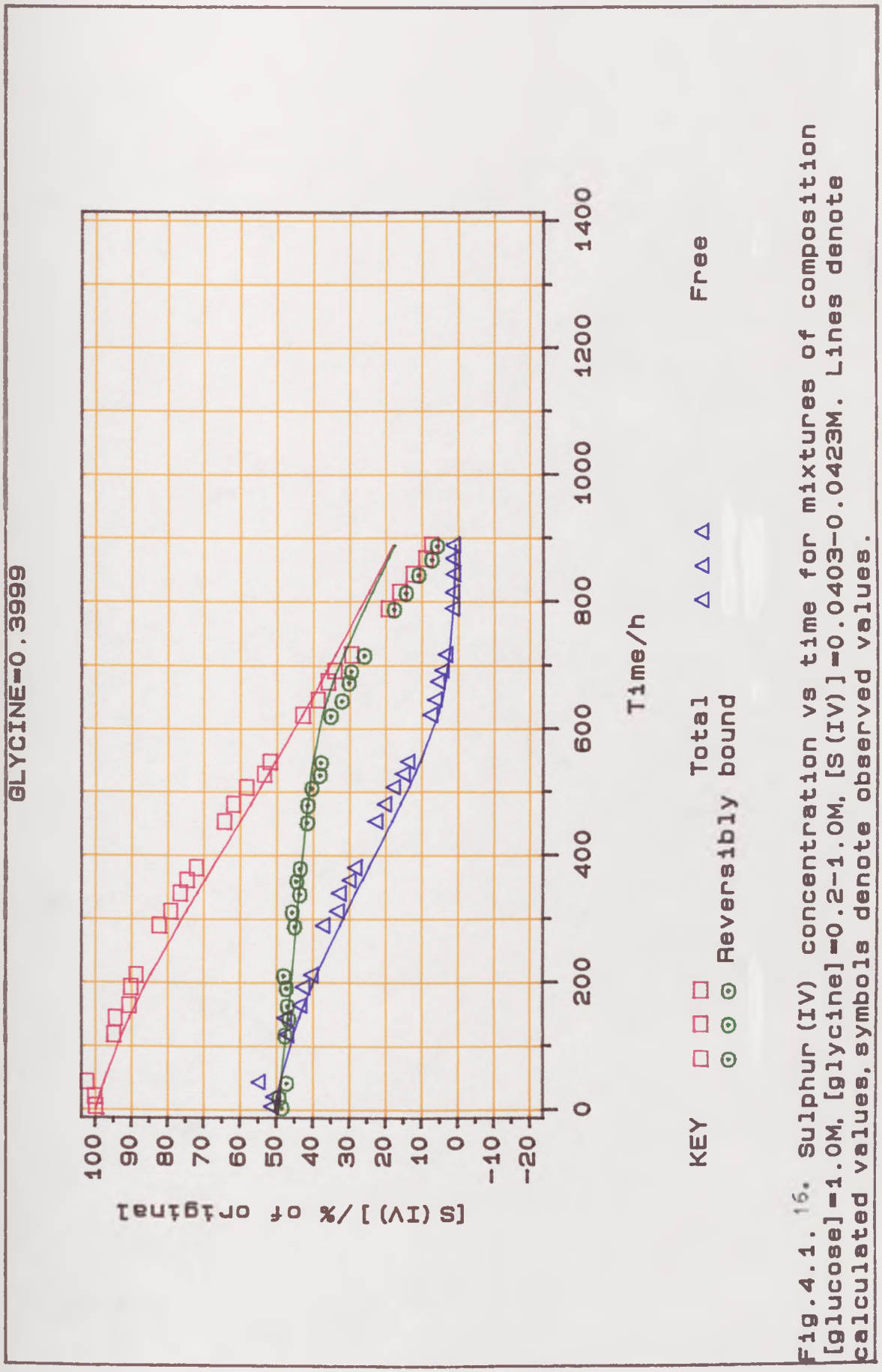


Fig.4.1.16. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M. Lines denote calculated values, symbols denote observed values.

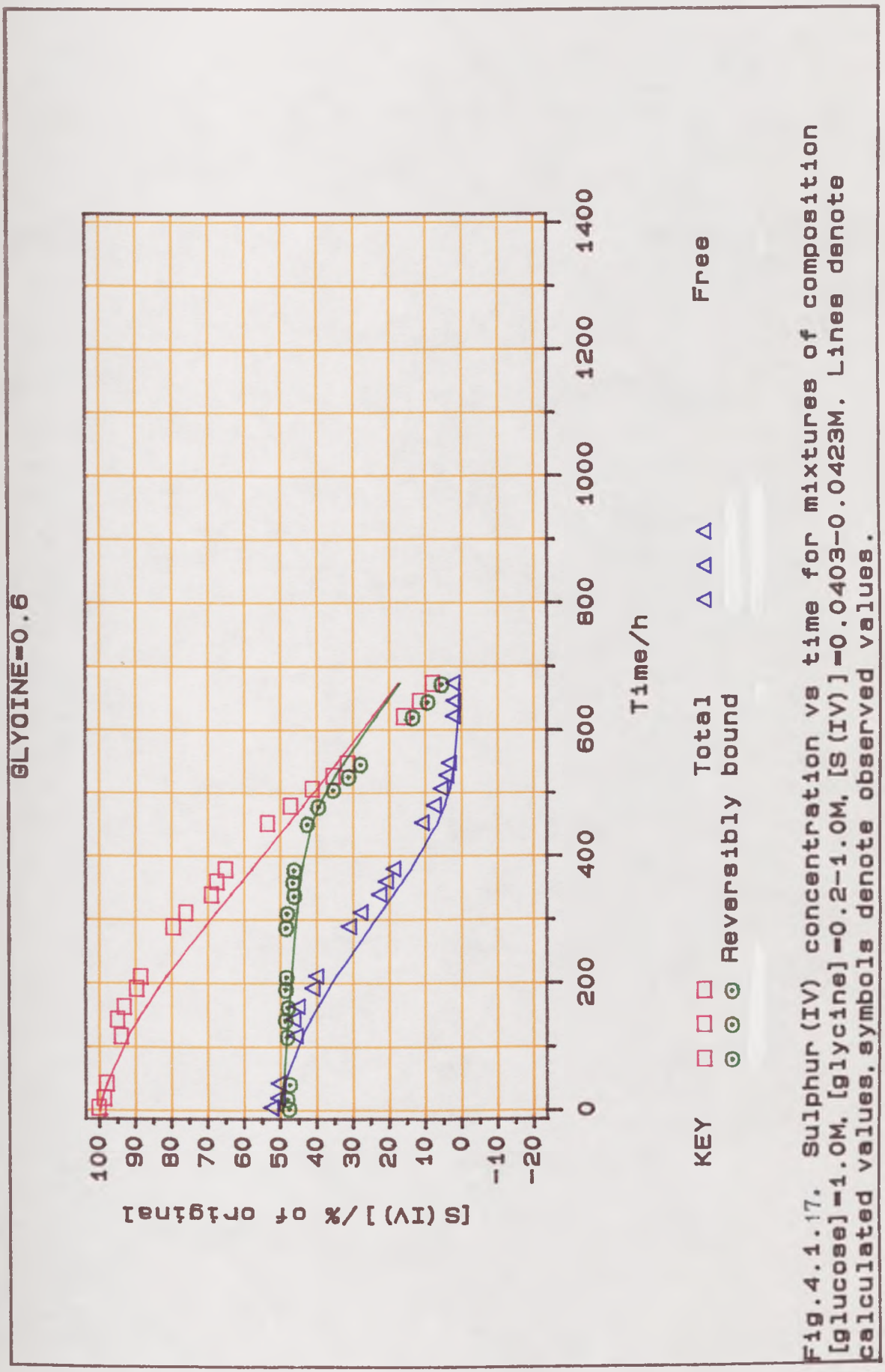


Fig.4.4.17. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M. Lines denote calculated values, symbols denote observed values.

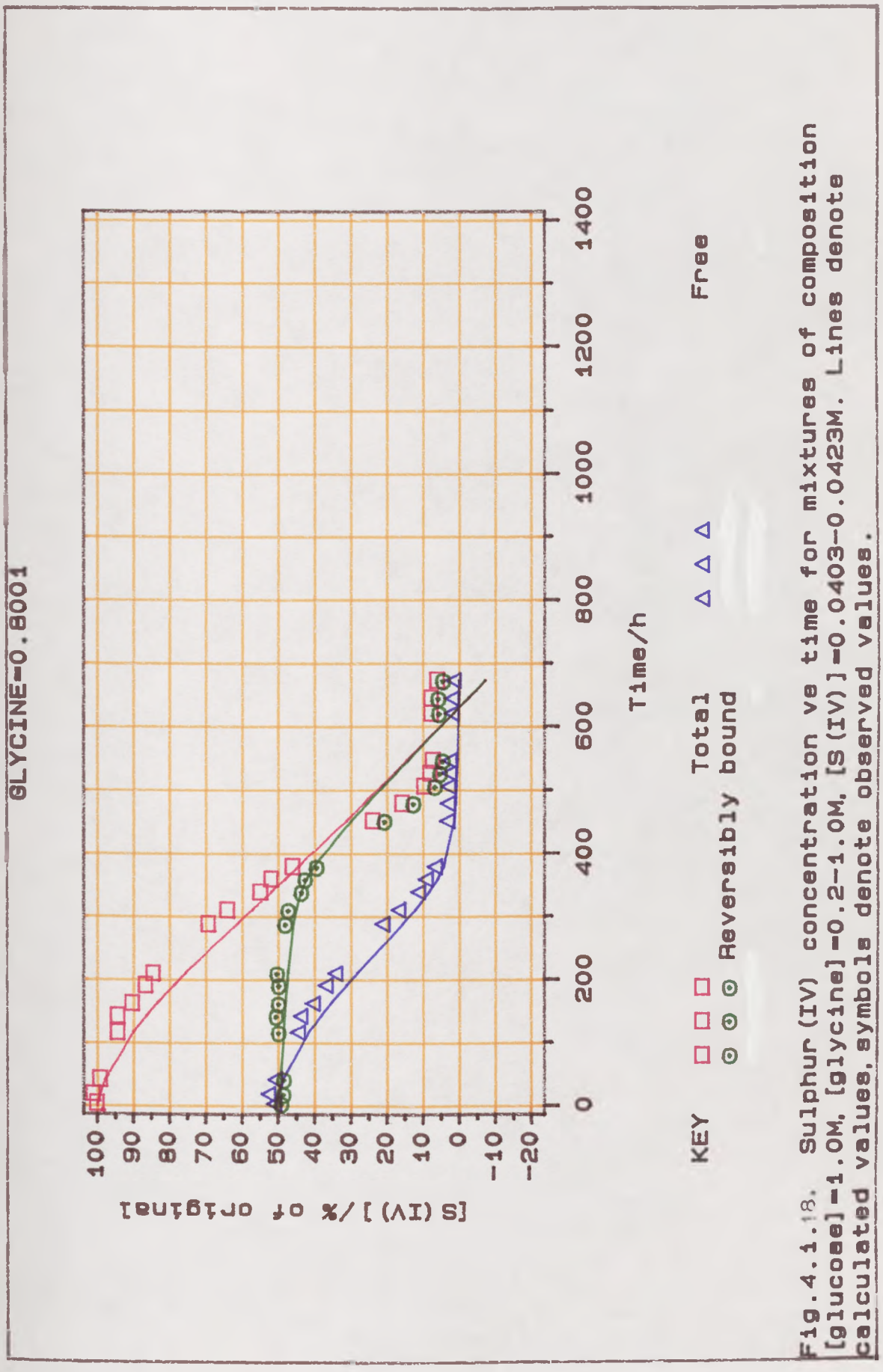


Fig.4.1.18. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M. Lines denote calculated values, symbols denote observed values.

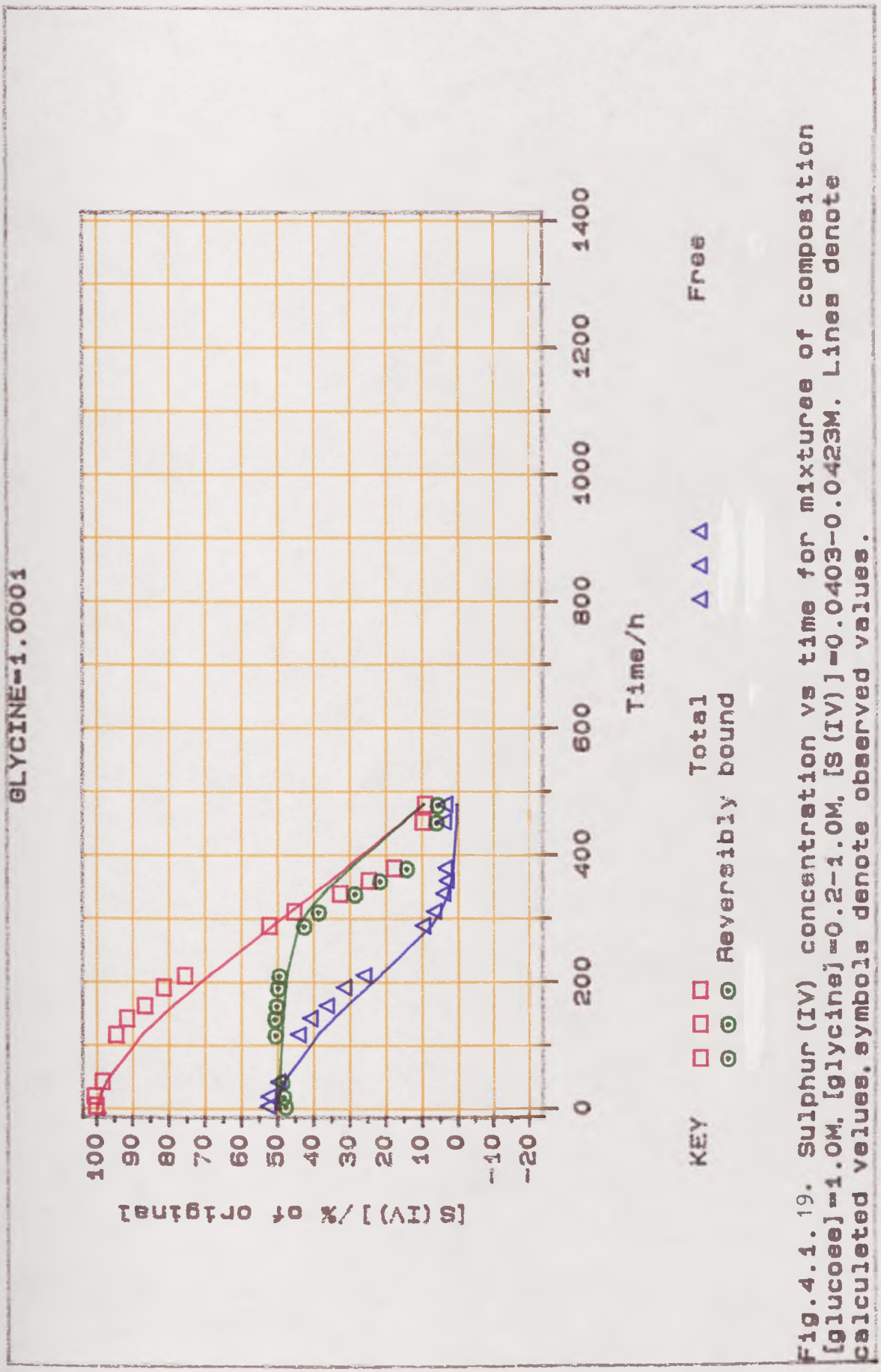


Fig.4.4.19. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.2-1.0M, [S(IV)]=0.0403-0.0423M. Lines denote calculated values, symbols denote observed values.

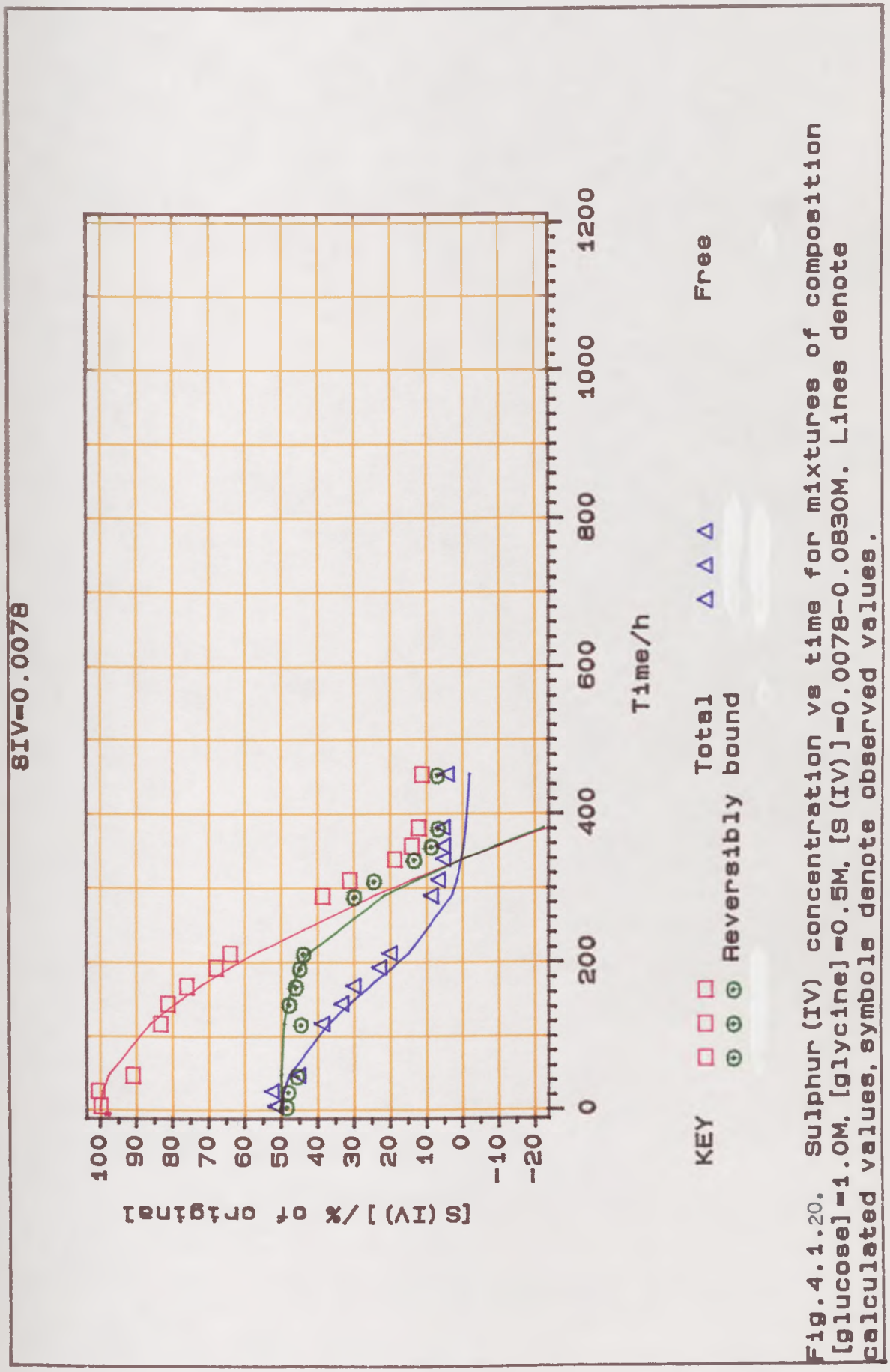


Fig.4.1.20. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

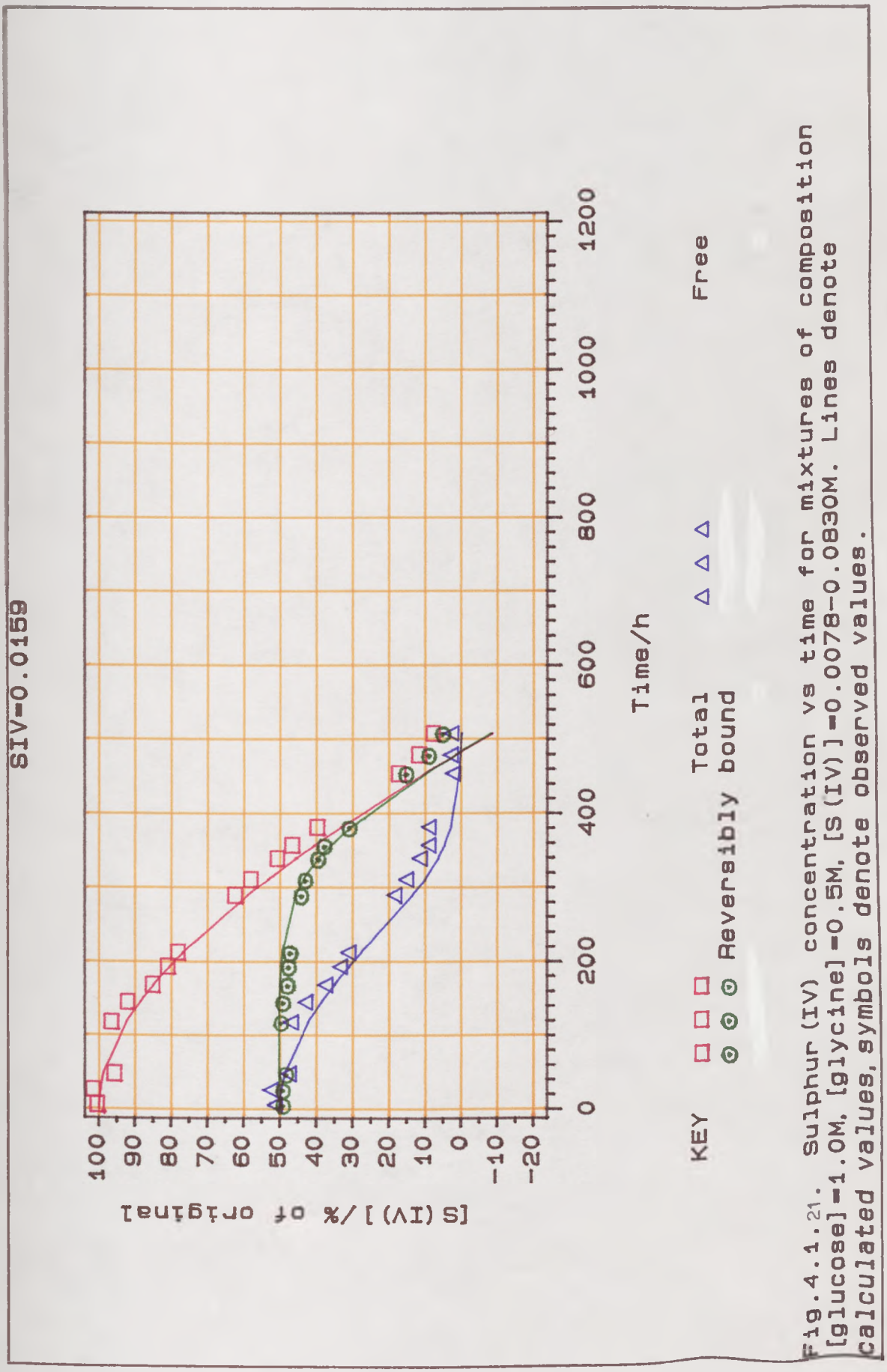


Fig.4.1.21. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

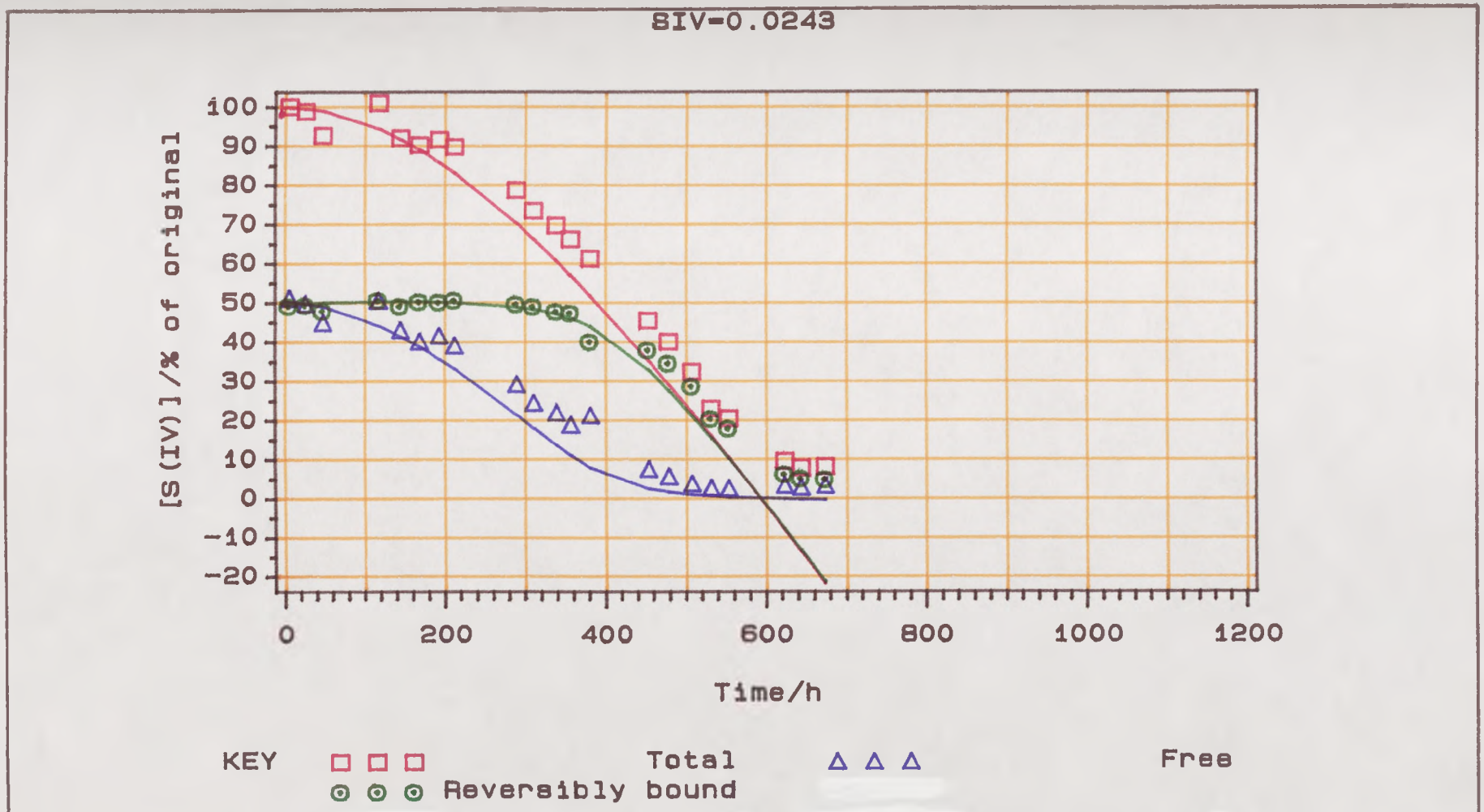


Fig.4.1.22. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

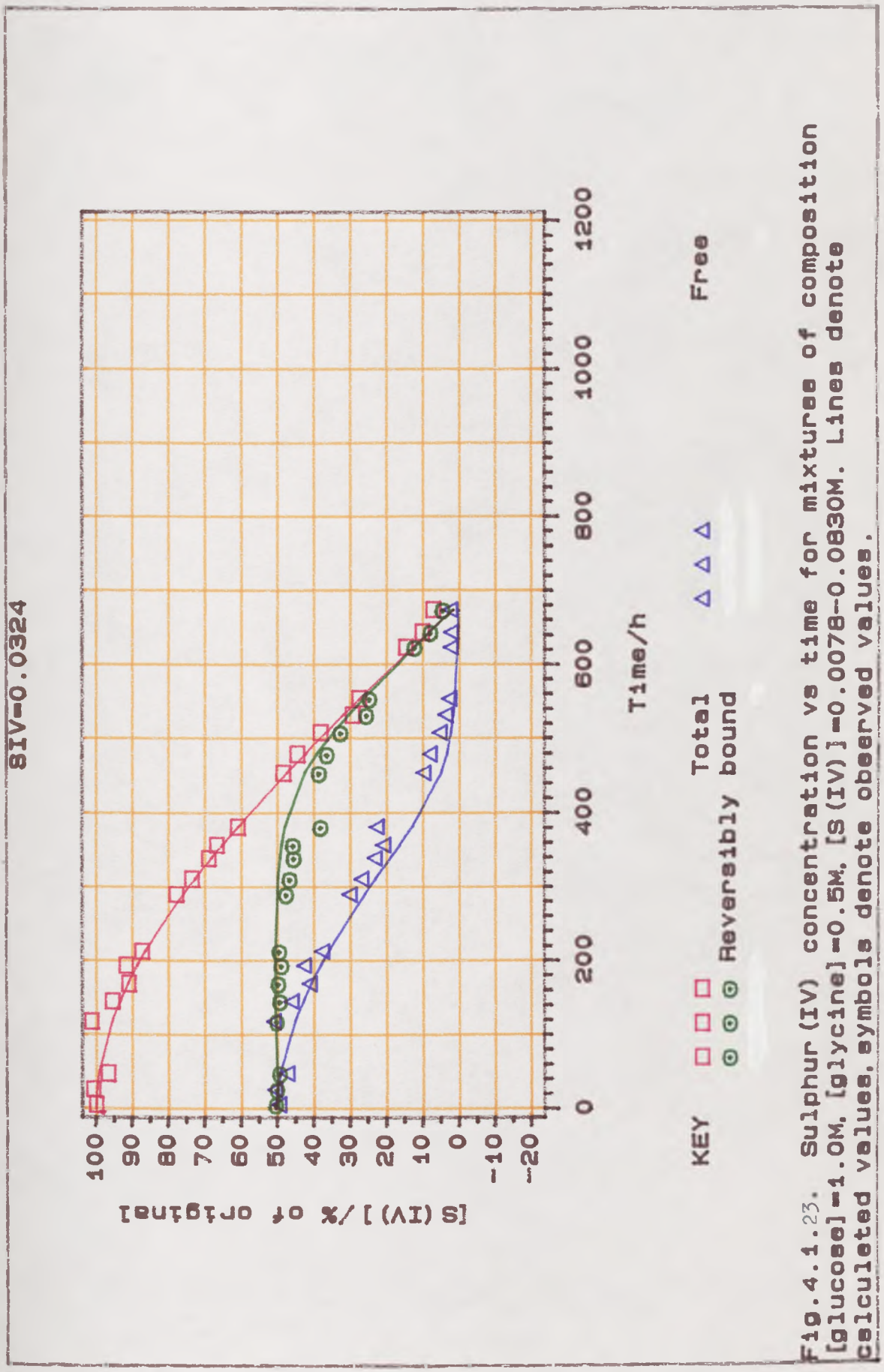


Fig.4.4.1.23. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

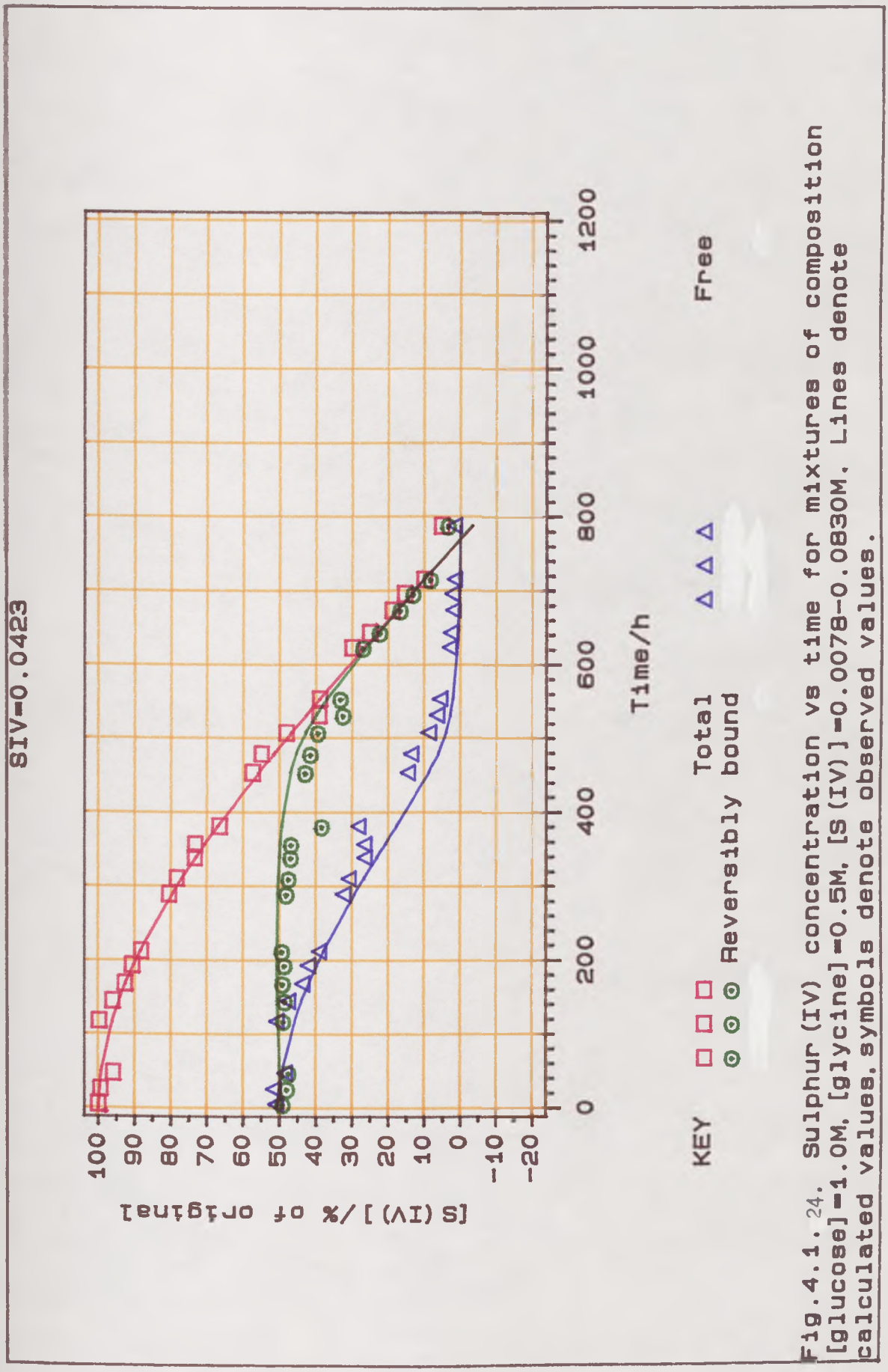


Fig.4.1.24. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

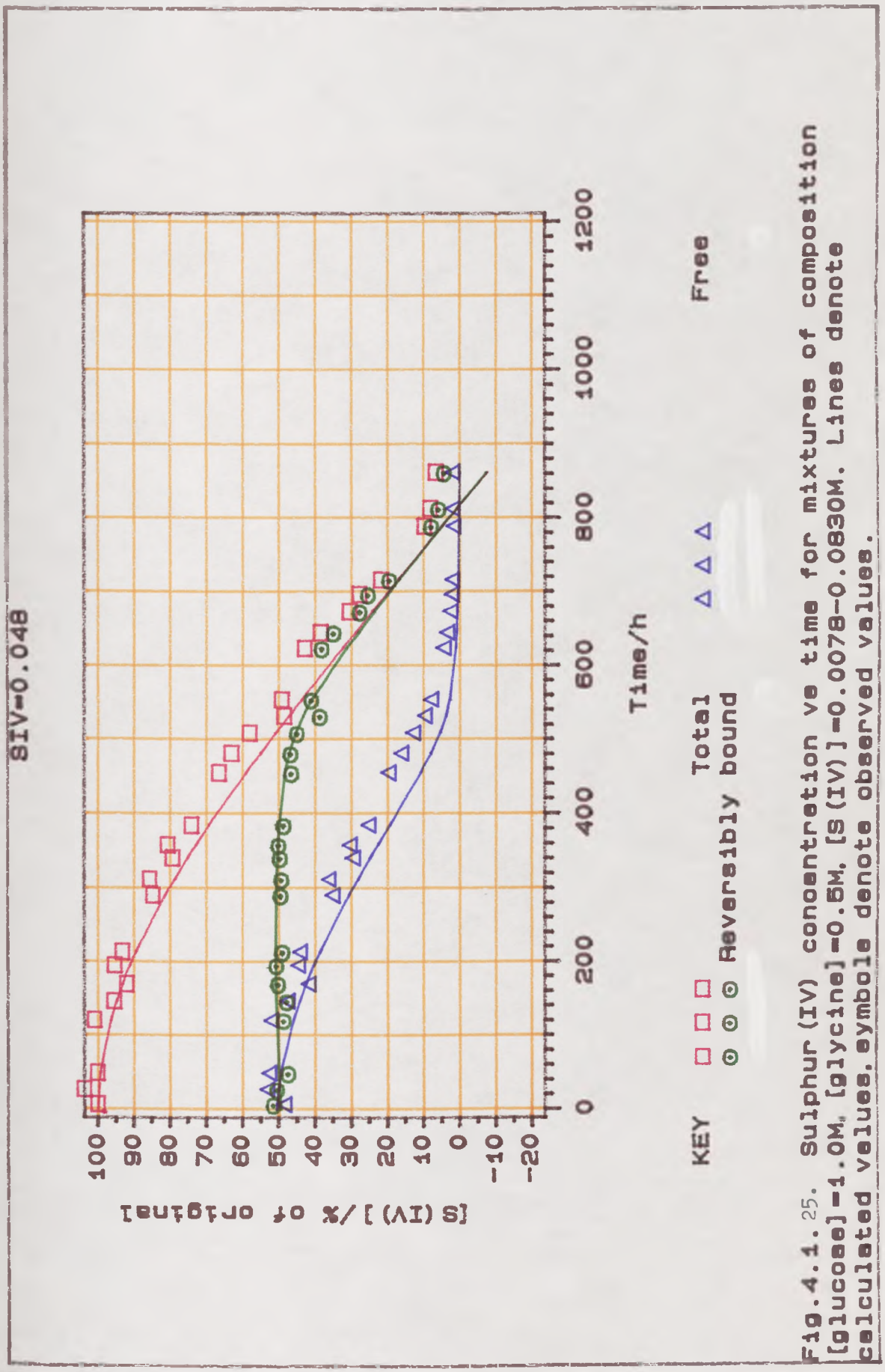


Fig.4.1.25. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

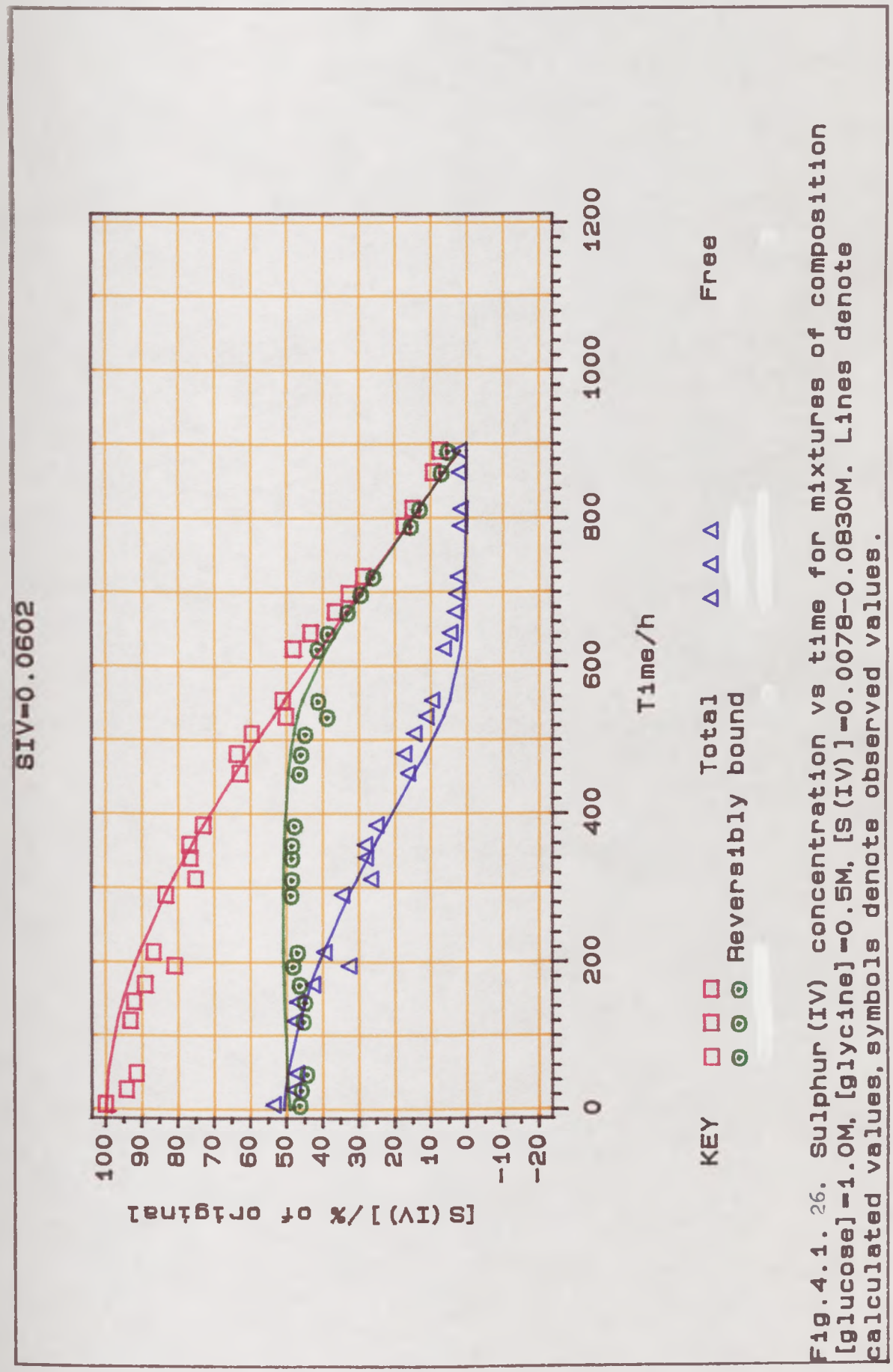


Fig.4.1.26. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

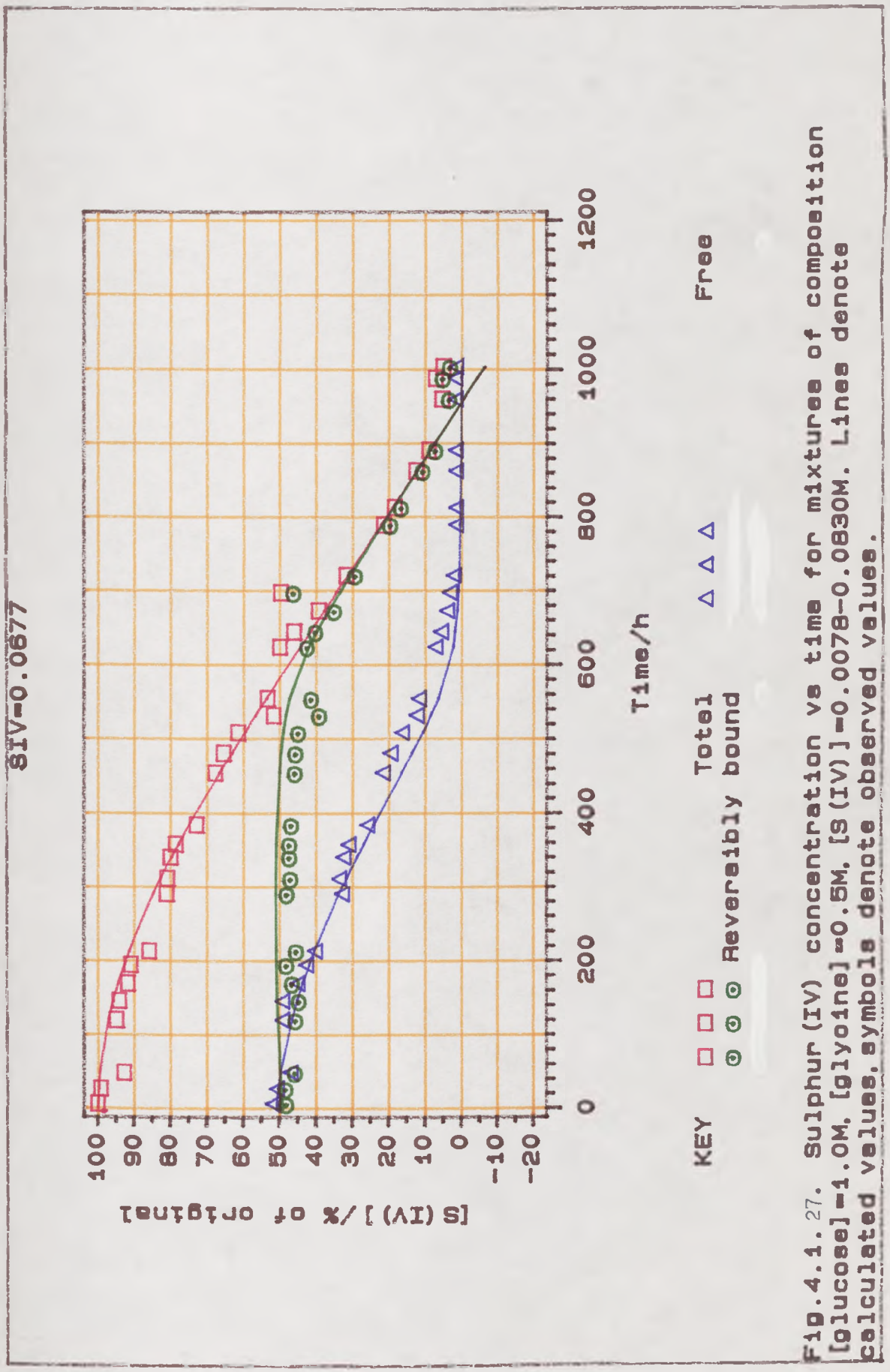


Fig.4.1.27. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

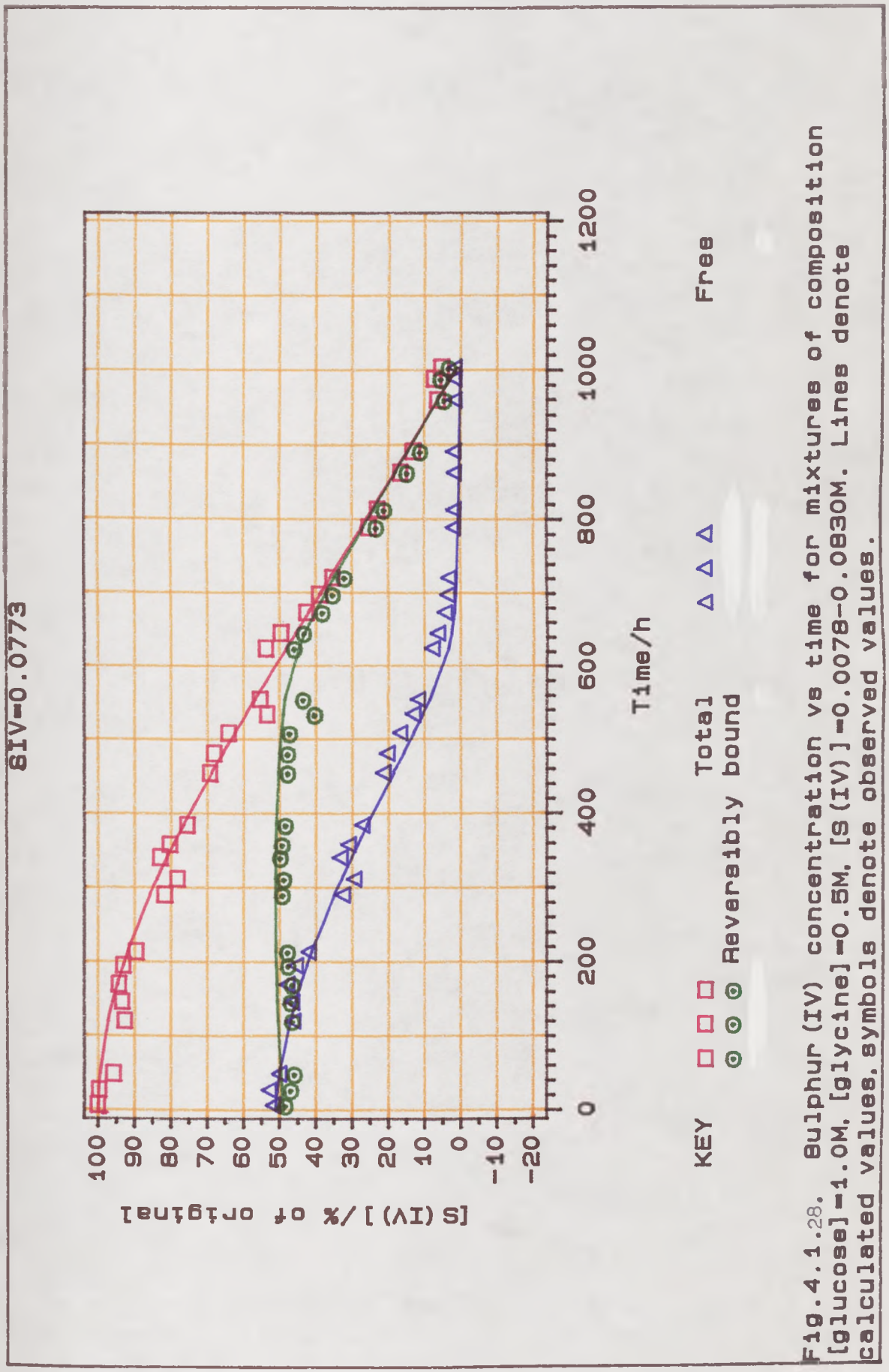


Fig.4.1.28. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

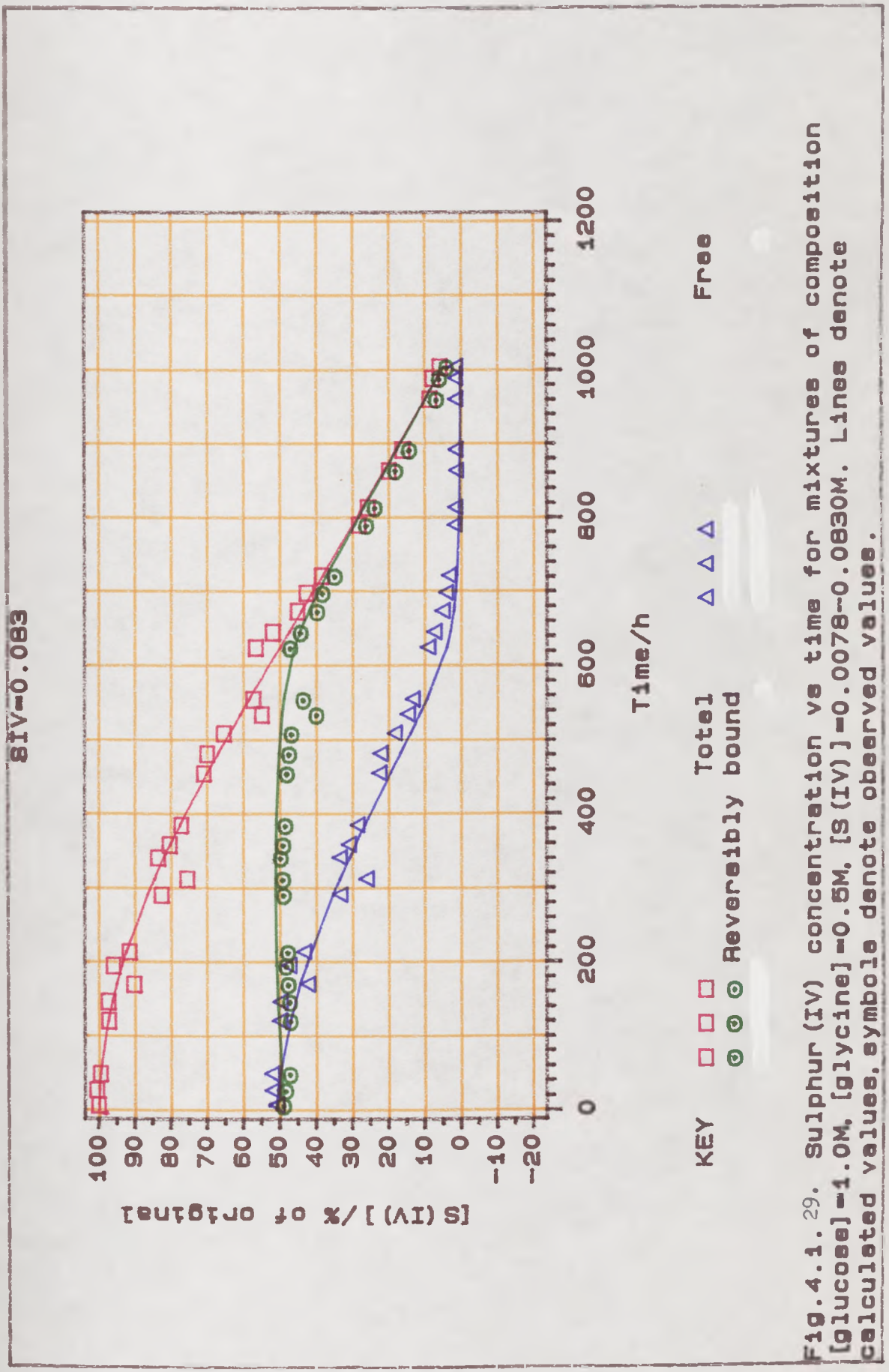


Fig.4.1.29. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0078-0.0830M. Lines denote calculated values, symbols denote observed values.

processes occurring in the reaction. Here it is assumed that the experimental results are reliable and that the combination of constants chosen for a kinetic run is the one that gives the best fit for those data; it is possible, however, that there is more than one combination of constants that fits the data.

In tables 4.1.4-4.1.6, the means of the average squared deviations for total, free and reversibly bound S(IV) concentrations have been calculated. This enables the quality of the fit between predicted and observed values of these concentrations to be assessed relative to one another. It can be seen that values of free S(IV) give a superior mean fit when compared to the fit for reversibly bound S(IV) in each kinetic run. This suggests that hydroxysulphonate formation is not described sufficiently accurately in the kinetic model. Alternatively, the equilibrium constants may have to be adjusted in order to obtain a good overall fit and this adjustment causes the fit for reversibly bound S(IV) to be less satisfactory than the fit for free S(IV). Addition of the means for total and free S(IV) concentration gives the sum of the means for the kinetic run. The effects of glucose, glycine and S(IV) concentrations on the quality of fit, relative to one another, can then be assessed. In addition, this also enables comparison of the data reported in this work to that of Wedzicha and Vacalis (1988).

From the total average values shown in table 4.1.4, it can be seen that the quality of fit essentially improves with increasing glucose concentration. This may be because the model system is simpler at high glucose concentration, due to the inhibition of S(IV) autoxidation.

In contrast to the findings for glucose, the best fit is obtained when there is a low concentration of glycine. However, the total

averages do not change greatly as the concentration increases by a factor of five. In comparison, the change seen for glucose when the concentration changes by the same factor was greater. This could be because the extent of S(IV) autoxidation remains relatively unchanged.

For reaction mixtures of varying S(IV) concentration, the best overall fit was obtained from a model without a term describing S(IV) autoxidation. As the largest total average occurs at 7.8 mM S(IV) this is likely to be due to S(IV) autoxidation being significant at this low S(IV) concentration. Above an S(IV) concentration of 0.0324 M the quality of fit is relatively independent of concentration. This could be because S(IV) autoxidation occurs only to a small and essentially constant extent, or because the S(IV)-catalysed Maillard reaction becomes the dominant pathway for the glucose-glycine-S(IV) reaction above this concentration and this pathway is relatively well described in the model of the reaction.

Using the original values of the constants shown in table 4.1.3, total, free and reversibly bound S(IV) concentrations were calculated. These are shown with the corresponding experimental values, when glucose concentration was varied, in Figs.4.1.4-4.1.8. It can be seen that the agreement between predicted and observed values is very poor, the calculated sum of the means, total plus free S(IV), being 1773.7 (1525.4 + 248.4). After adjustment of the original constants to obtain the maximum agreement between predicted and observed values of S(IV) concentration, the sums of the means calculated using the data in tables 4.1.4-4.1.6 are133.2 (i.e. 99.8 + 33.4), 53.0 (i.e. 39.3 + 13.7) and 106.6 (i.e. 91.0 + 15.6) per reaction mixture, when the concentrations of glucose, glycine and S(IV) respectively are varied. The lowest value or best fit is obtained when the

concentration of glycine is varied and this could be because the extent of autoxidation varies less in these reaction mixtures, than in mixtures where the concentrations of glucose or S(IV) were varied.

Table 4.1.3 shows that when glucose and glycine concentrations are varied, the value of the rate constant for S(IV) autoxidation is much larger than any of the other constants. In the first instance it seems as if by far the most important route for irreversible S(IV) loss will be by S(IV) autoxidation. However, the numerator of equation 4.1.7 involves a constant term of value 10^{-5}. When k_5 is multiplied by this term, the resultant value is of similar magnitude to the other rate constants. It can also be seen that in each case the constants giving the best fit are significantly different from the values used at the start of the fitting procedure. The values obtained are also dependent on the reagent being varied. If the computer model accurately described all the processes occurring in the glucose-glycine-S(IV) reaction, then it would be expected that only the rate constant for S(IV) autoxidation would have changed, owing to the variable extent to which this process occurs, in order to obtain a good fit. The fact that this is not the case suggests that the proposed mechanism for the reaction is incomplete and, therefore, constants require adjustment to compensate for the simplistic description of processes occurring in the reaction from the model. However, by adjustment of the constants it is possible to obtain a good fit of calculated values to the characteristic concentration/time profile for the glucose-glycine-S(IV) reaction, which suggests that the mechanism proposed so far is reasonable. It was shown in section 3.1.2.2, that the rate of irreversible S(IV) loss during the constant rate phase of the glucose-glycine-S(IV) reaction is dependent on whether or not the reaction mixture pH is maintained. There is an acid producing process occurring in the

glucose-glycine-S(IV) reaction and a process that is dependent on pH in the range 3.5-5.5; these may or may not be one and the same reaction. The explicit description of the acid producing process is absent from the model, whereas the pH-dependent process may constitute one of the reaction steps. If the model was improved to account for acid liberation and the pH dependency of the reaction, this may negate the need to adjust the constants in order to maximise the agreement between theoretical data and experimental results. On the other hand, if DH were to be formed directly from glucose this would constitute a process that would require inclusion in the kinetic model and might account for the observed difference between theory and experiment. The decomposition of hexoses such as D-glucose to 5-(hydroxymethyl)-2-furaldehyde in acid solution and to metasaccharinic acid in alkali represent two well known reactions (Feather and Harris, 1970). It is generally accepted (Speck, 1958; Anet, 1964) that DH or an enolic derivative thereof functions as an important intermediate in the formation of both compounds. In addition, Buera *et al* (1987) stated that when a sugar-amino acid solution at $a_w = 0.90$ is heated to 55 °C, the resultant browning is due to the contribution of two reactions, namely, Maillard browning and caramelisation. However, it was found that in a glucose-glycine system at pH 4.0 or 6.0, the contribution of caramelisation towards browning could be neglected. This is in agreement with the findings of this investigation, as from Fig.3.1.12 it can be seen that a plot of reaction rate for the S(IV)-inhibited Maillard reaction *vs* glycine concentration goes through the origin. This indicates that no significant reaction occurs in the absence of glycine at pH 5.5 and 55 °C.

4.2. Prediction of the concentrations of total, free and reversibly bound S(IV) at reduced water activity, using the kinetic model of the S(IV)-inhibited Maillard reaction.

4.2.1. Introduction.

By comparing Figs.4.1.4-4.1.8 to Figs.4.1.10-4.1.29, it can be seen that an acceptable level of agreement between predicted and observed values of S(IV) concentration is obtained after the original values of the rate and equilibrium constants, shown in table 4.1.3, are adjusted. However, as the preservative is present in foods of $a_w < 1$, it is important to see if such a level of agreement can be obtained at significantly reduced a_w. The quality of the fit may give useful information about the mechanism of the reaction at reduced a_w and an indication of the usefulness of this method for the measurement of the progress of the uncatalysed and S(IV)-catalysed Maillard browning reactions may be obtained.

4.2.2. Fitting theoretically generated data to experimental results obtained at reduced water activity.

The combinations of constants that gave the best correspondence between theoretical and experimental data for mixtures of varying S(IV) concentration at $a_w \approx 1$ and $a_w = 0.78$-0.79 are shown in table 4.2.1; the measure of fit for the data at reduced a_w being given in table 4.2.2. Theoretically generated, using adjusted rate and equilibrium constants, and experimentally obtained values of total, free and reversibly bound S(IV), at a_w 0.78-0.79, are shown as a function of time in Figs.4.2.1-4.2.5.

Constant	Value at water activity 1.0	Value at water activity 0.78-0.79
$k_1/M^{-1}h^{-1}10^{-4}$	1.91	5.34
$k_2/M^{-2}h^{-1}10^{-3}$	7.09	19.21
$k_3/h^{-1}10^{-4}$	5.10	5.10
$k_4/M^{-1}h^{-1}10^{-3}$	2.10	2.10
$k_5/M^{2}h^{-1}10^{-4}$	0.00	0.00
K_1/M	1.00	1.33
$K_2/M\,10^{-1}$	2.80	2.80
$K_3/M\,10^{-4}$	6.60	6.60

Table 4.2.1. Values of rate and equilibrium constants required to maximise the agreement between experimentally measured and theoretically calculated values of total, free and reversibly bound S(IV), for mixtures of composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0078-0.0830 M at a_w = 0.78-0.79.

During the first 100 h of the reaction, experimental and theoretical values show a high degree of agreement in each reaction mixture (Figs.4.2.1-4.2.5) except for the mixture containing 0.0153 M S(IV). After this time small deviations are observed, presumably because the model does not accurately describe all the processes occurring in the reaction.

The value of the dissociation constant for glucose hydroxysulphonate used to maximise the agreement between theoretical and experimental concentrations of S(IV) at $a_w \approx 1$ was 1.00 M (table 4.1.3), whereas at a_w = 0.78-0.79 this value was 1.33 M (table 4.2.1). The latter value corresponds exactly to that given by Wedzicha (1984a) infering that the adduct is less stable in glucose-glycine-S(IV) mixtures containing 46.9% w/w glycerol than in reaction mixtures not containing the polyol. Apart from this the only

S(IV) /M	Average squared deviation between calculated and measured values of			total average
	Total S(IV)	Free S(IV)	Reversibly bound S(IV)	
0.0153	78.5	8.1	48.9	135.6
0.0332	11.7	1.8	20.4	33.9
0.0503	18.8	3.6	27.8	50.1
0.0648	11.8	9.9	2.7	24.5
0.0819	16.5	8.4	16.5	41.5
MEAN	27.5	6.4	23.3	

Table 4.2.2. Average squared deviations between theoretical and experimental values of total, free and reversibly bound S(IV); total average squared deviations and mean of the average squared deviations for reaction mixtures of composition [glucose] = 1.0 M, [glycine] = 0.5 M, [S(IV)] = 0.0153-0.0819 M at a_w = 0.78-0.79.

difference between the constants at $a_w \approx 1$ and a_w 0.78-0.79 are the values of k_1 and k_2, indicating that the rate of DH formation is affected most by the change in a_w. However, as DH formation is the first rate determining step in the glucose-glycine-S(IV) reaction this is not unexpected.

The rate constants k_1 and k_2 refer to steps that occur early on in the uncatalysed and S(IV)-catalysed Maillard reactions. Values of k_1 and k_2 were obtained from the plot of rate *vs* initial S(IV) concentration at $a_w \approx 1$, these being 5.5×10^{-5} $M^{-1}h^{-1}$ and 2.0×10^{-3} M^{-2} h^{-1} respectively; each of these constants is multiplied by a factor of 3.5 in order to obtain the best fit between theoretical data and experimental results. The values of k_1 and k_2 obtained graphically by plotting rate *vs* $[S(IV)]_0$ at a_w 0.78-0.79 were

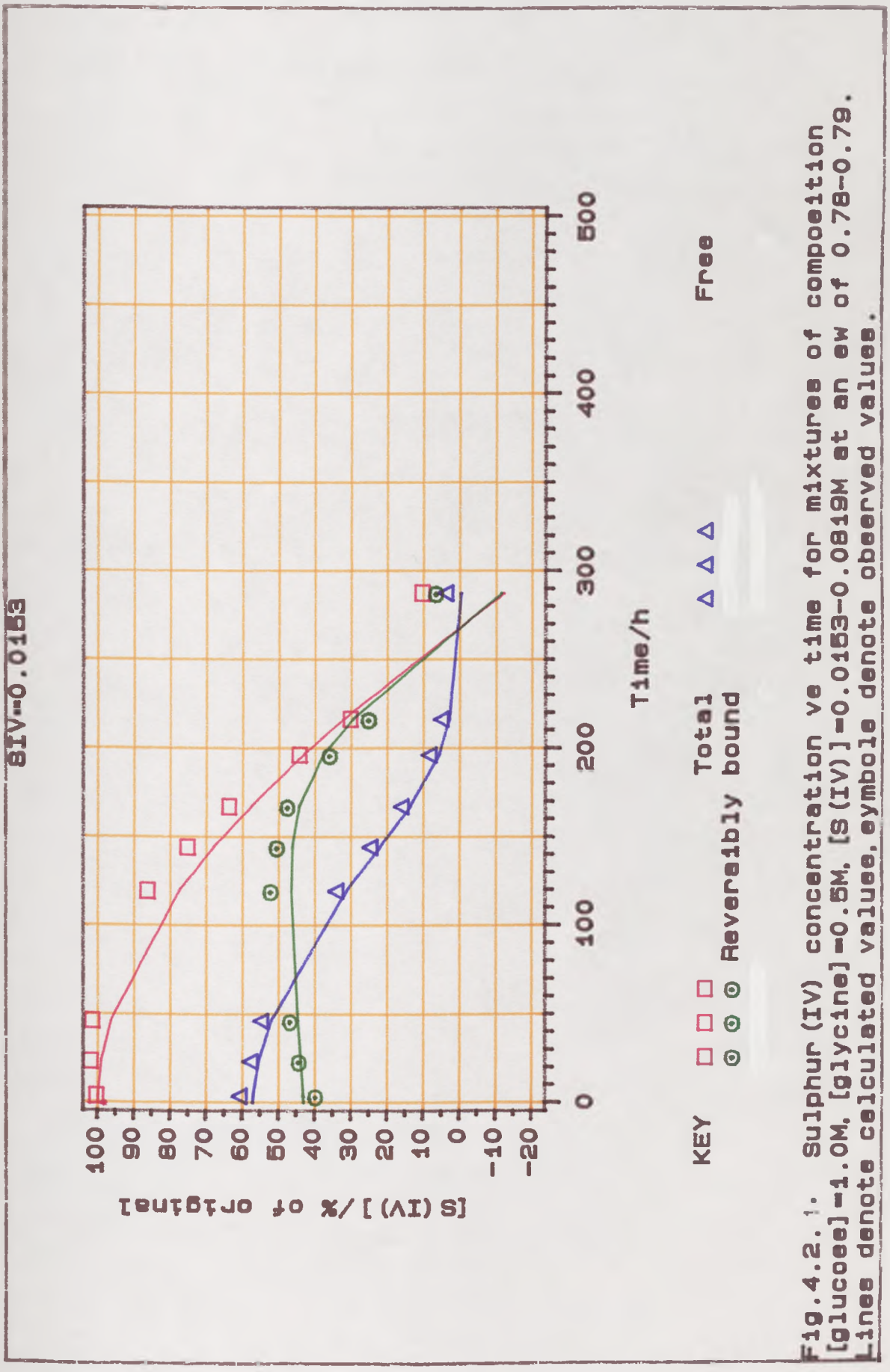


Fig.4.2.1. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=-0.0153-0.0819M at an sw of 0.78-0.79. Lines denote calculated values, symbols denote observed values.

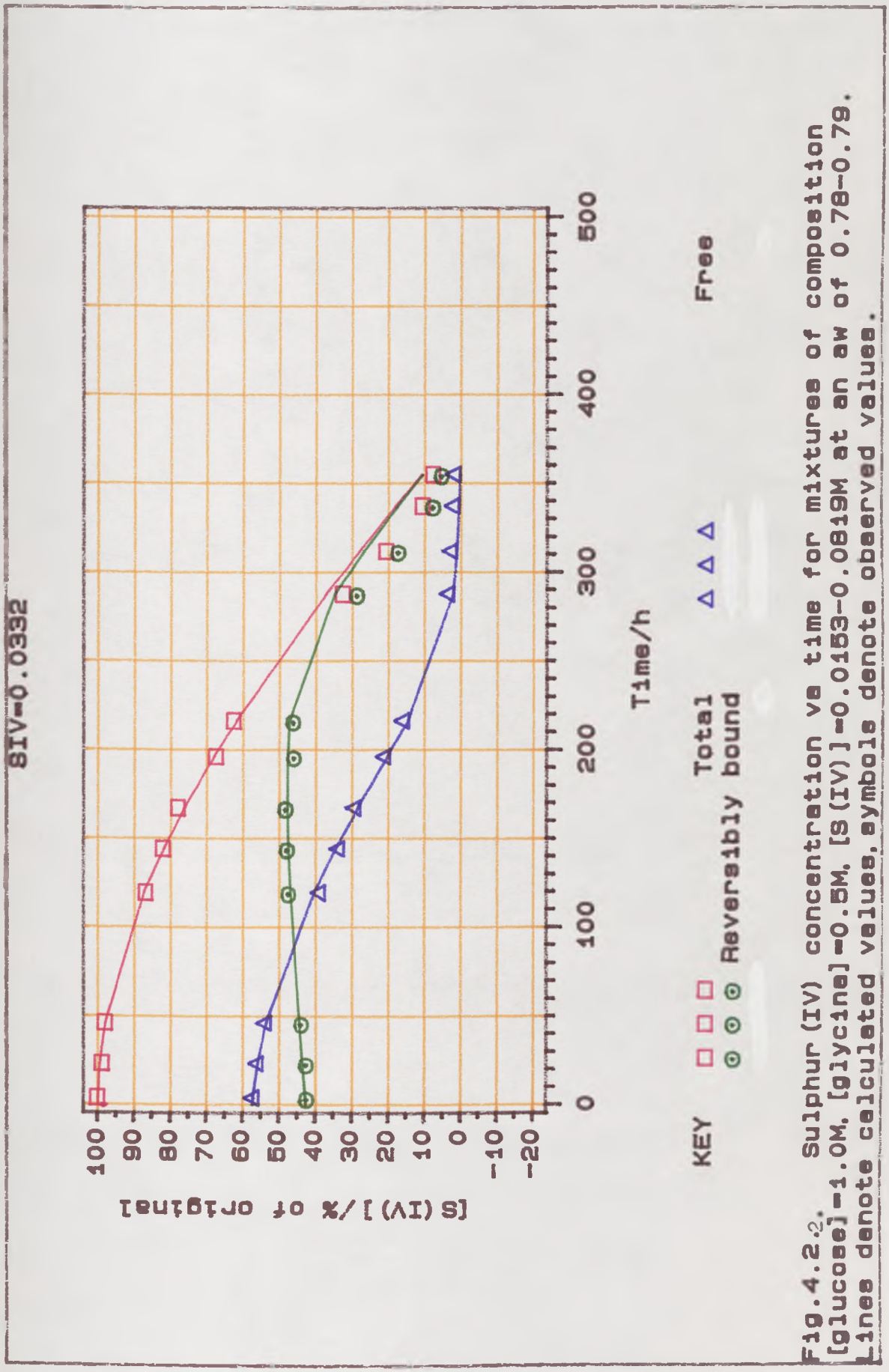


Fig.4.2.2. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0153-0.0819M at an aw of 0.78-0.79. Lines denote calculated values, symbols denote observed values.

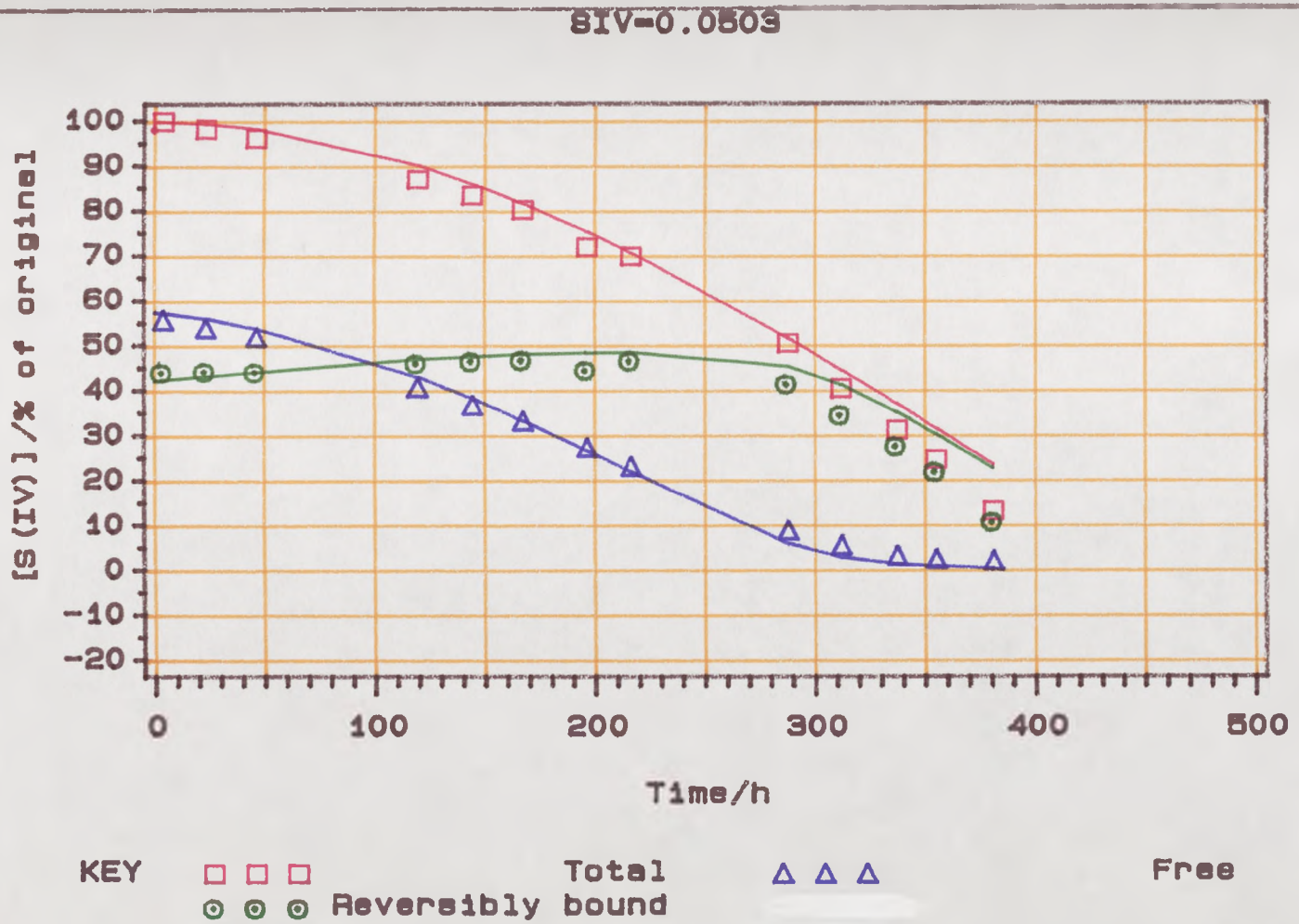


Fig.4.2.3. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0153-0.0819M at an aw of 0.78-0.79. Lines denote calculated values, symbols denote observed values.

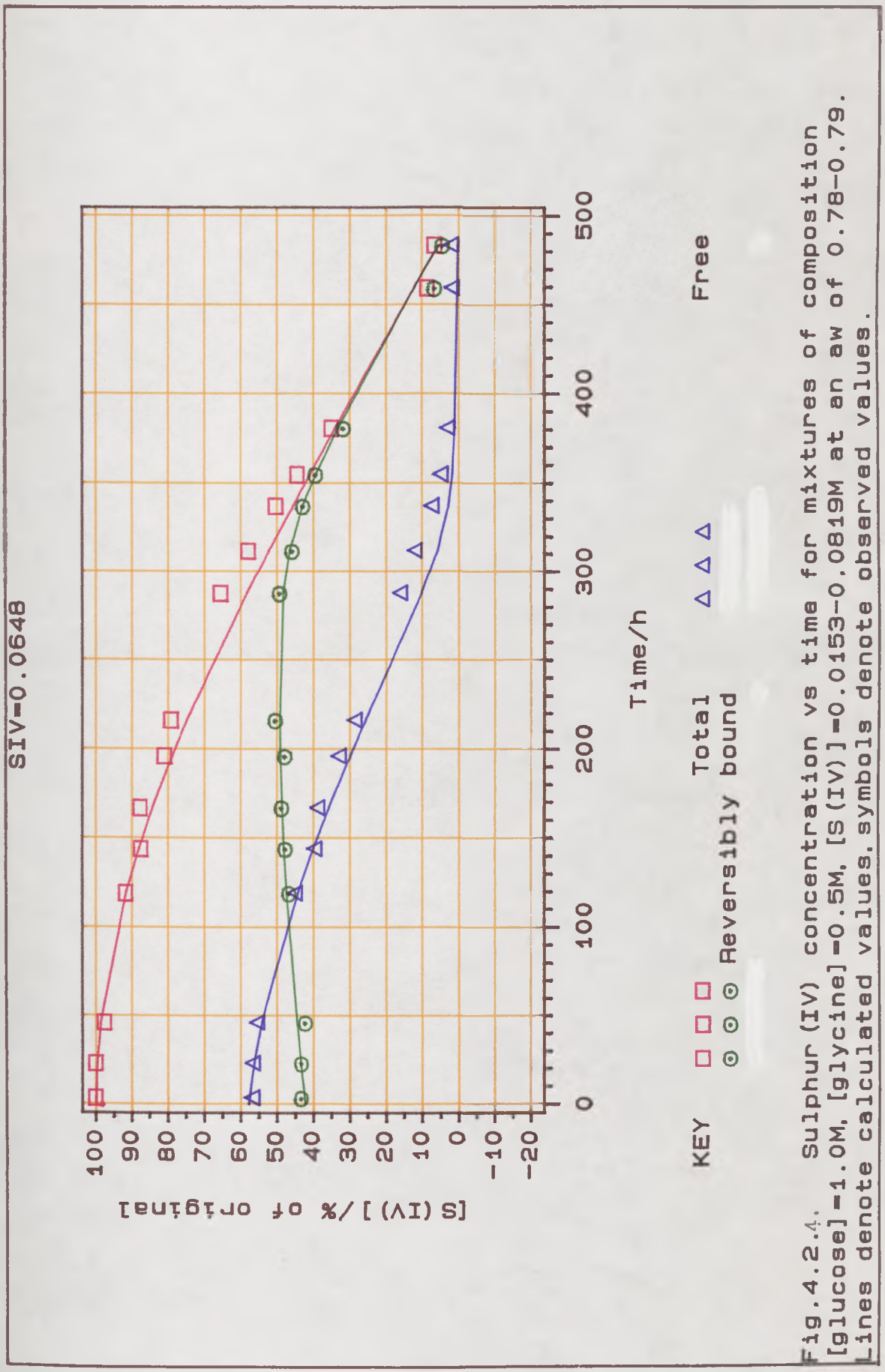


Fig.4.2.4. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0153-0.0819M at an aw of 0.78-0.79. Lines denote calculated values, symbols denote observed values.

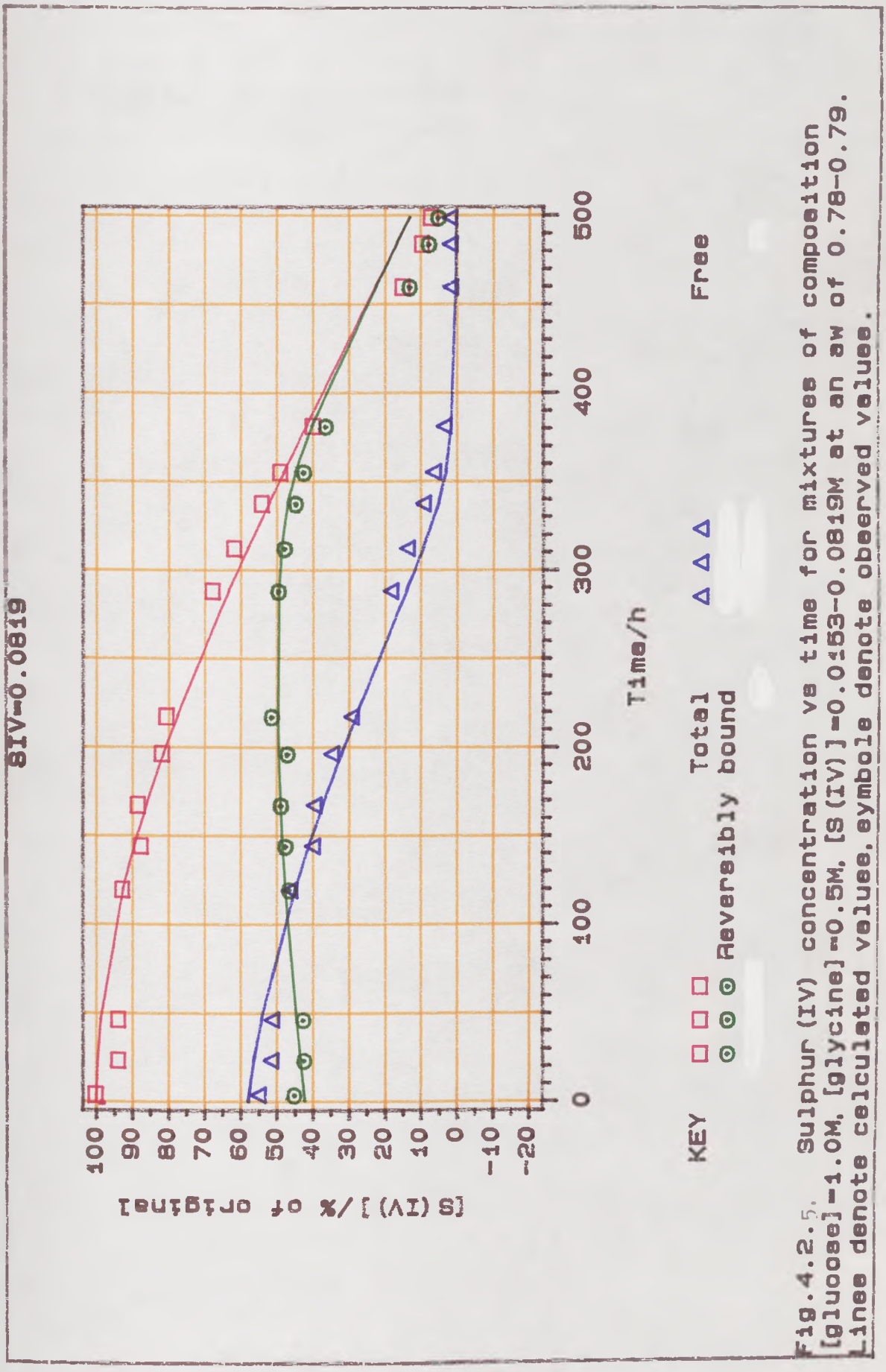


Fig.4.2.5. Sulphur(IV) concentration vs time for mixtures of composition [glucose]=1.0M, [glycine]=0.5M, [S(IV)]=0.0153-0.0819M at an aw of 0.78-0.79. Lines denote calculated values, symbols denote observed values.

1.3×10^{-4} $M^{-1}h^{-1}$ and 4.7×10^{-3} $M^{-2}h^{-1}$ respectively, each of these being multiplied by a factor of 4.05 to obtain the best fit. The amount by which these rate constants are adjusted in order to obtain values which, in conjunction with the other constants, give a good fit of theoretical data to experimental results is very similar in both cases, the small difference in scaling could be at least partly due to experimental error. The similarity in the scaling factors means that the relative contribution from k_1 and k_2 towards the overall fit remains essentially constant as the a_w changes from 1 to 0.78-0.79. The measured intercept and gradient of plots of rate *vs* $[S(IV)]_0$ are assumed to correspond to the rate constants k_1 and k_2 respectively. However, this may not be the case. For example, the intercept could be an overall measure of the rate of a number of inseparable processes. But if this were true, then, the intercepts obtained at $a_w \approx 1$ and 0.78-0.79 would probably require different scaling factors in order to obtain a fit, because it is likely that the supposed processes would be affected to different extents as the a_w is reduced. In addition, k_1 and k_2 are the constants for rate-determining processes in the glucose-glycine-S(IV) reaction. If this were not so, presumably there would be little or no change in the magnitude of the experimentally measured constants with reduced a_w. Hence, a larger scaling factor would be required for the rate constants at reduced a_w than the scaling factor at $a_w \approx 1$ in order to obtain a fit, as the measured rate of irreversible S(IV) loss is greater at this reduced a_w. This indicates that we are justified in assuming that the measured intercept and gradient correspond to the rate constants k_1 and k_2 and that these processes are rate-determining.

From table 4.2.2 it can be seen that, as in tables 4.1.4-4.1.6, the mean of the average squared deviations for free S(IV) is lower

than that for reversibly bound S(IV); showing that the fit of theoretical values to experimental values for free S(IV) is superior to the fit for reversibly bound S(IV). The values of the dissociation constants of the hydroxysulphonates of DH and DSH are significantly different to the literature values. Again, as in table 4.1.6, the poorest fit is obtained in the mixture containing the lowest S(IV) concentration. However, the calculated sums of the means, total plus free S(IV), at $a_w \approx 1$ and 0.78-0.79 are 106.6 (91.0 + 15.6) and 33.9 (i.e. 27.5 + 6.4) respectively when S(IV) concentration is varied. The fit is far better at reduced a_w than it is at $a_w \approx 1$, possibly because S(IV) autoxidation is completely suppressed in reaction mixtures containing 46.9% w/w glycerol.

4.3. Conclusion.

The kinetic model of the S(IV)-inhibited Maillard reaction shown in Fig.4.1.1, is an improvement on the model of Wedzicha and Vacalis (1988) for two reasons. Firstly, it allows for autoxidation of S(IV) which was shown to occur (section 3.1.2.6) in reaction mixtures containing glucose, glycine and S(IV). As oxygen is likely to be in contact with sulphited foods, it is possible that some autoxidation of S(IV) will occur in such instances. Indeed, Wedzicha and Herrera-Viloria (in press) concluded that a small fraction of S(IV) incorporated into vegetables is converted into sulphate ion during dehydration. Therefore, the inclusion of S(IV) autoxidation renders the kinetic model a more accurate description of the processes likely to be occurring in a "real" food situation. Secondly, it was demonstrated by Wedzicha and Kaban (1986), that DH forms a hydroxysulphonate adduct in the presence of glycine. The value of the dissociation constant for DH hydroxysulphonate used in the present

kinetic model allows for this. On the other hand, Wedzicha and Vacalis found that in order to obtain a satisfactory fit of theoretical data to experimental results, such an adduct of DH has to be left out.

When the original constants at $a_w \approx 1$, shown in table 4.1.3, are adjusted to obtain the maximum agreement between predicted and observed values of S(IV) concentration. The calculated sums of the means, total plus free S(IV), of the average squared deviations were 133.2 (99.8 + 33.4), 53.0 (39.3 + 13.7) and 106.6 (91.0 + 15.6) per reaction mixture, when the concentrations of glucose, glycine and S(IV) were varied respectively. When the original unadjusted constants were used to predict S(IV) concentrations a poor fit was obtained, this being illustrated in Figs.4.1.4-4.1.8, the sum of the means of the average squared deviations being 1773.7 (1525.4 + 248.4) when the concentration of glucose was varied. The best correspondence between theoretical data, calculated using the model in Fig.4.1.1, and experimental results is obtained when glycine concentration was varied. This is probably because in these mixtures the glucose concentration is constant and hence also the rate of S(IV) autoxidation; whereas when the concentrations of glucose or S(IV) were varied, the rate of S(IV) autoxidation is harder to quantify in the absence of direct measurements. For reaction mixtures containing glucose, glycine and S(IV), Wedzicha and Vacalis (1988) obtained a value of 41.1 for the sum of the means of the average squared deviations when S(IV) concentration was varied at $a_w \approx 1$. This is significantly better than the present value for the same variable, but only slightly better than the value obtained for glycine. However, the model used by these previous workers does not account for S(IV) autoxidation or the existence of DH hydroxysulphonate. In this work when the concentration of S(IV) was varied at a_w 0.78-0.79, a

considerable improvement in the quality of the fit was obtained. The sum of the means of the average squared deviations was 33.9 (27.5 + 6.4) at a_w 0.78-0.79, as compared to 106.6 (91.0 + 15.6) at $a_w \approx 1$. This significant improvement may have been brought about by the suppresion of S(IV) autoxidation in reaction mixtures containing 46.9% w/w glycerol. The improved fit is also better than that obtained by Wedzicha and Vacalis (1988). This could be due partly to the maintenance of reaction mixture pH in the current work, reducing the affect of H^+ liberation on the rate of the glucose-glycine-S(IV) reaction to a minimum. It was stated in section 3.1.2.3, that the values of k_1 and k_2 correspond to the rate constants for steps that occur early on in the uncatalysed and S(IV)-catalysed Maillard reactions. It was shown in section 1.3.1 that, in comparison to other methods that have been used to follow the progress of Maillard browning, studying the kinetics of the S(IV)-inhibited Maillard reaction offers a good method of obtaining the rate of the initial stages of the Maillard reaction. In addition, the rate of the initial stages of the S(IV)-catalysed Maillard reaction can also be obtained.

The model as shown in Fig. 4.1.1 is thought to be correct, but as it stands it is simplistic. It is evident that there is at least one process occurring in the glucose-glycine-S(IV) reaction, that is not described accurately or at all in the model. This is because the experimentally obtained rate and equilibrium constants used at the start of the fitting procedure have to be adjusted in order to increase the theoretical reaction rate, so that an acceptable fit is obtained. It was found that during the first 100 h of the reaction, both at reduced a_w and $a_w \approx 1$, a good fit of theoretical data to experimental results was obtained only after this adjustment. Despite this, at longer times deviations start to occur. In section 3.2.5 it was stated that it is unlikely that, and

contrary to earlier expectations, $S_2O_5^{2-}$ could catalyse the glucose-glycine reaction; from the evidence presented it seems that SO_3^{2-} is the more likely catalytic S(IV) species. Indeed, Wedzicha and Vacalis (1988) suggested that SO_3^{2-} could catalyse the formation of the ketoseamine in the glucose-glycine-S(IV) reaction and this agrees with the findings of section 3.1.2.3. Here it was stated that production of MFG from the first Schiff's base is most likely to be rate-determining step. According to Hodge (1953) formation of MFG from the Schiff's base is generally regarded to proceed as shown in Figs.4.3.1 and 4.3.2.

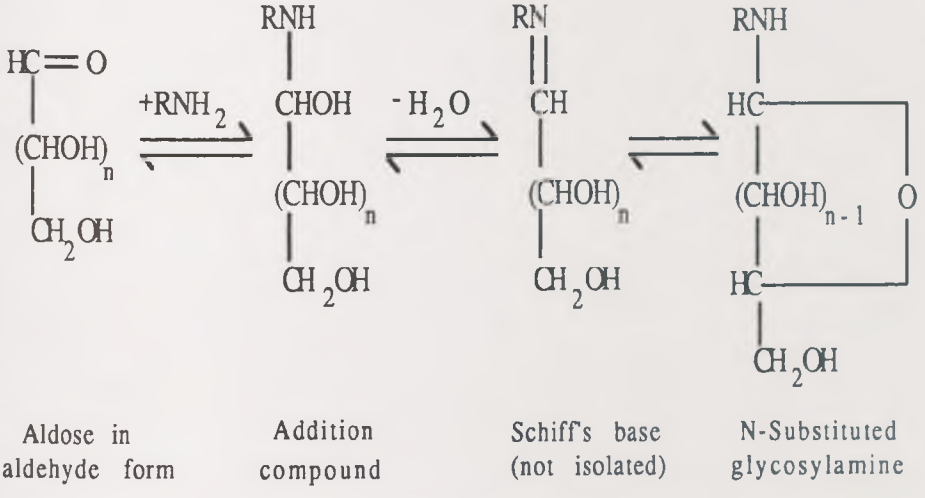


Fig.4.3.1. Glycosylamine (aldosylamine) production from an aldose and an amino acid during the early stages of the Maillard reaction.

The aldosylamine is unstable and undergoes a facile irreversible rearrangement, the Amadori rearrangement, to produce a more stable ketoseamine. This involves reversible protonation of the aldosylamine to form a cation of the Schiff's base, which loses a proton in an irreversible step to form an enol. The enol is in equilibrium with the ketoseamine which in the case of the glucose-glycine reaction, is MFG. The effect of SO_3^{2-} could be to facilitate the formation of the enol, by abstraction of a proton from

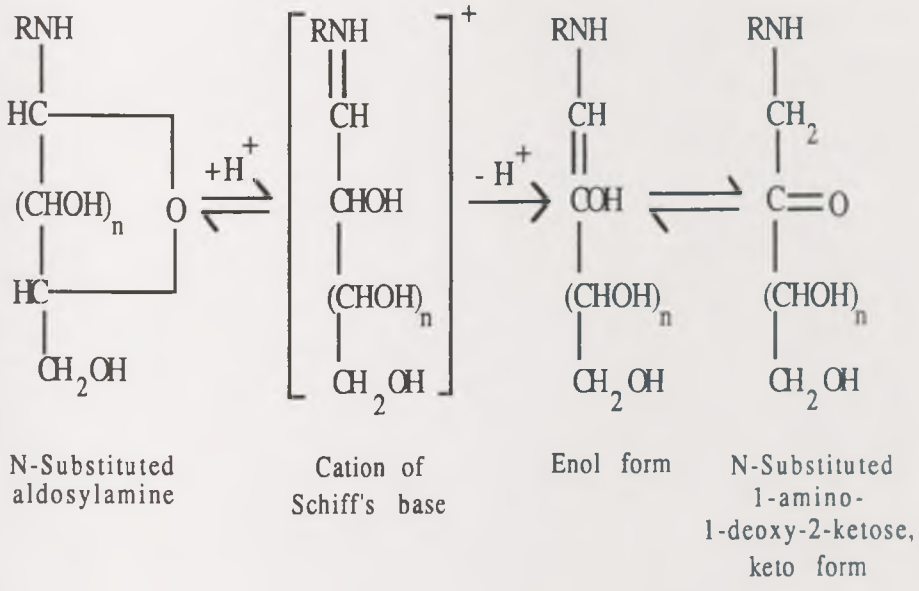


Fig.4.3.2. Ketoseamine production from the aldosylamine formed during the early stages of the Maillard reaction.

the cationic Schiff's base as shown in Fig.4.3.3.

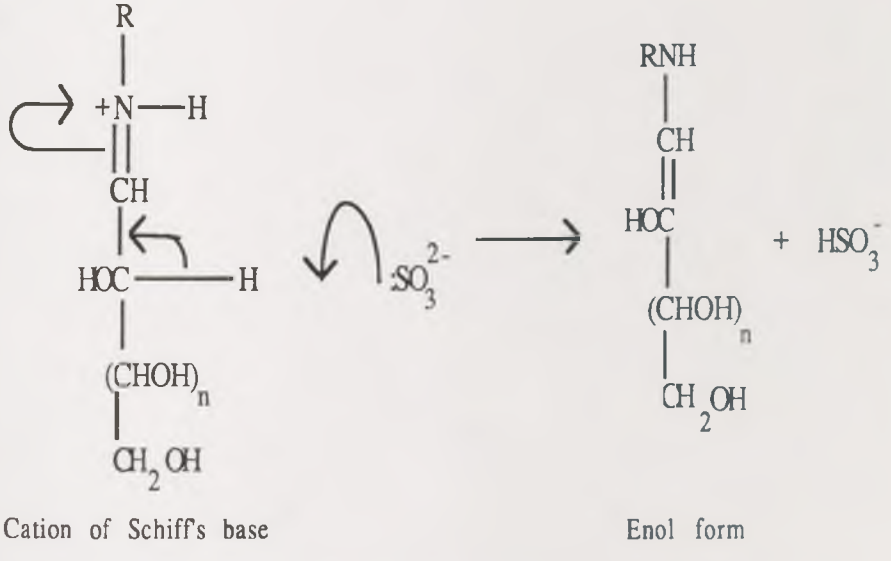


Fig.4.3.3. Proposed base catalysis of the Maillard reaction by SO_3^{2-} ion.

At pH 5.5 and 55 °C base catalysis by SO_3^{2-} would account for the first order effect of S(IV) on the rate of the glucose-glycine-S(IV) reaction. The formation of a transition state involving SO_3^{2-} and the cationic

Schiff's base would result in a reduction in the number of charged species and a delocalisation of charge over this activated complex, see Fig.3.2.24. Less solvation of the transition state would be required and hence this species would be favoured at low a_w, when the amount of water available for solvation is reduced. Base catalysis of the glucose-glycine reaction would also be in keeping with the findings of Potman and Van Wijk (1989). They stated that at pH 5.6 and 100 °C the first order catalysis of the Maillard reaction by phosphate was due the dihydrogen phosphate ion acting as a base and abstracting a proton during the Amadori rearrangement. With a pK of 7.18, HSO_3^- is neither a good acid nor a good base and at first sight it would seem that HSO_3^- is unlikely to be involved in acid-base catalysis. However, the pK of $H_2PO_4^-$ ion is 7.21. As the pK values of HSO_3^- and $H_2PO_4^-$ are of the same magnitude and it has been suggested that $H_2PO_4^-$ can base catalyse the Maillard reaction (Potman and Van Wijk, 1989), it is possible, therefore, that HSO_3^- could also base catalysis the Maillard reaction, although SO_3^{2-} seems the more likely catalyst. In addition, the reversible protonation of the aldosylamine would be susceptible to acid catalysis. Burton and McWeeny (1963) reported that the higher the level of acyclic sugar form the faster the rate of browning. The proportion of acyclic sugar form present was shown to be solvent dependent by Kaanane and Labuza (1989). However, no distinction has been drawn between cyclic and acyclic sugar forms in the present model because it is assumed that the proportion of acyclic form is relatively constant throughout the reaction. At 20 °C and pH 5.2-7.0 glucose is present predominantly in its cyclic form (Hayward and Angyal, 1977). However, the acyclic form of the aldose is usually referred to, as most studies (wertz *et al.*, 1981; Pigman and Anet, 1972) on the mutarotation of sugar agreed that the interconversion of

cyclic anomers of sugar proceeds via a central intermediate which is the aldehydo or keto form of sugar.

There are at least two processes by which acid is produced in the glucose-glycine-S(IV) reaction. One of these is proposed to be the result of S(IV)-autoxidation. The other occurs in the absence of S(IV) and involves glucose, glycine or a combination of the two. The second acid liberating process is not described explicitly in the model shown in Fig.4.1.1, however, it might be formed in one of the steps already in the model. The rate of irreversible S(IV) loss in glucose-glycine-S(IV) reaction mixtures containing 2 M glucose was shown to be dependent on whether or not reaction mixture pH is maintained (section 3.1.2.2). As S(IV)-autoxidation has been shown to be inhibited in a reaction mixture containing 2 M glucose, 0.5 M glycine and 0.0469 M S(IV), the effect of pH is unlikely to be due to a change in the rate of S(IV)-autoxidation. Instead, the effect is probably caused by at least one process occurring in the S(IV)-inhibited Maillard reaction of glucose, glycine and S(IV) that is pH dependent in the range 5.5-*c.* 4; this may or may not be the acid-producing glucose-glycine reaction. The pH dependence of the glucose-glycine-S(IV) reaction may be accounted for in three ways. Firstly, the fall in pH from 5.5 to *c.* 4 is likely to reduce the concentration of SO_3^{2-}, the suggested catalytic species, which would reduce the extent of catalysis and hence MFG formation. This would, therefore, account for the slower reaction rate observed when the pH of reaction mixtures is allowed to fall, in comparison to the rate observed if the pH is maintained. Secondly, it was stated in section 3.1.2.2, that the steady state concentration of DH could be obtained from the rate constants for DH formation and loss. Therefore, the lack of an effect of a drop in pH from 5.5 to *c.* 4 on this concentration,

could be because both of these steps are affected by pH to the same extent. A Schiff's base is formed prior to the formation of DH. In addition, Wedzicha and Kaban (1986) also suggested the involvement of a Schiff's base to account for the catalysis, by glycine, of the rate of DSH formation from DH. A drop in pH of the model system glucose-glycine-S(IV) may lead to protonation of the amine group of glycine, thus inhibiting nucleophilic attack on a carbonyl group of DH or glucose by the amino group. This would account for both the increase in reaction rate when the pH is maintained as compared to the rate obtained when the pH is allowed to fall and also the lack of an effect of the pH change on the steady state DH concentration. Thirdly, the first step of the facile irreversible rearrangement of the unstable aldosylamine, formed from glucose and glycine, to produce the more stable ketoseamine involves reversible formation of a cation of the Schiff's base by protonation of the aldosylamine. This step will be pH-dependent and may, therefore, be rate determining. It is speculated that reversible protonation of the Schiff's base of DH occurs, and could be pH dependent in the same way. However, as this occurs after the proposed rate-determining step, it is unlikely to account for the effect of pH on the glucose-glycine-S(IV) reaction. The second and third explanations suggest that the glucose-glycine Maillard reaction is pH dependent in the range 5.5-*c.* 4. This is in contrast to the findings of Wolfrom *et al* (1953), who stated that the Maillard reaction of xylose and glycine is independent of pH in the range pH 3-7. Clearly, confirmation of the pH dependence of the glucose-glycine reaction is required.

If the production of MFG from the cationic Schiff's base is the rate-determining step in the glucose-glycine reaction, then it should be possible to explain the effect of a_w on the rate of the reaction by

reference to this step. According to Labuza *et al* (1977), the occurrence of a rate of browning maximum as a_w increases from zero is probably due to four factors. (1) Water acting through product inhibition by retarding the initial glycosylamine reaction. (2) Water enhancing deamination reactions later on in the sequence as stated by Reynolds (1963). (3) Probably most importantly, water diluting the reactant components (Eichner, 1975; and Labuza, 1971). (4) The aqueous phase becoming less viscous with increasing water content and hence allowing greater mobility. Thus, as a_w is increased from zero, factors 2 and 4 serve to increase the rate which reaches a maximum and then begins to fall as factors 1 and 3 dominate. If the rate-determining step of the glucose-glycine reaction is MFG formation from the cationic Schiff's base, then reaction rate will be dependent on the concentration of this Schiff's base. It is generally accepted that the initial step in the glucose-glycine reaction is amine assisted dehydration. An addition compound is formed as a result of the nucleophilic attack on the carbonyl group of glucose by the amine group of glycine. This compound undergoes a reversible condensation producing the first Schiff's base and a mole of water (see Fig.4.3.1). Hence, as the concentration of water increases with a_w, the initial formation of this Schiff's base will be retarded as a result of the law of mass action. Therefore, the concentration of the cationic Schiff's base will also be reduced at every instant and so the reaction rate.

Improvements in the model along the lines described above may negate the need to adjust the constants, as compensation for the slower theoretical rate may not be required. Hence, the values used to obtain a satisfactory fit may be those obtained experimentally; if not, any adjustment of the constants may give an indication of the areas in which the model could be improved.

REFERENCES.

Adamu, D. J. M. (1986). The relationship between the reactivities of sulphur(IV) oxospecies in vegetables to their composition under processing conditions. Ph.D. Thesis, University of Leeds.

Anet, E. F. L. J. (1958). A new reaction of aldoses with primary amines and its significance for non-enzymic browning reactions. *Chem. Ind. (London)*, 1438-9.

Anet, E. F. L. J. (1959a). Chemistry of nonenzymic browning. VII. Crystalline di-D-fructose-glycine and some related compounds. *Aust. J. Chem.*, **12**, 280-7.

Anet, E. F. L. J. (1959b). Chemistry of nonenzymic browning. X. Difructoseamino acids as intermediates in browning reactions. *Aust. J. Chem.*, **12**, 491-6.

Anet, E. F. L. J. (1960). Degradation of carbohydrates. I. Isolation of 3-deoxyhexosones. *Aust. J. Chem.*, **13**, 396-403.

Anet, E. F. L. J. (1962). Degradation of carbohydrates. III. Unsaturated hexosones. *Aust. J. Chem.*, **15**, 503-9.

Anet, E. F. L. J. (1964). 3-deoxyglycosuloses (3-deoxyglycosones) and the degredation of carbohydrates. *Advan. Carbohyd. Chem.*, **19**, 181.

Arkhipova, G. P. and Chistyakova, I. I. (1971). Spectrophotometric study of aqueous bisulphite and pyrosulphite solutions. *Zh. Prikl. Khim.*, **44**, 2193-8. Through *Chemical Abstracts*, 76, 38033v.

Arnold, R. G. (1973). Inhibition of heat induced browning of milk by L-cystein. *J. Dairy Sci.*, **52**, 1857.

Bäckström, H. (1934). The chain mechanism in the autoxidation of sodium sulphite solutions. *Z. Phys. Chem.*, **B25**, 122-38.

Baird, N. C. and Taylor, K. F. (1981). Ab initio MOcalculations for the oxides, oxyacids, and oxyanions of S(IV) and S(VI). *J. Comput. Chem.*, **2**, 225.

Barrie, L. A. and Georgii, H. W. (1976). An experimental investigation of the absorption of sulphur dioxide by water droplets containing heavy metal ions. *Atmospheric Environment*, **10**, 743-9.

Basset, H. and Henry, A. J. (1935). The formation of dithionate by the oxidation of sulphurous acid and sulphites. *J. Chem. Soc.*,

914-29.

Basset, H. and Parker, W. (1951). The oxidation of sulphurous acid. *J. Chem. Soc.*, 1540-60.

Beemer, F. A. and Delleman, J. W. (1980). Combined deficiency of xanthine oxidase and sulphite oxidase; Opthalmological findings in a 3-week-old girl. *Metab. Pediat. Opthalmol.*, **4**, 49.

Beilke, S. and Lamb, D. (1975). Remarks on the rate of formation of bisulphite ions in aqueous solutions. *A. I. Ch. E. Journal*, **21**, 402-4.

Bell, R. P. (1973). *The proton in chemistry*, 2nd edn, Cornell University Press, Ithaca, New York.

Bello, J. and Bello, H. R. (1976). Chemical modification and cross linking of proteins by impurities in glycerol. *Arch. Biochem. Biophys.*, **172**, 608-610.

Benzing-Purdie, L. M. and Ratcliffe, C. I. (1986). A study of the Maillard reaction by ^{13}C and ^{15}N CP-MAS NMR: Influence of time, temperature and reactants on major products. In *Amino-carbonyl Reactions in Food and Biological Systems*, ed. Fujimaki, M., Namiki, M. and Kato, H. Elsevier Science Publishers, Amsterdam, pp 193-205.

Benzing-Purdie, L. M., Ripmeester, J. A. and Preston, C. M. (1983). Elucidation of the nitrogen forms in melanoidins and humic acid by nitrogen-15-cross polarisation-majic angle spinning nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.*, **31**, 913-915.

Bigelow, Z. (1898). *Phys. Ch.*, **26**, 493.

Bourne, D. W. A., Higuchi, T. and Pitman, I. H. (1974). Chemical equilibria in soluions of bisulphite salts. *J. Pharm. Sci.*, **63**, 865-8.

Britton, H. T. S. and Robinson, R. A. (1932). The use of the glass elecrode in titremetric work and precipitation reactions. The application of the principle of the solubility product to basic precipitates. *Trans. Faraday Soc.*, **28**, 531-45.

Buera, M. D. P., Chirife, J., Resnik, S. L. and Wetzler, G. (1987). Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. *J. Food Sci.*, **52**, 4, 1063-1067.

Bünau, G. and Eigen, M. (1962). On the kinetics of the iodine-sulphite reaction. *Z. Phys. Chem.*, **32**, 27-50.

Burton, H. S. and McWeeny, D. J. (1963). Non-enzymatic browning reactions: consideration of sugar stability. *Nature*, **197**, 266-268.

Burton, H. S., McWeeny, D. J. and Biltcliffe, D. O. (1962). Sulphites and aldose-amino reactions. *Chem. Ind. (London)*, 219-221.

Burton, H. S., McWeeny, D. J. and Biltcliffe, D. O. (1963). Non-enzymic browning: the role of unsaturated carbonyl compounds as intermediates and of SO_2 as an inhibitor of browning. *J. Sci. Food Agric.*, **14**, 911-920.

Buttery, R. G. and Ling, L. C. (1974). Alkylthiazoles in potato products. *J. Agric. Fd. Chem.*, **22**, 912-914.

Charbonnier, F., Faure, R. and Loiseleur, H. (1977). Refinement of the structure of tetraaquabis(methanesulphonato)copper(II) $[Cu(CH_3SO_3)_2(H_2O)_4]$. Evidence for a plane of symmetry in the methanesulphonato coordinate. *Acta Cryst.*, **B33**, 1845-8.

Chertkov, B. A. (1960). Oxidation of calcium sulfite in the process of scrubbing sulfur dioxide from flue gases. *Zhur. Priklad. Khim.*, **33**, 1708-14.

Chirife, J. and Fontan, C. F. (1980). A study of water activity lowering behaviour of polyethylene glycol in the intermediate moisture range. *J. Food Sci.*, **45**, 1717-19.

Chirife, J., Fontan, C. F. and Benmergui, E. A. (1980). The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. IV. a_w prediction in non electrolyte solutions. *J. Fd Technol.*, **15**, 59-70.

Choi, R. P., Koncus, A., O'Mally, C. and Fairbanks, B. (1949). A proposed method for the determination of color of dry products of milk. *J. Dairy Sci.*, **32**, 580.

Cole, S. J. (1967). The Maillard reaction in food products - carbon dioxide production. *J. Food Sci.*, **32**, 245.

Colthup, N. B., Daly, L. H. and Wiberley, S. E. (1975). *Introduction to infrared and raman spectroscopy*. Second edition. New York. San Francisco. London. Academic Press. pp. 111-112.

Connick, R. E., Tam, T. M. and Deuster, E. V. (1982). Equilibrium constant for the dimerization of bisulfite ion to form $S_2O_5^{2-}$. *Inorg. Chem.*, **21**, 103-107.

CRC. (1981-1982). *CRC handbook of chemistry and physics. A ready*

reference book of chemical and physical data. 62nd edition. Editor: Weast, R. C. Associate editor: Astle, M. J. CRC Press, INC.

Crowe, L. K., Jenness, R. and Coulter, S. T. (1948). *J. Dairy Sci.* **31**, 595.

Darykina, M. I. (1954). Action of phenolic inhibitors on the rate of oxidation of sodium bisulfite. *Trudy Moskov. Inzh.-Ekon. Inst., Khim. Ikhim. Proizvodstvo.* **1**, 143-5.

Davis, A. R. and Chatterjee, R. M. (1975). A vibrational-spectroscopic study of the SO_2·H_2O system. *J. solution. Chem.*, **4**, 399-412.

Dawes, I. W. and Edwards, R. A. (1966). Methyl substituted pyrazines as volatile reaction products of heated aqueous aldose-amino acid mixtures. *Chem. Ind. (london)*, 2203.

Deveze, D. and Rumpf, P. (1964). Spectrophotometric study of aqueous solutions of SO_2 in various acid buffers. *Compt. Rend.*, **258**, 6135-8.

Dulak, L., Chiang, G. and Gunnison, A. F. (1984). A sulphite oxidase-deficient rat model: reproduction toxicology of sulphide in the female. *Food Chem. Toxicol.*, **22**, 599-607.

Edwards, J. O. and Pearson, R. G. (1962). The factors determining nucleophilic reactivities. *J. Amer. Chem. Soc.*, **84**, 16-24.

Eichner, K. (1975). The influence of water content on non-enzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. In *Water relations of foods* : Proceedings of an international symposium held in Glasgow. Edited by Duckworth, R. B. Academic press inc (London). 417-434.

Eichner, K. and Karel, M. (1972). The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. *J. Agr. Food Chem.*, **20**, 218-223.

Einarsson, H. (1987a). The effect of time, temperature, pH and reactants on the formation of antibacterial compounds in the Maillard reaction. *Lebensm. Wiss. u Technol.*, **20**, 51-55.

Einarsson, H. (1987b). The effect of pH and temperature on the antibacterial effect of Maillard reaction products. *Lebensm. Wiss. u Technol.*, **20**, 56-58.

Ellis, A. J. and Anderson, D. W. (1961). Effect of pressure on the

first acid dissociation constants of 'sulphurous' and phosphoric acids. *J. Chem. Soc.*, 1765-7.

Ellis, G. P. and Honeyman, J. (1955). Glycosylamines. *Adv. Carbohydr. Chem.*, **10**, 95-168.

Falk, M. and Giguere, P. A. (1958). On the nature of sulphurous acid. *Can. J. Chem.*, **36**, 1121-5.

Feather, M. S. (1981). Amine-assisted sugar dehydration reactions. *Prog. Food Nutr. Sci.*, **5**, 37-45.

Feather, M. S. (1989). D-Glucose-L-alanine interactions: their role in Maillard polymer formation and Strecker degradation reactions. *Food Technol.* **29** *(Protein qual. eaf, process).* 263-289.

Feather, M. S. and Harris, J. F. (1970). On the mechanism of conversion of hexoses into 5-(hydroxymethyl)-2-furaldehyde and metasaccharinic aicd. *Carbohyd. Res.*, **15**, 304-309.

Feather, M. S. and Huang, R. D. (1985). Maillard polymers derived from D-glucose-1- and -6-^{14}C, glycine-1- and -2-^{14}C, and C-1- and S-methyl-^{14}C-methionine. *J. Carbohydr. Chem.*, **4**, 363-368.

Feather, M. S. and Nelson, D. (1984). Maillard polymers derived from D-glucose, D-fructose, 5-(hydroxymethyl)-2-furaldehyde, and glycine and methionine. *J. Agric. Food Chem.*, **32**, 1428-1432.

Ferretti, A. and Flanagan, V. P. (1972). Steam volatile constituents of nonfat dried milk. The role of the Maillard reaction in staling. *J. Agric. Fd. Chem.*, **20**, 695-698.

Fridovich, I. and Handler, P. (1958). Xanthine oxidase. III. Sulphite oxidation as an ultrasensitive assay. *J. Biol. Chem.*, **233**, 1578-80.

Fridovich, I. and Handler, P. (1959). Utilisation of the photosensitised oxidation of sulphite for manometric actinometry. *Biochim. Biophys. Acta*, **35**, 546-7.

Fridovich, I. and Handler, P. (1961). Detection of free radicals generated during enzymic oxidations by the initiation of sulphite oxidation. *J. Biol. Chem.*, **236**, 1836-40.

Frost, A. A. and Pearson, R. G. (1961). *Kinetics and Mechanism. A study of homogeneous chemical reactions.* 2nd edn. New York. London., John Wiley and Sons, Inc.

Fujimaki, M., Homma, S., Arakawa, N. & Inagaki, C. (1979). Growth response of rats fed with a diet containing non-dialysable

melanoidins. *Agric. Biol. Chem.*, **43**, 497-503.

Fuller, E. C. and Crist, R. H. (1941). The rate of oxidation of sulphite ions by oxygen. *J. Amer. Chem. Soc.*, **63**, 1644-50.

Ghiron, A. F., Quack, B., Mawhinney, T, P. and Feather, M. S. (1988). Studies on the role of 3-deoxy-d-erythro-glucosulose (3-deoxyglucosone) in nonenzymatic browning. Evidence for involvement in a Strecker degradation. *J. Agric. Food Chem.*, **36**, 677-680.

Golding, R. M. (1960). Ultraviolet absorption studies of the bisulfite-pyrosulfite equilibrium. *J. Chem. Soc.*, 3711-16.

Gomyo, T., Kato, H., Udaka, K., Horikoshi, M. and Fujimaki, M. (1972). Chemical studies on melanoidins. Part III. Effects of heating on chemical properties of melanoidin prepared from glycine-xylose system. *Agric. Biol. Chem.*, **36**, 125-132.

Goodall, D. C. (1984). The formation of inorganic sulphur oxoanion systems from sulphur dioxide in non-aqueous solvents. *Food Chem.*, **4**, 399-412.

Green, L. R. and Hine, J. (1974). The pH independent equilibrium constants and rate constants for formation of the bisulphite addition compound of isobutyraldehyde in water. *J. Org. Chem.*, **39**, 3896-901.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards-A. Physics and Chemistry.* **81A**, 1, 89-96.

Greenstock, C. L. and Miller, R. W. (1975). The oxidation of tiron by superoxide anion. Kinetics of the reaction in aqueous solution and in chloroplasts. *Biochim. Biophys. Acta*, **396**, 11-16.

Griffiths, P. R. (1972). *Laboratory methods in infrared spectroscopy.* Second edition. Editors: Miller, R. G. J. and Stace, B. C. London. New York. Rheine. Heyden & Son Ltd. pp. 84-86.

Gunnison, A. F. (1981). Sulphite toxicity: a critical review of *in vitro* and *in vivo* data. *Food Cosmet. Toxicol.*, **19**, 667-82.

Gunnison, A. F., Dulak, L., Chiang, G., Zaccardi, J. and Farrugella, T. J. (1981). A sulphite oxidase deficient rat model: subchronic toxicology. *Food Cosmet. Toxicol.*, **19**, 221-32.

Guthrie, J. P. (1979). Tautomeric equilibria and pK_a values for 'sulfurous' acid in aqueous solution: a thermodynamic analysis. *Can. J. Chem.*, **57**, 454-7.

Hägglund, E., Johnson, T. and Urban, H. (1930). Influence of sulphite and bisulphite solutions on sugar at high temperatures. *Ber.*, **63B**, 1387-95.

Halperin, J. and Taube, H. (1952). The transfer of oxygen atoms in oxidation reduction reactions. *J. Amer. Chem. Soc.*, **74**, 380-2.

Hayase, F., Kim, S. B. and Kato, H. (1984). Decolourisation and degradation products of the melanoidins by hydrogen peroxide. *Agric. Biol. Chem.*, **48**, 2711-2717.

Hayon, E., Treinin, A. and Wilf, J. (1972). Electronic spectra, photochemistry and autoxidation mechanism of sulphite-bisulphite-pyrosulphite systems. The SO_2^-, SO_3^-, SO_4^- and SO_5^- radicals. *J. Amer. Chem. Soc.*, **94**, 47-57.

Hayward, L. D. and Angyal, S. J. (1977). The circular dichroism of reducing sugars, and the proportion of carbonyl forms in aqueous solution thereof. *Carbohydr. Res.*, **53**, (1), 13-20.

Hegg, D. A. and Hobbs, P. A. (1978). Oxidation of sulphur dioxide in aqueous systems with particular reference to the atmosphere. *Atmospheric environment*, **12**, 241-53.

Henry, K., Kon, S. K., Lea, C. H. and White, J. C. D. (1948). Deterioration in storage of dried skim milk. *J. Dairy. Res.* **15**, 292-364.

Herlinger, A. W. and Long, T. V. (1969). An investigation of the structure of the disulphite ion in aqueous solution using Raman and infrared spectroscopies. *Inorg. Chem.*, **8**, 2661-5.

Herrera-Viloria, J. C. (1984). The state of sulphur(IV) oxospecies in dehydrated vegetables. PhD Thesis. University of Leeds.

Hine, J., Green, L. R., Meng Jr, P. C. and Thiagarajan, V. (1976). Rates and equilibria in hydration and bisulphite addition to 1,3-dimethoxyacetone. *J. Org. Chem.*, **41**, 3343-9.

Hodge, J. E. (1953). Dehydrated foods. Chemistry of browning reactions in model systems. *J. Agric. Food. Chem.*, **1**, 928-43.

Hodge, J. E. (1955). The Amadori rearrangement. *Adv. Carbohydr. Chem.*, **10**, 169-205.

Hodge, J. E. and Rist, C. E. (1952). N-Glycosyl derivatives of secondary amines. *J. Amer. Chem. Soc.*, **74**, 1494-7.

Hodge, J. E., Fisher, B. E. and Nelson, E. C. (1963). Dicarbonyls, reductones and heterocyclics produced by reactions of reducing sugars with amine salts. *Proc. Am. Soc. Brew. Chem.*, 84-8.

Hodge, J. E., Mills, F. D. and Fisher, B. E. (1972). Compounds of browned flavor derived from sugar-amine reactions. *Cereal Sci. Today*, **17**, 34-38.

Hølterman, A. (1966). The browning reaction. *Die Stärke*. **18**, 319-328.

Horner, D. A. and Connick, R. E. (1986). Equilibrium quotient for the isomerisation of bisulfite ion from HSO_3^- to SO_3H^-. *Inorg. Chem.*, **25**, 2414-2417.

Humphrey, R. E., Ward, M. H. and Hinze, W. (1970). Spectrophotometric determination of sulphite with 4,4-dithiopyridine and 5,5'-dithiobis(2-nitrobenzoic acid). *Anal. Chem.*, **42**, 698-702.

Huss Jr, A. and Eckert, C. A. (1977). Equilibria and ion activities in aqueous sulphur dioxide solutions. *J. Phys. Chem.*, **81**, 2268-70.

Institute of Food Technologists and Committee on Public Information (1976). Sulphites as food additives. *Nutr. Rev.*, **34**, 58-62.

Irreverre, F., Mudd, H., Heizer, W. D. and Laster, L. (1967). Sulphite oxidase deficiency. Studies of a patient with mental retardation, dislocated ocular lenses and abnormal urinary secretion of S-sulpho-L-cysteine, sulphite and thiosulphate. *Biochem. Med.*, **1**, 187-217.

Jaulmes, P. and Hamelle, G. (1961). The determination of sulphite ion iodimetrically and of aldehydes by the sulphite method. *Ann. Fals. Expert. Chim.*, **54**, 338-46.

Johansson, L. G., Lindqvist, O. and Vannerberg, N. G. (1980). The structure of caesium hydrogen sulphite. *Acta Cryst.*, **B36**, 2523-6.

Jones, L. H. and McLaren, E. (1958). Infrared absorption spectrum of SO_2 and CO_2 in aqueous solution. *J. Chem. Phys.*, **28**, 995.

Judd, D. B. and Wyszecki, G. (1963). *Color in business, science, and industry*. John Wiley and Sons, Inc. New York. London. pp 158-162.

Kaanane, A. and Labuza, T. P. (1989). The Maillard reaction in foods. In *Prog. Clin. Biol. Res.*, 304, (Maillard React. Aging, Diabetes, Nutr.), 301-27.

Kapsalis, J. G. (1973). *Moisture and food characteristics*. Activities Report. Research and Development Associates for Military Food and Packaging Systems, Inc. **25**, 60.

Kashtanov, L. I. and Ryzhov, V. P. (1936). The effect of catalysts

and of anticatalysts on the kinetics and mechanism of the oxidation of sulphur dioxide with ozone. *J. Gen. Chem. (U.S.S.R.).*, **6**, 732-47.

Kato, H. and Fujimaki, M. (1970). Formation of 1-alkyl-5-(hydroxymethyl)pyrrole-2-aldehydes in the browning reaction between hexoses and alkylamines. *Agric. Biol. Chem.*, **34**, 1071-7.

Kato, H. and Tsuchida, H. (1981). Estimation of melanoidin structure by pyrolysis and oxidation. *Prog. Food Nutr. Sci.*, **5**, 147-156.

Kato, H., Kim, S. B. and Hayase, F. (1986). Estimation of the partial chemical structures of melanoidins by oxidative degredation and ^{13}C CP-MAS NMR. In *Amino-carbonyl Reactions in Food and Biological Systems*, ed. M. Fujimaki, M. Namiki & H. Kato. Elsevier Science Publishers, Amsterdam, pp 215-223.

Kato, H., Lee, I. E., Chuyen, N. V., Kim, S. B. and Hayase, F. (1987). Inhibition of nitrosamine formation by non-dialysable melanoidins. *Agric. Biol. Chem.*, **51**, 1333-1338.

Kato, H., Noguchi, G. and Fujimaki, M. (1968). Reductone contents of non-dialysable melanoidins. *Agric. Biol. Chem.*, **32**, 916-919.

Kato, H., Yamamoto, M. and Fujimaki, M. (1969). Mechanisms of browning degradation of D-fructose in special comparison with D-glucose-glycine reaction. *Agric. Biol. Chem.*, **33**, 939-948.

Kim, S. B. and Park, Y. K. (1986). Decolourisation and degradation products of melanoidin on ozonolysis by active oxygen. II. Decolourisation and degradation products of melanoidin on ozonolysis. *Bull. Korean Fish Soc.*, **19**, 36-44.

Kim, S. B., Hayase, F. and Kato, H. (1985). Decolourisation and degradation products of melanoidins on ozonolysis. *Agric. Biol. Chem.*, **49**, 785-792.

Kirigaya, N., Kato, H. and Fujimaki, M. (1968). Studies on antioxidant activity of non-enzymic browning reaction products. Part 1. Relations of colour intensity and reductones with antioxidant activity of browning reaction products. *Agric. Biol. Chem.*, **32**, 287-290.

Kochler, P. E. and Odell, G. U. (1970). Factors affecting the formation of pyrazine compounds in sugar amine reactions. *J. Agric. Food. Chem.*, **18**, 895-8.

Kokesh, F. C. and Hall, R. E. (1975). A re-examination of the

equilibrium addition of bisulphite and sulphite ions to benzaldehyde. *J. Org. Chem.*, **40**, 1632-6.

Kolthoff, I. M., Belcher, R., Stenger, V. A. and Matsuyama, G. (1957). *Volumetric Analysis. Volume III. Titration methods: oxidation-reduction reactions*. Interscience Publishers, New York and London.

Kossiakoff, A. and Harker, D. (1938). The calculation of the ionisation constants of inoganic oxygen acids from their structures. *J. Amer. Chem. Soc.*, **60**, 2047-55.

Labuza, T. P. (1971). Properties of water as related to the keeping quality of foods. Paper presented at Third International Congress of Food Science and Technology. Washington, D. C. Aug. 9-14th. pp. 618-635.

Labuza, T. P. (1975). Interpretation of sorption data in relation to the state of constituent water. In *Water relations of foods* : Proceedings of an international symposium held in Glasgow. Edited by Duckworth, R. B. Academic press inc (London). 155-172.

Labuza, T. P. (1980). Effect of water activity on reaction kinetics of food deterioration. *Food Technol.*, **34**, 36.

Labuza, T. P. (1984). *Moisture sorption: Practical aspects of isotherm measurement and use*. The American Association of Cereal Chemists. St. Paul, Minnesota.

Labuza, T. P., Warren, R. M. and Warmbier, H. C. (1977). The physical aspects with respect to water and non-enzymatic browning. *Adv. exp. Biol.*, **86B**, 379-418.

Landrock, A. H. and Proctor, B. E. (1951). A new graphical interpolation method for obtaining humidity equilibria data, with special reference to its role in food packaging studies. *Food Technol.*, **5**, 332.

Larsson, L. O. and Kierkegaard, P. (1969). Crystal structure of sodium sulphite. Refinement allowing for the effect of crystal twinning. *Acta Chem. Scand.*, **23**, 2253-60.

Lay, A. (1970). Iodimetric determination of sulphur dioxide. *Mitt. Rebe. Wein Obstbau Früchteverwert (Klosterneuberg)*, **20**, 85-93.

Lay, A. (1971). New high speed process for determining the total sulphur dioxide content of wines as compared with other methods. *Mitt. Rebe. Wein Obstbau Früchteverwert (Klosterneuberg)*, **21**, 439-43.

Lea, C. H. (1951). Non-enzymatic browning. XXXVII. Text of papers. Second. Intl. Congress on Canned Foods, Paris, p. 1.

Lea, C. H. and Hanan, R. S. (1949). Studies of the reaction between proteins and reducing sugars in the "dry" state. 1. The effect of activity of water, of pH and of temperature on the primary reaction between caesein and glucose. *Biochim. Biophys. Acta.*, **3**, 313.

Lee, C. M., Sherr, B. and Koh, Y. N. (1984). Evaluation of kinetic parameters of a glucose-lysine Maillard reaction. *J. Agric. Food Chem.*, **32**, 379-382.

LeFevre, M. E., Wyssbrod, H. R. and Brodsky, W. A. (1970). Problems in the measurement of tissue respiration with the oxygen electrode. *Bioscience.*, **20**, 13, 761.

Lubieniecki-von Schelhorn, M. and Heiss, R. (1975). The influence of relative humidity on the thermal resistance of mold spores. In *Water relations of foods* : Proceedings of an international symposium held in Glasgow. Edited by Duckworth, R. B. Academic press inc (London). 339-346.

Maga, J. S. and Sizer, C. E. (1973). Pyrazines in foods. A review. *J. Agric. Fd. Chem.*, **21**, 22-30.

Maillard, L. C. (1912). Action of amino acids on sugars. Formation of melanoidins in a methodical way. *Compt. Rend.*, **154**, 66-68.

Maillard, L. C. (1916). Synthesis of humus-like substances by the interaction of amino acids and reducing sugars. *Annal. Chim. (Rome)*, **5**, 258-317.

Markova, J., Honischova, E. and Hampl, J. (1972). Non-enzymatic reactions in cereal production. Scientific Papers of Institute of Chem. Tech. Food. Prague E-33, Czechoslovakia.

Mason, H. M. and Walsh, G. (1928a). Note on the oxidation of sulphites by air. *Analyst (London)*, **53**, 142-4.

Mason, H. M. and Walsh, G. (1928b). Note on the titration of dilute sulphite solutions with standard iodine solutions. *Analyst (London)*, **53**, 144-9.

Mauron, J. (1981). The Maillard reaction in food; a critical review from the nutritional standpoint. *Prog. Food. Nutr. Sci.*, **5**, 5-35.

McCord, J. M. and Fridovich, I. (1969a). Superoxide dismutase. An enzymic function for erythrocuprein (haemocuprein). *J. Biol. Chem.*, **244**, 6049-55.

McCord, J. M. and Fridovich, I. (1969b). The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulphite, dimethylsulphoxide, and oxygen. *J. Biol. Chem.*, **244**, 6056-63.

McMurry, J. (1984). *Organic chemistry*. Brooks/cole publishing company. Monterey, California.

McWeeny, D. J. (1973). *The role of carbohydrates in non-enzymatic browning in molecular structure and functions of food carbohydrates*. (Birch and Green, ed.) Applied Science Publ.

McWeeny, D. J. (1981). Sulphur dioxide and the Maillard reaction in food. *Prog. Fd. Nutr. Sci.*, **5**, 395-404.

McWeeny, D. J. and Burton, H. S. (1963). Some possible glucose/glycine browning intermediates and their reactions with sulphite. *J. Sci. Food Agric.*, **14**, 291-302.

McWeeny, D. J., Biltcliffe, D. O., Powell, R. C. T. and Spark, A. A. (1969). The Maillard reaction and its inhibition by sulphite. *J. Food sci.*, **34**, 641-3.

McWeeny, D. J., Knowles, M. E. and Hearne, J. F. (1974). The chemistry of non-enzymic browning in foods and its control by sulphites. *J. Sci. Food Agric.*, **25**, 735-46.

McWeeny, D. J., Shepherd, M. J. and Bates, M. L. (1980). Physical loss and chemical reactions of SO_2 in strawberry jam production. *J. Food Technol.*, **15**, 613-17.

Meyer, B., Ospina, M. and Peter, L. B. (1980). Raman spectrometric determination of oxysulphur anions in aqueous systems. *Anal. Chim. Acta*, **117**, 301-11.

Meyer, B., Peter, L. and Shaskey-Rosenlund, C. (1979). Raman spectra of isotopic bisulphite and disulphite ions in alkali salts and aqueous solutions. *Spectrochim. Acta.*, **35A**, 345-54.

Meyer, B., Peter, L. and Spitzer, K. (1977). Trends in charge distributions in sulphanes, sulphanesulphonic acids, sulphanedisulphonic acids and sulphurous acid. *Inorg. Chem.*, **16**, 27-33.

Mitsubishi Chemical Industries Co. Ltd. (1980a). Antioxidants for sulphites and bisulphites. Jpn. Kokai Tokkyo Koho 80 54, 388 (cl. C09k15/32), 21 Apr. 1980, Appl. 78/126, 993, 16 Oct. 1978; 3 pp.

Mitsubishi Chemical Industries Co. Ltd. (1980b). Antioxidants for

sulphites and bisulphites. Jpn. Kokai Tokkyo Koho 80 54, 388 (cl. C09k15/08), 21 Apr. 1980, Appl. 78/126, 995, 16 Oct. 1978; 2 pp.

Monier-Williams, G. W. (1927). Determination of sulphur dioxide. *Analyst* , (London), **52**, 415-16.

Morozov, A. I., Sytilin, M. S. and Makolkin, I. A. (1972). Kinetics of the reaction of acrylonitrile with sodium sulphite at constant pH in an unbuffered medium. *Zh. Fiz. Khim.*, **46**, 1295-7.

Motai, H. (1974). Relationship between the molecular weight and colour intensity of colour components of melanoidin from glycine-xylose systems. *Agric. Biol. Chem.*, **38**, 2299-2304.

Motai, H. and Inoue, S. (1974). Conversion of colour components of melanoidin produced from the glycine-xylose system. *Agric. Biol. Chem.*, **38**, 233-9.

Murray, F. J., Schwetz, B. A., Crawford, A. A., Henck, J. W., Quast, J. F. and Staples, R. E. (1979). Embryotoxicity of inhaled sulphur dioxide and carbon monoxide in mice and rabbits. *J. Envir. Sci. Hlth.*, **C13**, 233.

Murrel, W. G. and Scott, W. J. (1966). The heat resistance of bacterial spores at various water activities. *J. Gen. Microbiol.* **43**, 411-425.

Nam, S. & Kim, M. L. (1984). Browning pattern and pigment of glucose/glycine model systems. *Korean J. Food Sci. Technol.*, **16**, 218-222.

Nord, A. G. (1973). Refinement of the crystal structure of Thenardite, Na_2SO_4(V). *Acta Chem. Scand.*, **27**, 814-22.

Norrish, R. S. (1966). An equation for the activity coefficients and equilibrium relative humidities of water in confectionery syrups. *J. Food Technol.*, **1**, 25-39.

Nursten, H. E. and O'Reilly, R. (1986). Coloured compounds formed by the interaction of glycine and xylose. *Food Chem.*, **20**, 45-60.

Obanu, Z. A., Ledward, D. A. and Lawrie, R. A. (1977). Reactivity of glycerol in intermediate moisture meats. *Meat Sci.*, **1**, 177-83.

Obretenov, T. D., Kuntcheva, M. J. and Panchev, I. N. (1986). Influence of reaction conditions on forming non-dialysable melanoidins from glucose and glycine. *Food Process. Preserv.*, **10**, 251-258.

Parks, O. W. (1967). Milk Flavour. In *Chemistry and Physiology of*

Flavours, pp. 296-314 (A. W. Schulz, E. A. Day and L. M. Libbey, eds.) Avi Publishing Co., Westport, Conneticut.

Paul, A. A. and Southgate, D. A. T. (1979). *McCance and Widdowson's The Composition of Foods*. Fourth revised and extended edition of MRC Special Report N.O. 297. London. Her Majesty's Stationary Office. Amsterdam, New York, Oxford. Elsevier/North-Holland Biomedical Press.

Pearson, R. G. (1968). Hard and soft acids and bases. H. S. A. B. Part 1. Fundamental principles. *J. Chem. Educ.*, **45**, 581-7.

Peiser, G. D. and Yang, S. F. (1979). Sulphite mediated destruction of β-carotene. *J. Agric. Food Chem.*, **27**, 446-9.

Petriella, C., Resnik, S. L., Lozano, R. D. and Chirife, J. (1985). Kinetics of deteriorative reactions in model food systems of high water activity: colour changes due to nonenzymic browning. *J. Food Sci.*, **50**, 622-626.

Pigman, W. and Anet, E. F. L. J. (1972). Mutarotation and actions of acids and bases. In *The carbohydrates, chemistry and biochemistry*. Pigman, W., Horton, D. (eds). Academic press, New York, San Francisco, London, pp. 165-194.

Potman, R. P. and van Wijk, T. A. (1989). Mechanistic studies of the Maillard reaction with emphasis on phosphate-mediated catalysis. *ACS Symp. Ser.* 1988 (Publ 1989). **409** (Therm. Gener. Aromas). 182-95.

Reardon, E. J. (1975). Dissociation contents of some monovalent sulphate ion pairs at 25 °C from stoichiometric activity coefficients. *J. Phys. Chem.*, **79**, 422-5.

Reynolds, T. M. (1963). Chemistry of nonenzymic browning. I. The reaction between aldoses and amines. *Adv. food res.*, **12**, 1-52.

Reynolds, T. M. (1965). Chemistry of non enzymic browning II. *Adv. Food Res.*, **14**, 167-283.

Roberts, A. C. and McWeeny, D. J. (1972). The use of sulphur dioxide in the food industry. A review. *J. Food Technol.*, 221-38.

Rochelle, G. T. and Owens, D. R. (1985). Sulphite oxidation inhibited by thiosulphate. *Coal Sci. Technol. (Process Util. High Sulphur Coals).*, **9**, 611-24.

Rockland, L. B. (1957). A new treatment for hygroscopic equilibria: Application to walnuts (Juglans regia) and other foods. *Food Res.*, **22**, 604.

Rockland, L. B. and Nishi, S. K. (1980). Influence of water activity on food product quality and stability. *Food. Technology.*, 42-51, 59.

Rubinsztain, Y., Ioselis, P., Ikan, R. and Aizenshtat, Z. (1984). Investigations on the structural units of melanoidins. *Org. Geochem.*, **6**, 791-804.

Sapotnitski, S. A. and Glushchenko, N. V. (1962). Oxidation of sulphites by air in dilute acidified solution. *J. Appl. Chem. (USSR)*, **35**, 2104-7.

Scanlan, R. A., Lindsay, R. C., Libbey, L. M. and Day, E. A. (1968). Heat induced volatile compounds in milk. *J. Dairy Sci.* **51**, 1001-1007.

Schaeffer, K. and Köhler, W. (1918). Optical study of the constitution of hyposulphurous acid, its salts and ester. *Z. Anorg. Allgem. Chem.*, **104**, 212-50.

Schmidkunz, H. (1963). Chemilumineszenz der Sulphitoxidation. PhD Thesis, University of Frankfurt.

Schönberg, A. and Moubacher, R. (1952). The Strecker degradation of α-amino acids. *Chem. Rev.*, **50**, 261-77.

Schroeter, L. C. (1963). Kinetics of air oxidation of sulphurous acid salts. *J. Pharm. Sci.*, **52**, 559-63.

Schroeter, L. C. (1966). *Sulfur dioxide: applications in foods, beverages and pharmaceuticals*, Pergamon press, London.

Scientific Committee for Food (1981). EUR 7421. Reports of the Scientific Committee for Food (Eleventh Series). Commission of the European Communities, Food Science and Techniques, Luxemburg: Office for Official Publications of the European Communities, pp. 47-8.

Seow, C. C. and Cheah, P. B. (1985). Reactivity of sorbic acid and glycerol in non-enzymatic browning in liquid intermediate moisture model systems. *Food Chemistry.*, **18**, 71-80.

Shapiro, R. (1977). Genetic effects of bisulphite (sulphur dioxide). *Mutation Res.*, **39**, 149-75.

Sillen, L. G. (1964). Stability constants of metal ion complexes. Section 1. Inorganic ligands, Special Publ. No. 17, The Chemical Society, London.

Simon, A. (1947). *Angew. Chem.*, **59**, 247.

Simon, A. and Kriegsmann, H. (1956). Ionic equilibriums in aqueous

solutions of acid sulfites. *Chem. Ber.*, **89**, 2442-6.

Simon, A. and Pischtschan, A. (1961). The state of H_2O in liquid SO_2. *J. Prakt. Chem.*, **14**, 196-207.

Simon, A. and Waldmann, K. (1955). Constitution of acid sulfites. I. Aqueous disulfite (pyrosulfite) solutions and their behaviour in relation to concentration and degree of basicity. *Z. Anorg. U. Allgem. Chem.*, **281**, 113-34.

Simon, A., Waldman, K. and Steger, E. (1956). The constitution of acid sulphite V. The structure of disulphite. *Z. Annorg. Allgem. Chem.*, **288**, 131-47.

Slae, S. and Shapiro, R. (1978). Kinetics and mechanism of the deamination of 1-methyl-5,6-dihydrocytosine. *J. Org. Chem.*, **43**, 1721-6.

Sloan, A. E. and Labuza, T. P. (1976). Prediction of water activity lowering ability of food humectants at high a_w. *J. Food Sci.*, **41**, 532-5.

Smith, R, M. and Martell, A. E. (1976). *Critical stability constants. Vol. 4. Inorganic complexes.* Plenum Press, New York.

Song, P. S., Chichester, C. O. and Stadtman, F. H. (1966). Kinetic behaviour and mechanism of inhibition in the Maillard reaction. I. Kinetic behaviour of the reaction between D-glucose and glycine. *J. Food Sci.*, **31**, 906-913.

Souci, S. W., Fachmann, W. and Kraut, H. (1981). Food composition and Tables 1981/82. Wessenschaftliche Verlag, Stuttgart.

Spark, A. A. (1969). Role of amino acids in non-enzymic browning. *J. Sci. Food Agric.*, **20**, 308-316.

Speck Jr, J. C. (1958). The Lobry De Bruyn-Alberda Van Ekenstein transformation. *Advan. Carbohyd. Chem.*, **13**, 63.

Steelhammer, J. C. and Hutta, P. J. (1982a). Method and composition for inhibiting the amount of oxidation of sulphite in a sulphite containing aqueous gas scrubber medium. Eur. pat. Appl. EP 56, 180 (cl. B01D53/34), 21 Jul. 1982, US Appl. 223, 591, 09 Jan. 1981; 43 pp.

Steelhammer, J. C., Morrison, I. D. and Wittenbrook, L. S. (1982b). Polyethyleneamines as sulfite antioxidants. U.S. US 4, 310, 438 (cl. 252-401; C09K15/16), 12 Jan. 1982, Appl. 25, 304, 30 Mar. 1979; 5 pp.

Stewart, T. D. and Donnally, L. H. (1932a). The aldehyde bisulphite

compounds II. The effect of varying hydrogen ion and of varying temperature upon the equilibrium between benzaldehyde and bisulphite ion. *J. Amer. Chem. Soc.*, **54**, 3555-8.

Stewart, T. D. and Donnally, L. H. (1932b). The aldehyde bisulphite compounds I. The rate of dissociation of benzaldehyde sodium bisulphite as measured by its first order reaction with iodine. *J. Amer. Chem. Soc.*, **54**, 2333-40.

Stoll, M., Winter, M., Gautschi, F., Flament, I. and Willhalm, B. (1967). Recherches sur les arômes. Sur l'arôme de café. I. *Helv. Chim. Acta.*, **50**, 628-694.

Strömberg, A., Gropen, O., Wahlgren, U. and Lindqvist, O. (1983). Ab initio calculations on the sulfite ion, SO_3^{2-}, and hydrogen sulfite ion, HSO_3^- or SO_2OH^-. *Inorg. Chem.*, **22**, 1129-1133.

Sykes, P. (1965). *A Guidebook to Mechanisms in Organic Chemistry*, 2nd edn, Longmans, London.

Sykes, P. (1986). *A guidebook to mechanism in organic chemistry.* Sixth edition. Longman. London and New York.

Taguchi, K. and Sampei, Y. (1986). The formation of clay mineral and $CaCO_3$ association reactions of melanoidins. *Org. Geochem.*, **10**, 1081-1089.

Takeuchi, H., Nishioka, Y., Fujishiro, M. and Muramatsu, K. (1987). Physiological effects of non-dialysable melanoidin in rats. *Agric. Biol. Chem.*, **51**, 969-976.

Tarr, H. L. (1954). The Maillard reaction in flesh foods. *Food Tech.*, **1**, 15.

Tartar, H. V. and Garretson, H. H. (1941). The thermodynamic ionisation constants of sulphurous acid at 25 °C. *J. Amer. Chem. Soc.*, **63**, 808-16.

Teng, T. and Lenzi, F. (1974). Water activity data representation of aqueous solutions at 25 °C. *Can. J. Chem. Eng.*, **52**, 387-391.

Thalacker, R. (1975). Content of fermentatively produced sulphur dioxide in full flavoured and heavy beers. *Mitteilungsbl. GDCh-Fachgruppe Lebensmittelchem. Gerichtl. Chem.*, **29**, 129-32.

Thomas, G. O. and Monk, C. B. (1956). Spectroscopic studies of electrolytic dissociation. Part 2. Some thiosulphates in 50 % ethanol. *Trans Faraday Soc.*, **52**, 685-9.

Til, H. P., Ferron, V. J. and Groot, A. P. de (1972a). The toxicity of sulphite. I. Long-term feeding and multigeneration studies in rats.

Food Cosmet. Toxicol., **10**, 291-310.

Til, H. P., Ferron, V. J. and Groot, A. P. de and Van der Wal, P. (1972b). The toxicity of sulphite II. Short- and long-term feeding studies in pigs. *Food Cosmet. Toxicol.*, **10**, 463-73.

Troller, J. A. (1980). Influence of water activity on microorganisms found in foods. *Food Technol.*, (In press).

Troller, J. A. and Christian, J. H. B. (1978). *Water Activity and Food.* Academic Press, New York.

Urone, P. F. and Boggs, W. E. (1951). Acid-bleached fuchsin in determination of sulphur dioxide in the atmosphere. *Anal. Chem.*, **23**, 1517-19.

Vacalis, N. (1986). Formation of 3-deoxyhexosulose in the sulphite inhibited Maillard reaction. PhD Thesis. University of Leeds.

Vas, K. (1949). The equilibrium between glucose and sulphurous acid. *J. Soc. Chem. Ind.*, **68**, 340-3.

Vogel, A. I. (1961). *Textbook of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis,* 3rd edn, Longman's, London. pp. 446-447.

Vogel, A. I. (1989a). *Vogel's textbook of quantitative chemical analysis.* 5th ed./ revised by Jeffery, G. H., Bassett, J., Mendham, J. and Denney, R. C. Longman's, London.

Vogel, A. I. (1989b). *Vogel's textbook of practical organic chemistry.* 5th ed./ revised by Furniss, B. S., Hannaford, A. J., Smith, P. W. G. and Tatchell, A. R. Longman's, London. pp. 254-273.

Waldradt, J. P., Pittet, A. O., Kinlin, T. E., Muralidhara, R. and Sanderson, A. (1971). Volatile components of roasted peanuts. *J. Agric. Fd. Chem.*, **19**, 972-979.

Walker, R., Mendoza-Garcia, M. A., Ioannides, C. and Quattrucci, E. (1983a). Acute toxicity of 3-deoxy-4-sulphohexosulose in rats and mice and *in vitro* mutagenicity in the Ames test. *Food Chem. Toxicol.*, **21**, 299-303.

Walker, R., Mendoza-Garcia, M. A., Quattrucci, E. and Zerilli, M. (1983b). Metabolism of 3-deoxy-4-sulphohexosulose, a reaction product of sulphite in foods, by rat and mouse. *Food Chem. Toxicol.*, **21**, 291-7.

Warmbier, H. C., Schnickels, R. A. and Labuza, T. P. (1976). Effect of glycerol on nonenzymatic browning in a solid intermediate moisture model food system. *J. Food Sci.*, **41**, 528-531.

Wedzicha, B. L. (1984a). A kinetic model for the sulphite inhibited Maillard reaction. *Food Chemistry.*, **14**, 173-187.

Wedzicha, B. L. (1984b). *Chemistry of sulphur dioxide in foods.* London: Elsevier applied science publishers LTD.

Wedzicha, B. L. (1987). Review: Chemistry of sulphur dioxide in vegetable dehydration. *Int. J. Food sci. Technol.*, **22**, 433-450.

Wedzicha, B. L. and Chishya, B. E. (1983). The stability constant for the interaction between bovineserum albumin and magnesium N-phenyl-8-aminonaphthalene sulphonate. *Food Chem.*, **10**, 179-193.

Wedzicha, B. L. and Garner, D. N. (1991). The formation and reactivity of osuloses in the sulphite-Inhibited Maillard reaction of glucose and glycine. *Food Chemistry.*, **39**, 73-86.

Wedzicha, B. L. and Goddard, S. J. (1989). Chemical basis of the level of use of sulphur dioxide in food. *Proceedings FIE89*, Expoconsult Publishers, Maarssen, The Netherlands. p37-41.

Wedzicha, B. L. and Goddard, S. J. (1991). The state of sulphur dioxide at high concentration and low water activity. *Food. Chemistry.*, **40**, 119-136.

Wedzicha, B. L. and Herrera-Viloria, J. C. (in press). Formation of sulphate ion during the dehydration of sulphited vegetables.

Wedzicha, B. L. and Kaban, J. (1986). Kinetics of the reaction between 3-deoxyhexosulose and S(IV) oxospecies in the presence of glycine. *Food Chem.*, **22**, 209-223.

Wedzicha, B. L. and Lamikanra, O. (1983). Sulphite mediated destruction of β-carotene: the partial characterisation of reaction products. *Food Chem.*, **10**, 4, 275-83.

Wedzicha, B. L. and McWeeny, D. J. (1974). Non-enzymic browning reactions of ascorbic acid and their inhibition. The production of 3-deoxy-4-sulphopentosulose in mixtures of ascorbic acid, glycine and bisulphite ion. *J. Sci. Food Agric.*, **25**, 577-587.

Wedzicha, B. L. and McWeeny, D. J. (1974). Non-enzymic browning reactions of ascorbic acid and their inhibition. The identification of 3-deoxy-4-sulphopentosulose in dehydrated, sulphited cabbage after storage. *J. Sci. Food Agric.*, **25**, 589-592.

Wedzicha, B. L. and McWeeny, D. J. (1975). Concentrations of some sulphonates derived from sulphite in certain foods and preliminary studies on the nature of other sulphite derived products. *J. Sci.*

Food Agric., **26**, 327-35.

Wedzicha, B. L. and Smith, G. (1987). Geometry of 1,2-dihydroxy-1,2-disulphonates. *Food Chem.*, **25**, 165-174.

Wedzicha, B. L. and Vacalis, N. (1988). Kinetics of the sulphite-inhibited Maillard reaction: The effect of sulphite ion. *Food. Chem.*, **27**, 259-271.

Wedzicha, B. L., Bellion, I. R. and Goddard, S. J. (1991). Inhibition of browning by sulfites. In *Nutritional and toxicological consequences of food processing*. M. Friedman (ed). Advances in experimental medicine and biology volume 289, Plenum Press, New York., pp 217-236.

Wedzicha, B. L., Kaban, J. and Aldous, D. G. (1985). The dissociation constant of the hydroxysulphonate of 3,4-dideoxy-4-sulphohexosulose in the sulphite-inhibited Maillard reaction of glucose and glycine. *Food Chemistry.*, **17**, 125-129.

Weier, T. E., Stocking, C. R., Barbour, M, G. and Rost, T, L. (1982). *Botany: an introduction to plant biology*. Sixth edition. USA. John Wiley & Sons, Inc.

Wertz, P. W., Garver, J. C. and Anderson, L. (1981). Anatomy of a complex mutarotation - kinetics of tautomerization of α-D-galactopyranose and β-D-galactopyranose in water. *J. Amer. Chem. Soc.*, **103**, 13, 3916-22.

Weygand, F. (1939). Preparation of N-glucosides of aniline and substituted anilines. *Ber.*, **72B**, 1663-7.

Wilson, R. A., Mussinan, C. J., Katl, I. and Sanderson, A. (1973). Isolation and identification of some sulphur chemicals present in pressure-cooked beef. *J. Agric. Fd. Chem.*, **21**, 873-875.

Winter, E. R. S. and Briscoe, H. V. A. (1951). Oxygen atom transfer during the oxidation of aqueous sodium sulphite. *J. Amer. Chem. Soc.*, **73**, 496-7.

Winton, A. L. and Winton, K. B. (1935). *The structure and composition of foods*. Vol. II. John Wiley and sons, New York.

Wolff, S. P. and Dean, R. T. (1987). Monosaccharide autoxidation: a potential source of oxidative stress in diabetes? Model reactions with nucleotides and protein. *Bioelectrochemistry and bioenergetics.*, **18**, 283-293.

Wolf, M., Walker, J. E. and Kapsalis, J. G. (1972). Water vapour sorption hysteresis in dehydrated food. *J. agric. Fd Chem.*, **20**,

1073.

Wolfrom, M. L., Kashimura, N. and Horton, D. (1974). Factors affecting the Maillard browning reaction between sugars and amino acids. Studies on the non-enzymic browning of dehydrated orange juice. *J. Agric. Food Chem.*, **22**, 796-800.

Wolfrom, M. L., Kolb, D. K. and Langer Jr, A. W. (1953). Chemical interactions of amino compounds and sugars. VII. pH dependency. *J. Amer. Chem. Soc.*, **75**, 3471-3.

Würdig, G. and Schlotter, H. A. (1971). Occurrence of sulphur dioxide-forming yeasts in a natural grape-must yeast mixture. *Deut. Lebensm. Rundschau*, **67**, 86-91.

Yang, S. F. (1970). Sulphoxide formation from methionine or its sulphide analogues during aerobic oxidation of sulphite. *Biochim.*, **9**, 5008-14.

Yang, S. F. (1973). Destruction of tryptophan during the aerobic oxidation of sulphite ions. *Environ. Res.*, **6**, 395-402.

Yoshihiro, Y., Kuroiwa, S. and Nakamura, M. (1961). The role of 5-(hydroxymethyl)-2-furaldehyde in melanoidin formation in heated glucose solution. *Kogyo Kagaku Zasshi*, **64**, 551-5.

Young, P. R. and Jencks, W. P. (1977). Nonenforced catalysis of the bisulphite carbonyl addition reaction by hydrogen bonding. *J. Amer. Chem. Soc.*, **99**, 1206-14.

APPENDIX 1

FILE: IANM1SS FORTRAN A LEEDS UNIVERSITY COMPUTING SERVICE VM/370 5.0 CMS

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      PROGRAM IANM1SS                                                    IAN00010
                                                                         IAN00020
C A PROGRAM TO CALCULATE THE THEORETICAL CONCENTRATIONS OF TOTAL,FREE & IAN00030
C REVERSIBLY BOUND S(IV),AT THE TIMES WHEN EXPERIMENTAL MEASUREMENTS    IAN00040
C WERE MADE.                                                           IAN00050
C THE RATE AND EQUILIBRIUM CONSTANTS ARE VARIED SYSTEMATICALLY, UNTILL IAN00060
C THEORETICAL AND EXPERIMENTAL RESULTS SHOW THE MAXIMUM DEGREE OF      IAN00070
C CORRELATION;THESE RESULTS ARE THEN PLOTTED.                          IAN00080
C THE QUALITY OF THE FIT IS MEASURED AS THE SUM OF THE                 IAN00090
C SQUARES OF THE DEVIATIONS,THIS BEING CALCULATED USING THE PROGRAM    IAN00100
C IANM1TB FORTRAN.                                                     IAN00110
                                                                       IAN00120
C                                                                      IAN00130
C DECLARING VARIABLES AND ARRAYS.                                      IAN00140
C                                                                      IAN00150
                                                                       IAN00160
      REAL*8  GLUC,GLY,SIV,RK1,RK2,RK3,RK4,RK5,EK1,EK2,EK3,TIME,DSH,DH, IAN00170
     *        SGLUC,SDH,SDSH,S4,DC,G,E,F,B,TTLS4,FRS4,FREES4,RBS4,      IAN00180
     *        TOCK,TOTTIM,RVBNDEXP,FREEEXP,TOTEXP,GLUCINL,GLYINL,       IAN00190
     *        DT,CLOCK,TOTTHAR,FRTHAR,RBTHAR,TIMEXAR,TOTEXAR,FREXAR,    IAN00200
     *        RBEXAR,TICKTHAR                                           IAN00210
      INTEGER I,N,J,L,A,C,RNMIX,IN,M,COUNT,THEODATA,EXPDATA              IAN00220
C                                                                      IAN00230
C INTRODUCING ARRAYS TO STORE DATA.                                    IAN00240
C                                                                      IAN00250
      DIMENSION TOTTHAR(50),FRTHAR(50),RBTHAR(50),TIMEXAR(50),         IAN00260
     *          TOTEXAR(50),FREXAR(50),RBEXAR(50),TICKTHAR(50)         IAN00270
      READ(1,*)L                                                       IAN00280
      THEODATA=1                                                       IAN00290
      EXPDATA= 2                                                       IAN00300
C                                                                      IAN00310
C STATING VALUES OF RATE AND EQUILIBRIUM CONSTANTS.                    IAN00320
C                                                                      IAN00330
                                                                       IAN00340
C                                                                      IAN00350
C VALUES USED,WITHOUT ADJUSTMENT,WHEN GLUCOSE CONCENTRATION WAS VARIED IAN00360
C AT AW=1                                                              IAN00370
C                                                                      IAN00380
                                                                       IAN00390
C     RK1= 5.45570E-5                                                  IAN00400
C     RK2= (2.02627E-3/5.45570E-5)*RK1                                 IAN00410
C     RK3= 1.7E-3                                                      IAN00420
C     RK4= (7.0E-3/1.7E-3)*RK3                                         IAN00430
C     EK1= 1.33                                                        IAN00440
C     EK2= 0.004                                                       IAN00450
C     EK3= 0.0044                                                      IAN00460
                                                                       IAN00470
                                                                       IAN00480
                                                                       IAN00490
                                                                       IAN00500
C                                                                      IAN00510
C ADJUSTED VALUES USED WHEN GLUCOSE CONCENTRATION WAS VARIED AT AW=1   IAN00520
C                                                                      IAN00530
                                                                       IAN00540
C     RK1= 5.45570E-5*2                                                IAN00550
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FILE: IANM1SS  FORTRAN  A   LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

C      RK2= (2.02627E-3/5.45570E-5)*RK1                                    IAN00560
C      RK3= 1.7E-3*0.85                                                    IAN00570
C      RK4= (7.0E-3/1.7E-3)*RK3                                            IAN00580
C      RK5= 15                                                             IAN00590
C      EK1= 1                                                              IAN00600
C      EK2= 0.004*70                                                       IAN00610
C      EK3= 0.0044*0.15                                                    IAN00620
                                                                           IAN00630
                                                                           IAN00640
                                                                           IAN00650
C                                                                          IAN00660
C ADJUSTED VALUES USED WHEN GLYCINE CONCENTRATION WAS VARIED AT AW=1       IAN00670
C                                                                          IAN00680
                                                                           IAN00690
C      RK1= 5.45570E-5*1.6                                                 IAN00700
C      RK2= (2.02627E-3/5.45570E-5)*RK1                                    IAN00710
C      RK3= 1.7E-3*0.6                                                     IAN00720
C      RK4= (7.0E-3/1.7E-3)*RK3                                            IAN00730
C      RK5= 25                                                             IAN00740
C      EK1= 1                                                              IAN00750
C      EK2= 0.004*70                                                       IAN00760
C      EK3= 0.0044*0.15                                                    IAN00770
                                                                           IAN00780
                                                                           IAN00790
                                                                           IAN00800
C                                                                          IAN00810
C ADJUSTED VALUES USED WHEN S(IV) CONCENTRATION WAS VARIED AT AW=1         IAN00820
C                                                                          IAN00830
                                                                           IAN00840
C      RK1= 5.45570E-5*3.5                                                 IAN00850
C      RK2= (2.02627E-3/5.45570E-5)*RK1                                    IAN00860
C      RK3= 1.7E-3*0.3                                                     IAN00870
C      RK4= (7.0E-3/1.7E-3)*RK3                                            IAN00880
C      RK5= 0                                                              IAN00890
C      EK1= 1                                                              IAN00900
C      EK2= 0.004*70                                                       IAN00910
C      EK3= 0.0044*0.15                                                    IAN00920
                                                                           IAN00930
                                                                           IAN00940
                                                                           IAN00950
C                                                                          IAN00960
C ADJUSTED VALUES USED WHEN S(IV) CONCENTRATION WAS VARIED AT REDUCED AWIAN00970
C                                                                          IAN00980
                                                                           IAN00990
       RK1= 1.31925E-4*4.05                                                IAN01000
       RK2= (4.74373E-3/1.31925E-4)*RK1                                    IAN01010
       RK3= 1.7E-3*0.3                                                     IAN01020
       RK4= (7.0E-3/1.7E-3)*RK3                                            IAN01030
       RK5= 0                                                              IAN01040
       EK1= 1.33                                                           IAN01050
       EK2= 0.004*70                                                       IAN01060
       EK3= 0.0044*0.15                                                    IAN01070
                                                                           IAN01080
                                                                           IAN01090
C                                                                          IAN01100
```

FILE: IANM1SS FORTRAN A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

```
C  LOOP 10.THERE ARE L REACTION MIXTURES.                                  IAN01110
C                                                                         IAN01120
      DO 10 I=1,L                                                         IAN01130
         GLUC=0                                                           IAN01140
         S4=0                                                             IAN01150
         COUNT=0                                                          IAN01160
         DSH=0                                                            IAN01170
         DH=0                                                             IAN01180
         TOCK=0                                                           IAN01190
         N=0                                                              IAN01200
         SGLUC=0                                                          IAN01210
         SDH=0                                                            IAN01220
         SDSH=0                                                           IAN01230
         DT=0.05                                                          IAN01240
         READ(1,*) M,GLUC,GLY,SIV,TOTTIM,RNMIX                            IAN01250
         GLUCINL=GLUC                                                     IAN01260
         GLYINL=GLY                                                       IAN01270
         S4=SIV                                                           IAN01280
C                                                                         IAN01290
C  CALCULATING THE NUMBER OF TIME PERIODS,N,OF LENGTH 50H AND CYCLES,IN,  IAN01300
C  OF LOOP 20 REQUIRED SO THAT THE CALCULATION COVERS THE ENTIRE REACTIONIAN01310
C                                                                         IAN01320
         A=INT(TOTTIM/50.0)                                               IAN01330
         N=A+1                                                            IAN01340
         IN=N*1000                                                        IAN01350
C                                                                         IAN01360
C  CALCULATING THE CONCENTRATION OF GLUCOSE HYDROXYSULPHONATE AT TIME     IAN01370
C  ZERO.                                                                  IAN01380
C                                                                         IAN01390
         B=(S4+GLUC+EK1)                                                  IAN01400
         SGLUC=(B-(SQRT(B*B-(4*S4*GLUC))))*0.5                            IAN01410
C                                                                         IAN01420
C  CALCULATING THEORETICAL VALUES OF TOTAL,FREE AND REVERSIBLY BOUND      IAN01430
C  S(IV) AT TIME ZERO.                                                    IAN01440
C                                                                         IAN01450
         TOTTHAR(1)=100                                                   IAN01460
         FRTHAR(1)=((S4-SGLUC)/SIV)*100                                   IAN01470
         RBTHAR(1)=(SGLUC/SIV)*100                                        IAN01480
         TICKTHAR(1)=0.0                                                  IAN01490
                                                                          IAN01500
90       READ(1,*)RVBNDEXP,FREEEXP,TOTEXP,CLOCK                          IAN01510
                                                                          IAN01520
C                                                                         IAN01530
C  AFTER LINE 90 HAS READ ITS FIRST SET OF DATA,LOOP 20 IS                IAN01540
C  CONTINUED AND NOT RESTARTED WHEN THE SECOND SET IS READ.               IAN01550
C                                                                         IAN01560
         COUNT=COUNT+1                                                    IAN01570
         IF(COUNT.GT.1) GOTO 20                                           IAN01580
         DO 20 J=1,IN                                                     IAN01590
C                                                                         IAN01600
C  CALCULATING THE AMOUNTS OF REACTANTS,INTERMEDIATES AND PRODUCTS.       IAN01610
C  DC IS THE CHANGE IN THE AMOUNT OF REACTANT OR INTERMEDIATE DURING THE  IAN01620
C  TIME PERIOD DT.                                                        IAN01630
C                                                                         IAN01640
                                                                          IAN01650
```

FILE: IANM1SS FORTRAN A LEEDS UNIVERSITY COMPUTING SERVICE VM/370 5.0 CMS

```
                FREES4=S4-SGLUC-SDH-SDSH                                        IAN01660
                DC=(RK1*(GLUC-SGLUC)*GLY*DT) +  (RK2*(GLUC-SGLUC)*GLY           IAN01670
     *              *FREES4*DT)                                                 IAN01680
                DH=DH+DC                                                        IAN01690
                GLUC=GLUC-DC                                                    IAN01700
                                                                                IAN01710
                DC=(RK3*(DH-SDH)*DT)+(RK4*(DH-SDH)*GLY*DT)                      IAN01720
                DH=DH-DC                                                        IAN01730
                DSH=DSH+DC                                                      IAN01740
                S4=S4-DC                                                        IAN01750
                                                                                IAN01760
                DC=(RK5*FREES4*1E-5*DT)/(GLUC + 1E-5)                           IAN01770
                S4=S4-DC                                                        IAN01780
                DC=(RK5*FREES4*1E-5*DT)/(GLY  + 1E-5)                           IAN01790
                S4=S4-DC                                                        IAN01800
C                                                                               IAN01810
C CALCULATING THE LEVELS OF REVERSIBLY BOUND REACTANTS,INTERMEDIATES            IAN01820
C AND PRODUCTS.                                                                 IAN01830
C                                                                               IAN01840
40              G=SGLUC                                                         IAN01850
                E=SDH                                                           IAN01860
                F=SDSH                                                          IAN01870
                                                                                IAN01880
                B=(GLUC+S4+EK1-SDH-SDSH)                                        IAN01890
                SGLUC=(B-SQRT(B*B-4*GLUC*(S4-SDH-SDSH)))*0.5                    IAN01900
                B=(DH+S4+EK2-SDSH-SGLUC)                                        IAN01910
                SDH=(B-SQRT(B*B-4*DH*(S4-SDSH-SGLUC)))*0.5                      IAN01920
                B=(DSH+S4+EK3-SDH-SGLUC)                                        IAN01930
                SDSH=(B-SQRT(B*B-4*DSH*(S4-SDH-SGLUC)))*0.5                     IAN01940
                  IF (E.EQ.0.OR.F.EQ.0.OR.G.EQ.0) GOTO40                        IAN01950
                    IF (ABS((SGLUC-G)/G).LT.0.001) GOTO 50                      IAN01960
                          GOTO40                                                IAN01970
 50                 IF (ABS((SDH-E)/E).LT.0.001) GOTO 60                       IAN01980
                          GOTO40                                                IAN01990
 60                 IF (ABS((SDSH-F)/F).LT.0.001) GOTO 30                      IAN02000
                          GOTO40                                                IAN02010
 30                 TOCK=TOCK+0.05                                             IAN02020
C                                                                               IAN02030
C IF THE THEORETICAL TIME EXCEEDS THE TOTAL REACTION TIME + 0.05,THEN           IAN02040
C THERE ARE NO MORE DATA POINTS TO BE READ FOR THIS MIXTURE AND LOOP 20          IAN02050
C IS EXITED WITHOUT COMPLETING IN CYCLES.THIS SAVES COMPUTER TIME.              IAN02060
C                                                                               IAN02070
                    IF (TOCK.GT.TOTTIM+0.05) GOTO 80                            IAN02080
C                                                                               IAN02090
C IF THE THEORETICAL TIME IS GREATER THAN OR EQUAL TO THE LAST                  IAN02100
C EXPERIMENTAL TIME READ;THEN LOOP 20 IS EXITED TO CALCULATE RESULTS.           IAN02110
C THE NEXT SET OF DATA POINTS IS READ BY LINE 90 AND LOOP 20 IS REJOINEDIAN02120
C WITHOUT IT BEING RESTARTED.                                                   IAN02130
C                                                                               IAN02140
                    IF (TOCK.GE.CLOCK) GOTO 70                                  IAN02150
20              CONTINUE                                                        IAN02160
C                                                                               IAN02170
C CALCULATING THEORETICAL CONCENTRATIONS,AS A % OF THE ORIGINAL,OF TOTALIAN02180
C FREE AND REVERSIBLY BOUND S(IV).                                              IAN02190
C                                                                               IAN02200
```

FILE: IANM1SS FORTRAN A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

```
70          TTLS4=S4*100/SIV                                                IAN02210
            FRS4=(S4-SGLUC-SDH-SDSH)*100/SIV                                IAN02220
            RBS4=((SGLUC+SDH+SDSH)/SIV)*100                                 IAN02230
C                                                                           IAN02240
C STORING THEORETICAL AND EXPERIMENTAL DATA IN ARRAYS.                      IAN02250
C                                                                           IAN02260
            TOTTHAR(COUNT+1)=TTLS4                                          IAN02270
            FRTHAR(COUNT+1)=FRS4                                            IAN02280
            RBTHAR(COUNT+1)=RBS4                                            IAN02290
            TICKTHAR(COUNT+1)=TOCK                                          IAN02300
C                                                                           IAN02310
            TOTEXAR(COUNT)=TOTEXP                                           IAN02320
            FREXAR(COUNT)=FREEEXP                                           IAN02330
            RBEXAR(COUNT)=RVBNDEXP                                          IAN02340
            TIMEXAR(COUNT)=CLOCK                                            IAN02350
C                                                                           IAN02360
C IF THE LAST DATA POINT HAS BEEN READ PRINT OUT THE RESULTS,               IAN02370
C OTHERWISE READ THE NEXT SET OF DATA.                                      IAN02380
C                                                                           IAN02390
            IF (COUNT.EQ.M) GOTO 80                                         IAN02400
            GOTO 90                                                         IAN02410
C                                                                           IAN02420
C SENDING THE THEORETICAL AND EXPERIMENTAL DATA POINTS TO DATA FILES        IAN02430
C READY FOR PLOTTING.                                                       IAN02440
C                                                                           IAN02450
80        DO 120 C=1,M+1                                                    IAN02460
           WRITE(2,1000)TICKTHAR(C),TOTTHAR(C),FRTHAR(C),                   IAN02470
     *          RBTHAR(C),RNMIX,GLUCINL,GLYINL,SIV,THEODATA                IAN02480
1000        FORMAT(F6.1,1X,F5.1,1X,F5.1,1X,F5.1,1X,I2,1X,F6.4,1X,F6.4,      IAN02490
     *          1X,F6.4,1X,I2)                                             IAN02500
120       CONTINUE                                                          IAN02510
          DO 130 C=1,M                                                      IAN02520
            WRITE(3,1200)TIMEXAR(C),TOTEXAR(C),FREXAR(C),                   IAN02530
     *          RBEXAR(C),RNMIX,GLUCINL,GLYINL,SIV,EXPDATA                 IAN02540
1200        FORMAT(F6.1,1X,F5.1,1X,F5.1,1X,F5.1,1X,I2,1X,F6.4,1X,F6.4,      IAN02550
     *          1X,F6.4,1X,I2)                                             IAN02560
130       CONTINUE                                                          IAN02570
 10     CONTINUE                                                           IAN02580
        STOP                                                               IAN02590
        END                                                                IAN02600
```

FILE: IANM1TB FORTRAN A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

```
      PROGRAM IANM1TB                                                    IAN00010
                                                                         IAN00020
C A PROGRAM TO CALCULATE THE THEORETICAL CONCENTRATIONS OF TOTAL,FREE &  IAN00030
C REVERSIBLY BOUND S(IV),AT THE TIMES WHEN EXPERIMENTAL MEASUREMENTS     IAN00040
C WERE MADE.                                                             IAN00050
C THE RATE AND EQUILIBRIUM CONSTANTS ARE VARIED SYSTEMATICALLY, UNTILL   IAN00060
C THECRETICAL AND EXPERIMENTAL RESULTS SHOW THE MAXIMUM DEGREE OF        IAN00070
C CORRELATION.                                                           IAN00080
C THE AVERAGE SQUARED DEVIATIONS BETWEEN PREDICTED AND OBSERVED VALUES   IAN00090
C OF TOTAL,FREE AND REVERSIBLY BOUND S(IV) ARE CALCULATED FOR EACH       IAN00100
C REACTION MIXTURE.THE MEANS OF THESE VALUES FOR TOTAL AND FREE S(IV)    IAN00110
C ARE CALCULATED,THESE BEING ADDED TO PRODUCE THE SUM OF THE MEANS FOR   IAN00120
C THE KINETIC RUN AS A WHOLE.                                            IAN00130
                                                                         IAN00140
                                                                         IAN00150
                                                                         IAN00160
C                                                                        IAN00170
C DECLARING VARIABLES AND ARRAYS.                                        IAN00180
C                                                                        IAN00190
                                                                         IAN00200
      REAL*8  GLUC,GLY,SIV,RK1,RK2,RK3,RK4,RK5,EK1,EK2,EK3,DSH,DH,       IAN00210
     *        SGLUC,SDH,SDSH,S4,DC,G,E,F,B,TILS4,FRS4,FREES4,RBS4,       IAN00220
     *        TOTDEV,FRDEV,RBDEV,TOTEXP,FREEEXP,RVBNDEXP,                IAN00230
     *        TOTDIFF,FRDIFF,PBDIFF,TOTS4DVN,FRS4DVN,PBS4DVN,            IAN00240
     *        TOCK,TOTTIM,SUM1,SUM2,SUM3,DT,CLOCK,                       IAN00250
     *        TOTDVN,FREEDVN,RVBNDDVN,TICK,GLUCINL,GLYINL                IAN00260
     *        SUMTOTDV,SUMFREDV,MNSUMTOT,MNSUMFRE,SUMOFMNS               IAN00270
      INTEGER I,N,J,L,A,C,RNMIX,COUNT,M,IN                               IAN00280
C                                                                        IAN00290
C INTRODUCING ARRAYS TO STORE DATA.                                      IAN00300
C                                                                        IAN00310
      DIMENSION TOTDVN(50),FREEDVN(50),RVBNDDVN(50),TICK(50)             IAN00320
      READ(1,*)L                                                         IAN00330
                                                                         IAN00340
C                                                                        IAN00350
C STATING VALUES OF RATE AND EQUILIBRIUM CONSTANTS RESPECTIVLY.          IAN00360
C                                                                        IAN00370
                                                                         IAN00380
C                                                                        IAN00390
C VALUES USED,WITHOUT ADJUSTMENT,WHEN GLUCOSE CONCENTRATION WAS VARIED   IAN00400
C AT AW=1                                                                IAN00410
C                                                                        IAN00420
                                                                         IAN00430
C     RK1= 5.45570E-5                                                    IAN00440
C     RK2= (2.02627E-3/5.45570E-5)*RK1                                   IAN00450
C     RK3= 1.7E-3                                                        IAN00460
C     RK4= (7.0E-3/1.7E-3)*RK3                                           IAN00470
C     EK1= 1.33                                                          IAN00480
C     EK2= 0.004                                                         IAN00490
C     EK3= 0.0044                                                        IAN00500
                                                                         IAN00510
                                                                         IAN00520
                                                                         IAN00530
                                                                         IAN00540
C                                                                        IAN00550
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FILE: IANM1TB  FORTRAN  A   LEEDS UNIVERSITY COMPUTING SERVICE VM/4PO 5.0 CMS

C  ADJUSTED VALUES USED WHEN GLUCOSE CONCENTRATION WAS VARIED AT AW=1     IAN00560
C                                                                         IAN00570
                                                                          IAN00580
C      RK1= 5.45570E-5*2                                                  IAN00590
C      RK2= (2.02627E-3/5.45570E-5)*RK1                                   IAN00600
C      RK3= 1.7E-3*0.85                                                   IAN00610
C      RK4= (7.0E-3/1.7E-3)*RK3                                           IAN00620
C      RK5= 15                                                            IAN00630
C      EK1= 1                                                             IAN00640
C      EK2= 0.004*70                                                      IAN00650
C      EK3= 0.0044*0.15                                                   IAN00660
                                                                          IAN00670
                                                                          IAN00680
                                                                          IAN00690
C                                                                         IAN00700
C  ADJUSTED VALUES USED WHEN GLYCINE CONCENTRATION WAS VARIED AT AW=1     IAN00710
C                                                                         IAN00720
                                                                          IAN00730
C      RK1= 5.45570E-5*1.6                                                IAN00740
C      RK2= (2.02627E-3/5.45570E-5)*RK1                                   IAN00750
C      RK3= 1.7E-3*0.6                                                    IAN00760
C      RK4= (7.0E-3/1.7E-3)*RK3                                           IAN00770
C      RK5= 25                                                            IAN00780
C      EK1= 1                                                             IAN00790
C      EK2= 0.004*70                                                      IAN00800
C      EK3= 0.0044*0.15                                                   IAN00810
                                                                          IAN00820
                                                                          IAN00830
                                                                          IAN00840
C                                                                         IAN00850
C  ADJUSTED VALUES USED WHEN S(IV) CONCENTRATION WAS VARIED AT AW=1       IAN00860
C                                                                         IAN00870
                                                                          IAN00880
C      RK1= 5.45570E-5*3.5                                                IAN00890
C      RK2= (2.02627E-3/5.45570E-5)*RK1                                   IAN00900
C      RK3= 1.7E-3*0.3                                                    IAN00910
C      RK4= (7.0E-3/1.7E-3)*RK3                                           IAN00920
C      RK5= 0                                                             IAN00930
C      EK1= 1                                                             IAN00940
C      EK2= 0.004*70                                                      IAN00950
C      EK3= 0.0044*0.15                                                   IAN00960
                                                                          IAN00970
                                                                          IAN00980
                                                                          IAN00990
C                                                                         IAN01000
C  ADJUSTED VALUES USED WHEN S(IV) CONCENTRATION WAS VARIED AT REDUCED AWIAN01010
C                                                                         IAN01020
                                                                          IAN01030
       RK1= 1.31925E-4*4.05                                               IAN01040
       RK2= (4.74373E-3/1.31925E-4)*RK1                                   IAN01050
       RK3= 1.7E-3*0.3                                                    IAN01060
       RK4= (7.0E-3/1.7E-3)*RK3                                           IAN01070
       RK5= 0                                                             IAN01080
       EK1= 1.33                                                          IAN01090
       EK2= 0.004*70                                                      IAN01100
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FILE: IANM1TB  FORTRAN  A   LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

      EK3= 0.0044*0.15                                                  IAN01110
                                                                        IAN01120
C                                                                       IAN01130
C  RESETTING THE VARIABLES THAT ARE USED TO CALCULATE THE SUM OF THE    IAN01140
C  AVERAGE SQUARED DEVIATIONS FOR TOTAL AND FREE S(IV) CONCENTRATIONS   IAN01150
C                                                                       IAN01160
                                                                        IAN01170
      SUMTOTDV=0                                                        IAN01180
      SUMFREDV=0                                                        IAN01190
      SUMOFMNS=0                                                        IAN01200
      WRITE(2,1400)                                                     IAN01210
1400  FORMAT('KEY FOR ABBREVIATIONS.',///,'COUNT=THE DATA POINT NUMBER.',IAN01220
     *'TH=THEORETICAL VALUES.   EX=EXPERIMENTAL VALUES.',//,            IAN01230
     *'TOT=TOTAL [S(IV)]. FR=FREE [S(IV)]. RB=REVERSIBLY BOUND [S(IV)].'IAN01240
     *,//,'EACH [S(IV)] IS AS A % OF THE ORIGINAL [S(IV)].'             IAN01250
     *,//,'TIM=TIME. RNMIX=REACTION MIXTURE N.O. A=N-1.',//,            IAN01260
     *'N=N.O. OF TIME SEGMENTS OF 50H. IN=N.O. OF CYCLES OF LOOP 20.',//,IAN01270
     *'DT=STEP TIME LENGTH. M=N.O. OF DATA POINTS. TOCK=TH TIME.',//,   IAN01280
     *'AV SQ DVN=AVERAGE SQUARED DEVIATION PER PAIR OF TH & EX VALUES.',IAN01290
     */,'TOT AV SQ DVN=TOTAL AV SQ DVN.',                               IAN01300
     */,'RK=RATE CONSTANT. EK=EQUILIBRIUM CONSTANT.')                   IAN01310
C                                                                       IAN01320
C  LOOP 10.THERE ARE L REACTION MIXTURES.                               IAN01330
C                                                                       IAN01340
      DO 10 I=1,L                                                       IAN01350
         GLUC=0                                                         IAN01360
         S4=0                                                           IAN01370
         COUNT=0                                                        IAN01380
         SUM1=0                                                         IAN01390
         SUM2=0                                                         IAN01400
         SUM3=0                                                         IAN01410
         DSH=0                                                          IAN01420
         DH=0                                                           IAN01430
         TOCK=0                                                         IAN01440
         N=0                                                            IAN01450
         SGLUC=0                                                        IAN01460
         SDH=0                                                          IAN01470
         SDSH=0                                                         IAN01480
         DT=0.05                                                        IAN01490
         READ(1,*) M,GLUC,GLY,SIV,TOTTIM,RNMIX                          IAN01500
         WRITE(2,1200)                                                  IAN01510
1200      FORMAT(/,'COUNT',3X,'TOT TH',3X,'TOT EX',4X,'FR TH',4X,'FR EX'IAN01520
     *     ,4X,'RB TH',4X,'RB EX',4X,'TIM TH',4X,'TIM EX',4X,'RNMIX')   IAN01530
C                                                                       IAN01540
C  CALCULATING THE NUMBER OF TIME PERIODS,N,OF LENGTH 50H AND CYCLES,IN, IAN01550
C  OF LOOP 20 REQUIRED SO THAT THE CALCULATION COVERS THE ENTIRE REACTIONIAN01560
C                                                                       IAN01570
         A=INT(TOTTIM/50.0)                                             IAN01580
         N=A+1                                                          IAN01590
         IN=N*1000                                                      IAN01600
         GLUCINL=GLUC                                                   IAN01610
         GLYINL=GLY                                                     IAN01620
         S4=SIV                                                         IAN01630
C                                                                       IAN01640
C  CALCULATING THE CONCENTRATION OF GLUCOSE HYDROXYSULPHONATE AT TIME   IAN01650
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FILE: IANM1TB  FORTRAN  A   LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

C ZERO.                                                                 IAN01660
C                                                                       IAN01670
         B=(S4+GLUC+EK1)                                                IAN01680
         SGLUC=(B-(SQRT(B*B-(4*S4*GLUC))))*0.5                          IAN01690
90       READ(1,*)RVBNDEXP,FREEEXP,TOTEXP,CLOCK                        IAN01700
C                                                                       IAN01710
C AFTER LINE 90 HAS READ ITS FIRST SET OF DATA,LOOP 20 IS               IAN01720
C CONTINUED AND NOT RESTARTED WHEN THE SECOND SET IS READ.              IAN01730
C                                                                       IAN01740
         COUNT=COUNT+1                                                  IAN01750
         IF(COUNT.GT.1)GOTO 20                                          IAN01760
         DO 20 J=1,IN                                                   IAN01770
               FREES4=S4-SGLUC-SDH-SDSH                                 IAN01780
C                                                                       IAN01790
C CALCULATING THE AMOUNTS OF REACTANTS,INTERMEDIATES AND PRODUCTS.      IAN01800
C DC IS THE CHANGE IN THE AMOUNT OF REACTANT OR INTERMEDIATE DURING THE IAN01810
C TIME PERIOD DT.                                                       IAN01820
C                                                                       IAN01830
               DC=(RK1*(GLUC-SGLUC)*GLY*DT) +  (RK2*(GLUC-SGLUC)*G_Y    IAN01840
     *             *FREES4*DT)                                          IAN01850
               DH=DH+DC                                                 IAN01860
               GLUC=GLUC-DC                                             IAN01870
                                                                        IAN01880
               DC=(RK3*(DH-SDH)*DT)+(RK4*(DH-SDH)*GLY*DT)               IAN01890
               DH=DH-DC                                                 IAN01900
               DSH=DSH+DC                                               IAN01910
               S4=S4-DC                                                 IAN01920
                                                                        IAN01930
               DC=(RK5*FREES4*1E-5*DT)/(GLUC + 1E-5)                    IAN01940
               S4=S4-DC                                                 IAN01950
               DC=(RK5*FREES4*1E-5*DT)/(GLY  + 1E-5)                    IAN01960
               S4=S4-DC                                                 IAN01970
                                                                        IAN01980
C                                                                       IAN01990
C CALCULATING THE LEVELS OF REVERSIBLY BOUND REACTANTS,INTERMEDIATES    IAN02000
C AND PRODUCTS.                                                         IAN02010
C                                                                       IAN02020
40             G=SGLUC                                                  IAN02030
               E=SDH                                                    IAN02040
               F=SDSH                                                   IAN02050
                                                                        IAN02060
               B=(GLUC+S4+EK1-SDH-SDSH)                                 IAN02070
               SGLUC=(B-SQRT(B*B-4*GLUC*(S4-SDH-SDSH)))*0.5             IAN02080
               B=(DH+S4+EK2-SDSH-SGLUC)                                 IAN02090
               SDH=(B-(SQRT(B*B-4*DH*(S4-SDSH-SGLUC))))*0.5             IAN02100
               B=(DSH+S4+EK3-SDH-SGLUC)                                 IAN02110
               SDSH=(B-SQRT(B*B-4*DSH*(S4-SDH-SGLUC)))*0.5              IAN02120
                  IF (E.EQ.0.OR.F.EQ.0.OR.G.EQ.0) GOTO40                IAN02130
                     IF (ABS((SGLUC-G)/G).LT.0.001) GOTO 50             IAN02140
                        GOTO40                                          IAN02150
 50                  IF (ABS((SDH-E)/E).LT.0.001) GOTO 60               IAN02160
                        GOTO40                                          IAN02170
 60                  IF (ABS((SDSH-F)/F).LT.0.001) GOTO 30              IAN02180
                        GOTO40                                          IAN02190
 30                  TOCK=TOCK+0.05                                     IAN02200
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FILE: IANM1TB  FORTRAN  A   LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

C                                                                       IAN02210
C IF THE THEORETICAL TIME EXCEEDS THE TOTAL REACTION TIME + 0.05,THEN   IAN02220
C THERE ARE NO MORE DATA POINTS TO BE READ FOR THIS MIXTURE AND LOOP 20 IAN02230
C IS EXITED WITHOUT COMPLETING IN CYCLES.THIS SAVES COMPUTER TIME.      IAN02240
C                                                                       IAN02250
                      IF (TOCK.GT.TOTTIM+0.05) GOTO 80                  IAN02260
C                                                                       IAN02270
C IF THE THEORETICAL TIME IS GREATER THAN OR EQUAL TO THE LAST          IAN02280
C EXPERIMENTAL TIME READ;THEN LOOP 20 IS EXITED TO CALCULATE RESULTS.   IAN02290
C THE NEXT SET OF DATA POINTS IS READ BY LINE 90 AND LOOP 20 IS REJOINEDIAN02300
C WITHOUT IT BEING RESTARTED.                                           IAN02310
C                                                                       IAN02320
                      IF (TOCK.GE.CLOCK) GOTO 70                        IAN02330
 20         CONTINUE                                                    IAN02340
C                                                                       IAN02350
C CALCULATING THEORETICAL CONCENTRATIONS,AS A % OF THE ORIGINAL,OF TOTAL IAN02360
C FREE AND REVERSIBLY BOUND S(IV).                                      IAN02370
C                                                                       IAN02380
 70         TTLS4=S4*100/SIV                                           IAN02390
            FRS4=(S4-SGLUC-SDH-SDSH)*100/SIV                           IAN02400
            RBS4=((SGLUC+SDH+SDSH)/SIV)*100                            IAN02410
C                                                                       IAN02420
C CALCULATING THE SQUARES OF THE DEVIATIONS & STORING THEM IN ARRAYS.   IAN02430
C                                                                       IAN02440
            TOTDIFF=TTLS4-TOTEXP                                       IAN02450
            TOTDEV=TOTDIFF*TOTDIFF                                     IAN02460
            FRDIFF=FRS4-FREEEXP                                        IAN02470
            FRDEV=FRDIFF*FRDIFF                                        IAN02480
            RBDIFF=RBS4-RVBNDEXP                                       IAN02490
            RBDEV=RBDIFF*RBDIFF                                        IAN02500
                                                                       IAN02510
            TOTDVN(COUNT)=TOTDEV                                       IAN02520
            FREEDVN(COUNT)=FRDEV                                       IAN02530
            RVBNDDVN(COUNT)=RBDEV                                      IAN02540
            TICK(COUNT)=TOCK                                           IAN02550
      WRITE(2,1000)COUNT,TTLS4,TOTEXP,FRS4,FREEEXP,PBS4,RVBNDEXP,TOCK  IAN02560
     *             ,CLOCK,RNMIX                                        IAN02570
1000  FORMAT(I3,5X,F5.1,4X,F5.1,4X,F5.1,4X,F5.1,4X,F5.1,4X,F5.1,4X,     IAN02580
     *       F6.1,4X,F6.1,5X,I3)                                       IAN02590
C                                                                       IAN02600
C IF THE LAST DATA POINT HAS BEEN READ ADD UP THE DEVIATIONS,           IAN02610
C OTHERWISE READ THE NEXT SET OF DATA.                                  IAN02620
C                                                                       IAN02630
      IF (COUNT.EQ.M) GOTO 80                                          IAN02640
         GOTO 90                                                       IAN02650
80    CONTINUE                                                         IAN02660
C                                                                       IAN02670
C ADDING UP THE DEVIATIONS FOR TOTAL,FREE & REVERSIBLY BOUND S(IV)      IAN02680
C & DIVIDING BY THE NUMBER OF DATA POINTS.SUMMING THE AVERAGE SQUARED   IAN02690
C DEVIATIONS FOR TOTAL AND FREE S(IV).                                  IAN02700
C                                                                       IAN02710
      DO 120 C=1,COUNT                                                 IAN02720
120      SUM1=SUM1+TOTDVN(C)                                           IAN02730
         TOTS4DVN=SUM1/COUNT                                           IAN02740
         SUMTOTDV=SUMTOTDV + TOTS4DVN                                  IAN02750
```

FILE: IANM1TB FORTRAN A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

```
                                                                      IAN02760
      DO 130 C=1,COUNT                                                IAN02770
130      SUM2=SUM2+FREEDVN(C)                                         IAN02780
         FRS4DVN=SUM2/COUNT                                           IAN02790
         SUMFREDV=SUMFREDV + FRS4DVN                                  IAN02800
                                                                      IAN02810
      DO 140 C=1,COUNT                                                IAN02820
140      SUM3=SUM3+RVBNDDVN(C)                                        IAN02830
         RBS4DVN=SUM3/COUNT                                           IAN02840
                                                                      IAN02850
                                                                      IAN02860
      WRITE(2,1100)A,N,IN,DT,COUNT,M,TOCK,TOTTIM,TOTS4DVN,FRS4DVN,    IAN02870
     *             RBS4DVN,RNMIX,GLUCINL,GLYINL,SIV                   IAN02880
1100  FORMAT(/,'A=',I4,/,'N =',I4,/,'IN =',I7,/,'DT =',F7.5,'H',      IAN02890
     *   /,'COUNT =',                                                 IAN02900
     *   I4,/,'M =',1X,I4,/,'TOCK =',1X,F8.1,'H',/,'TOTAL TIME =',F8.1, IAN02910
     *   'H',/,'AV SQ DVN TOTS4 =',1X,F8.1,/,'AV SQ DVN FRS4 =',1X,   IAN02920
     *   F8.1,/,'AV SQ DVN RBS4 =',1X,F8.1,/,'RNMIX =',1X,I3,/,       IAN02930
     *   'FOR THE MIXTURE OF COMPOSITION [GLUCOSE] =',1X,F3.1,'M',    IAN02940
     *   2X,'[GLYCINE] =',1X,F3.1,'M',2X,'[S(IV)] =',1X,F6.4,'M',///) IAN02950
                                                                      IAN02960
 10   CONTINUE                                                        IAN02970
                                                                      IAN02980
C                                                                     IAN02990
C CALCULATING THE MEANS OF THE AVERAGE SQUARED DEVIATIONS FOR TOTAL AND IAN03000
C FREE S(IV),AND THE SUM OF THESE TWO MEANS.                          IAN03010
C                                                                     IAN03020
                                                                      IAN03030
      MNSUMTOT=SUMTOTDV/L                                             IAN03040
      MNSUMFRE=SUMFREDV/L                                             IAN03050
                                                                      IAN03060
      SUMOFMNS=MNSUMTOT + MNSUMFRE                                    IAN03070
                                                                      IAN03080
      WRITE(2,1300)MNSUMTOT,MNSUMFRE,SUMOFMNS,                        IAN03090
     *             RK1,RK2,RK3,RK4,RK5,EK1,EK2,EK3                    IAN03100
1300  FORMAT(/,                                                       IAN03110
     * 'THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR TOTAL S(IV) =', IAN03120
     * 1X,F8.1,/,                                                     IAN03130
     * 'THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR FREE S(IV) =', IAN03140
     * 1X,F8.1,/,                                                     IAN03150
     * 'THE SUM OF THE MEANS FOR THE KINETIC RUN AS A WHOLE =',1X,F8.1, IAN03160
     * //,'RK1=',F12.10,'ME-1HE-1',4X,                               IAN03170
     * 'RK2=',F12.10,'ME-2HE-1',4X,'RK3=',F12.10,'HE-1',/,           IAN03180
     * 'RK4=',F12.10,'ME-1HE-1',4X,'RK5=',F7.1,'MHE-1',              IAN03190
     *  /,'EK1=',1X,F9.6,'M',4X,'EK2=',1X,F9.6,'M',4X,'EK3=',1X,F9.6, IAN03200
     * 'M')                                                           IAN03210
      STOP                                                            IAN03220
      END                                                             IAN03230
```

```
FILE: IANM1    SAS      A   LEEDS UNIVERSITY COMPUTING SERVICE VM/4PO 5.0 CMS

      cms fi IN1 DISK sulfrn19 expltsiv;
      data EX;
      keep time  yout key   glucose rnmix datatype glycine siv;
      infile in1;
      input time  totalEX freeEX rvbndex rnmix glucose glycine siv
            datatype;
      yout=totalex;key=1;output;
      yout=freeex;key=2;output;
      yout=rvbndex;key=3;output;

      cms fi IN2 DISK sulfrn19 thpltsiv;
      data TH;
      keep time glucose rnmix datatype glycine siv yout key;
      infile in2;
      input time totalth freeth rvbndth rnmix glucose glycine siv
            datatype;
      yout=totalth;key=4;output;
      yout=freeth;key=5;output;
      yout=rvbndth;key=6;output;


      % include gdevices;
      i3192g;
/*    ibmpc;
      hp7550;
      plotfile r12kmsiv; */

      PROC PRINT DATA=EX;TITLE 'EXPERIMENTAL DATA';
      PROC PRINT DATA=TH;TITLE 'THEORETICAL DATA ';


      DATA BOTH;
      MERGE TH EX ;
      by rnmix datatype;

      proc sort;
/*    by glucose;
      by glycine; */
      by siv;

      proc format;
           value  lend
              1='Total'
              2='Free '
              3='Reversibly bound'
              4=' '
              5='.'
              6=',';
      run;


      PROC PRINT data=both;TITLE 'BOTH SETS OF DATA';

/*    proc gplot DATA=BOTH ;     */
      proc gplot DATA=BOTH  gout=km.catalg;
```

```
FILE: IANM1    SAS      A   LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

/*     by glucose;
       by glycine;  */
       by siv;
       title;

       axis1  label=(a=90 '[S(IV)]/% of original')
              order=-20 to 100 by  10
              length=9  CM;

       axis2  label=('Time/h')
              order=0 to  500 by 100
              length=16 CM ;
/*
footnote1 j=l
'Fig.4.1.     Sulphur(IV) concentration vs time for mixtures of composition ';
footnote2 j=l
'[glucose]=0.4-2.0M,[glycine]=0.5M,[S(IV)]=0.056-0.0438M. Lines denote ';
footnote3 j=l
'calculated values,symbols denote observed values. Here the constants have not';
footnote4 j=l
'been corrected,hence a poor quality fit has been obtained.';

footnote1 j=l
'Fig.4.1.     Sulphur(IV) concentration vs time for mixtures of composition ';
footnote2 j=l
'[glucose]=0.4-2.0M,[glycine]=0.5M,[S(IV)]=0.056-0.0438M. Lines denote';
footnote3 j=l
'calculated values,symbols denote observed values.';


footnote1 j=l
'Fig.4.1.     Sulphur(IV) concentration vs time for mixtures of composition ';
footnote2 j=l
'[glucose]=1.0M,[glycine]=0.2-1.0M,[S(IV)]=0.046-0.042?M. Lines denote';
footnote3 j=l
'calculated values,symbols denote observed values.';

footnote1 j=l
'Fig.4.1.     Sulphur(IV) concentration vs time for mixtures of composition ';
footnote2 j=l
'[glucose]=1.0M,[glycine]=0.5M,[S(IV)]=0.037?-0.0?30M. Lines denote';
footnote3 j=l
'calculated values,symbols denote observed values.';

       */
footnote1 j=l
'Fig.4.2.     Sulphur(IV) concentration vs time for mixtures of composition ';
footnote2 j=l
'[glucose]=1.0M,[glycine]=0.5M,[S(IV)]=0.0153-0.0?19M at an aw of 0.7?-0.79.';
footnote3 j=l
'Lines denote calculated values,symbols denote observed values.';
```

```
FILE: IANM1    SAS      A   LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

      plot   yout*time=key
             /frame overlay vaxis=axis1 haxis=axis2
             href=0 to   500 by   50 chref=orange
             vref=-20 to 100 by 10 cvref=orange;
            format key  land.;

      symbol1 v=square c=red;
      symbol2 v=triangle c=purple;
      symbol3 v=- c=green;
      symbol4 v=none i=join c=red;
      symbol5 v=none i=join c=purple;
      symbol6 v=none i=join c=green;
```

APPENDIX 2

FILE: S4R11TBL NOFITGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/4?0 5.0 CMS

KEY FOR ABBREVIATIONS.

COUNT=THE DATA POINT NUMBER.TH=THEORETICAL VALUES. EX=EXPERIMENTAL VALUES.
TOT=TOTAL [S(IV)]. FR=FREE [S(IV)]. RB=REVERSIBLY BOUND [S(IV)].
EACH [S(IV)] IS AS A % OF THE ORIGINAL [S(IV)].
TIM=TIME. RNMIX=REACTION MIXTURE N.O. A=N-1.
N=N.O. OF TIME SEGMENTS OF 50H. IN=N.O. OF CYCLES OF LOOP 20.
DT=STEP TIME LENGTH. M=N.O. OF DATA POINTS. TOCK=TH TIME.
AV SQ DVN=AVERAGE SQUARED DEVIATION PER PAIR OF TH & EX VALUES.
TOT AV SQ DVN=TOTAL AV SQ DVN.
RK=RATE CONSTANT. EK=EQUILIBRIUM CONSTANT.

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	99.9	77.0	72.7	23.0	27.2	6.8	6.8	1
2	100.0	98.3	76.4	69.8	23.6	28.4	21.6	21.6	1
3	100.0	101.1	75.4	72.8	24.5	28.3	45.0	45.0	1
4	99.7	86.0	72.4	61.0	27.3	25.0	119.3	119.3	1
5	99.6	85.0	71.4	60.8	28.2	24.2	144.3	144.3	1
6	99.5	74.9	70.5	50.4	29.0	24.5	165.1	165.1	1
7	99.3	75.2	69.3	51.7	30.0	23.5	193.7	193.7	1
8	99.1	80.6	68.5	56.1	30.6	24.5	213.0	213.0	1
9	98.4	75.0	65.1	52.6	33.2	22.4	291.5	291.5	1
10	98.1	60.7	64.2	40.4	33.9	20.4	313.0	313.0	1
11	97.7	74.1	62.9	53.5	34.8	20.6	342.3	342.3	1
12	97.5	59.2	62.1	39.2	35.4	20.0	362.4	362.4	1
13	97.2	58.6	61.2	38.9	36.0	19.7	382.2	382.2	1
14	96.0	51.6	58.0	33.6	38.0	18.0	454.9	454.9	1
15	95.5	43.7	56.8	28.3	38.7	15.3	481.6	481.6	1
16	95.0	47.6	55.6	30.1	39.4	17.5	508.9	508.9	1
17	94.6	37.8	54.7	23.0	39.9	14.8	527.8	527.8	1
18	94.1	39.3	53.7	23.2	40.4	16.0	549.6	549.6	1
19	92.5	31.6	50.4	15.9	42.1	15.7	623.7	623.7	1
20	91.9	28.7	49.3	13.7	42.6	14.9	647.4	647.4	1
21	91.1	25.7	48.0	10.2	43.1	15.5	675.4	675.4	1
22	90.6	26.7	47.2	11.8	43.5	14.9	694.1	694.1	1
23	90.0	23.6	46.0	8.7	43.9	14.9	719.0	719.0	1
24	87.8	17.6	42.8	4.9	45.1	12.7	790.7	790.7	1
25	87.0	17.6	41.6	4.9	45.4	12.8	816.6	816.6	1
26	86.1	15.7	40.3	4.0	45.8	11.7	845.0	845.0	1
27	85.3	12.3	39.2	2.5	46.1	9.8	869.2	869.2	1
28	84.5	13.0	38.2	2.4	46.3	10.5	890.9	890.9	1
29	81.9	10.3	35.1	1.6	46.9	8.7	960.7	960.7	1

A= 19
N = 20
IN = 20000
DT =0.05000H
COUNT = 29
M = 29
TOCK = 960.7H
TOTAL TIME = 960.7H
AV SQ DVN TOTS4 = 2457.2
AV SQ DVN FRS4 = 737.3
AV SQ DVN RBS4 = 518.7
RNMIX = 1

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FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 0.4M [GLYCINE] = 0.5M [S(IV)] = 0.0365M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	99.9	62.5	56.0	37.4	43.9	5.9	5.9	2
2	100.0	97.1	61.7	55.6	38.3	41.5	20.7	20.7	2
3	99.9	97.4	60.4	56.0	39.5	41.4	44.0	44.0	2
4	99.5	92.9	56.3	50.4	43.2	42.6	118.3	118.3	2
5	99.2	88.6	54.8	46.7	44.4	41.9	144.1	144.1	2
6	99.0	85.8	53.7	44.0	45.3	41.8	164.1	164.1	2
7	98.6	89.1	52.0	46.1	46.5	43.1	192.7	192.7	2
8	98.3	87.1	50.9	44.6	47.3	42.5	212.0	212.0	2
9	96.7	82.5	46.5	39.7	50.3	42.8	290.5	290.5	2
10	96.2	77.0	45.2	34.7	51.0	42.3	312.0	312.0	2
11	95.5	71.3	43.6	30.1	51.8	41.1	339.5	339.5	2
12	94.9	72.0	42.4	29.0	52.5	43.0	361.5	361.5	2
13	94.3	75.6	41.3	32.6	53.0	43.0	381.2	381.2	2
14	91.8	67.5	37.1	25.3	54.7	42.2	453.9	453.9	2
15	90.7	64.7	35.6	22.8	55.2	42.0	480.6	480.6	2
16	89.6	62.9	34.0	21.7	55.6	41.2	507.9	507.9	2
17	88.8	60.3	33.0	19.0	55.8	41.2	526.8	526.8	2
18	87.8	56.7	31.7	16.6	56.0	40.1	548.5	548.5	2
19	84.0	48.4	27.6	9.7	56.4	38.7	622.5	622.5	2
20	82.7	44.0	26.3	7.9	56.3	36.1	646.5	646.5	2
21	81.0	41.5	24.8	6.0	56.2	35.5	674.7	674.7	2
22	79.9	39.3	23.9	5.2	56.0	34.1	693.1	693.1	2
23	78.3	35.0	22.6	4.2	55.7	30.8	718.1	718.1	2
24	73.3	23.2	19.0	2.4	54.4	20.9	789.8	789.8	2
25	71.4	19.2	17.7	1.6	53.7	17.6	815.6	815.6	2
26	69.2	14.3	16.4	1.5	52.8	12.8	844.0	844.0	2
27	67.3	10.9	15.4	1.3	51.9	9.6	868.3	868.3	2
28	65.5	9.1	14.4	1.3	51.0	7.8	889.9	889.9	2

A= 17
N = 18
IN = 18000
DT =0.05000H
COUNT = 28
M = 28
TOCK = 889.9H
TOTAL TIME = 889.9H
AV SG DVN TOTS4 = 1028.6
AV SG DVN FRS4 = 166.8
AV SG DVN RBS4 = 410.1
RNMIX = 2
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 0.8M [GLYCINE] = 0.5M [S(IV)] = 0.0398M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	52.7	45.9	47.3	54.2	4.4	4.4	3
2	100.0	99.0	51.8	46.5	48.2	52.5	19.2	19.2	3

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3	99.9	98.7	50.3	46.4	49.6	52.2	42.5	42.5	3
4	99.2	98.5	45.7	40.5	53.5	58.0	116.8	116.8	3
5	98.8	90.5	44.0	37.6	54.8	52.9	142.7	142.7	3
6	98.4	87.8	42.8	35.7	55.7	52.1	162.6	162.6	3
7	97.8	86.9	41.0	34.1	56.8	52.8	191.3	191.3	3
8	97.4	87.7	39.8	35.3	57.6	52.4	210.5	210.5	3
9	94.9	78.7	34.9	28.3	60.0	50.5	289.0	289.0	3
10	94.1	73.2	33.5	23.3	60.5	49.9	310.5	310.5	3
11	92.9	63.1	31.9	16.3	61.1	46.7	338.0	338.0	3
12	91.9	65.0	30.5	17.2	61.4	47.8	360.0	360.0	3
13	91.0	64.4	29.3	17.4	61.7	47.0	379.8	379.8	3
14	86.9	52.3	24.9	9.5	62.0	42.8	452.4	452.4	3
15	85.2	46.4	23.4	7.4	61.8	39.0	479.1	479.1	3
16	83.3	41.4	21.8	5.4	61.5	36.0	506.4	506.4	3
17	81.9	36.7	20.8	3.7	61.1	33.0	525.4	525.4	3
18	80.2	32.3	19.6	3.1	60.7	29.2	547.0	547.0	3
19	73.9	16.3	15.7	1.8	58.2	14.5	621.1	621.1	3
20	71.6	10.7	14.5	1.6	57.1	9.2	645.0	645.0	3
21	68.8	8.0	13.2	1.7	55.6	6.3	673.2	673.2	3
22	66.9	6.4	12.3	1.9	54.5	4.5	691.6	691.6	3

A= 13
N = 14
IN = 14000
DT =0.050000H
COUNT = 22
M = 22
TOCK = 691.6H
TOTAL TIME = 691.6H
AV SQ DVN TOTS4 = 1208.7
AV SQ DVN FRS4 = 132.2
AV SQ DVN RBS4 = 610.3
RNMIX = 3
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.2M [GLYCINE] = 0.5M [S(IV)] = 0.0414M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	99.9	45.3	39.5	54.7	60.4	6.6	6.6	4
2	100.0	98.4	44.4	38.8	55.6	59.6	21.4	21.4	4
3	99.8	97.3	42.9	38.3	57.0	59.0	44.7	44.7	4
4	98.9	93.1	38.1	33.1	60.8	60.0	119.1	119.1	4
5	98.4	89.2	36.4	29.4	61.9	59.8	144.9	144.9	4
6	97.9	87.1	35.1	27.8	62.7	59.3	164.8	164.8	4
7	97.0	87.7	33.3	28.6	63.7	59.1	193.5	193.5	4
8	96.4	86.0	32.1	26.7	64.3	59.3	212.8	212.8	4
9	93.1	72.3	27.2	17.4	65.9	54.9	291.3	291.3	4
10	91.9	67.5	25.9	14.0	66.1	53.5	312.8	312.8	4
11	90.3	56.8	24.2	8.9	66.2	47.9	340.3	340.3	4
12	89.0	55.9	22.9	7.9	66.1	48.0	362.2	362.2	4
13	87.6	50.5	21.7	6.2	65.9	44.3	382.0	382.0	4
14	81.9	30.0	17.6	2.6	64.4	27.4	454.7	454.7	4
15	79.5	21.2	16.1	2.3	63.4	18.9	481.4	481.4	4
16	76.8	12.9	14.7	2.2	62.2	10.8	508.7	508.7	4

FILE: S4R11TBL NOFITGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

17	74.9	9.5	13.7	2.0	61.2	7.4	527.6	527.6	4
18	72.6	7.4	12.7	1.9	59.9	5.5	549.2	549.2	4
19	63.6	6.9	9.4	2.0	54.2	4.9	623.3	623.3	4
20	60.4	8.2	8.4	1.7	52.0	6.6	647.3	647.3	4
21	56.5	7.5	7.4	1.7	49.1	5.8	675.4	675.4	4

A= 13
N = 14
IN = 14000
DT =0.05000H
COUNT = 21
M = 21
TOCK = 675.4H
TOTAL TIME = 675.4H
AV SQ DVN TOTS4 = 1529.3
AV SQ DVN FRS4 = 102.7
AV SQ DVN RBS4 = 939.2
RNMIX = 4
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.6M [GLYCINE] = 0.5M [S(IV)] = 0.0438M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.1	39.9	33.9	60.1	66.2	5.4	5.4	5
2	100.0	100.6	38.9	33.1	61.1	67.5	20.3	20.3	5
3	99.8	100.7	37.4	35.2	62.4	65.4	43.6	43.6	5
4	98.6	92.7	32.5	27.1	66.1	65.7	117.9	117.9	5
5	97.8	91.5	30.8	26.5	67.1	65.0	143.7	143.7	5
6	97.2	87.8	29.5	24.0	67.7	63.8	163.7	163.7	5
7	96.0	81.4	27.6	19.4	68.4	62.0	192.3	192.3	5
8	95.2	78.4	26.4	17.9	68.8	60.5	211.6	211.6	5
9	90.6	57.1	21.5	7.2	69.1	49.9	290.1	290.1	5
10	89.0	49.9	20.2	5.5	68.9	44.4	311.6	311.6	5
11	86.9	37.4	18.6	3.3	68.3	34.1	339.1	339.1	5
12	84.9	30.0	17.3	2.5	67.7	27.5	361.1	361.1	5
13	83.1	21.5	16.2	1.9	66.9	19.6	380.8	380.8	5
14	75.2	8.5	12.3	2.4	62.9	6.0	453.5	453.5	5
15	71.8	8.3	11.0	2.1	60.8	6.2	480.2	480.2	5

A= 9
N = 10
IN = 10000
DT =0.05000H
COUNT = 15
M = 15
TOCK = 480.2H
TOTAL TIME = 480.2H
AV SQ DVN TOTS4 = 1403.0
AV SQ DVN FRS4 = 102.9
AV SQ DVN RBS4 = 827.9
RNMIX = 5
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 2.0M [GLYCINE] = 0.5M [S(IV)] = 0.0434M

FILE: S4R11TBL NOFITGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR TOTAL S(IV) = 1525.4
THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR FREE S(IV) = 248.4
THE SUM OF THE MEANS FOR THE KINETIC RUN AS A WHOLE = 1773.7

RK1=0.0000545570ME-1HE-1 RK2=0.0020262692ME-2HE-1 RK3=0.0016999999HE-1
RK4=0.0069999982ME-1HE-1 RK5= 0.0MHE-1
EK1= 1.330000M EK2= 0.004000M EK3= 0.004400M

FILE: SULFRN11 TABLGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

KEY FOR ABBREVIATIONS.

COUNT=THE DATA POINT NUMBER.TH=THEORETICAL VALUES. EX=EXPERIMENTAL VALUES.
TOT=TOTAL [S(IV)]. FR=FREE [S(IV)]. RB=REVERSIBLY BOUND [S(IV)].
EACH [S(IV)] IS AS A % OF THE ORIGINAL [S(IV)].
TIM=TIME. RNMIX=REACTION MIXTURE N.O. A=N-1.
N=N.O. OF TIME SEGMENTS OF 50H. IN=N.O. OF CYCLES OF LOOP 20.
DT=STEP TIME LENGTH. M=N.O. OF DATA POINTS. TOCK=TH TIME.
AV SQ DVN=AVERAGE SQUARED DEVIATION PER PAIR OF TH & EX VALUES.
TOT AV SQ DVN=TOTAL AV SQ DVN.
RK=RATE CONSTANT. EK=EQUILIBRIUM CONSTANT.

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.7	99.9	71.7	72.7	28.0	27.2	6.8	6.8	1
2	98.9	98.3	70.9	69.8	27.9	28.4	21.6	21.6	1
3	97.4	101.1	69.6	72.8	27.8	28.3	45.0	45.0	1
4	91.7	86.0	63.6	61.0	28.1	25.0	119.3	119.3	1
5	89.5	85.0	61.2	60.8	28.3	24.2	144.3	144.3	1
6	87.5	74.9	59.0	50.4	28.5	24.5	165.1	165.1	1
7	84.8	75.2	55.9	51.7	28.9	23.5	193.7	193.7	1
8	82.9	80.6	53.7	56.1	29.2	24.5	213.0	213.0	1
9	74.9	75.0	44.3	52.6	30.6	22.4	291.5	291.5	1
10	72.7	60.7	41.7	40.4	31.0	20.4	313.0	313.0	1
11	69.7	74.1	38.1	53.5	31.6	20.6	342.3	342.3	1
12	67.6	59.2	35.6	39.2	32.0	20.0	362.4	362.4	1
13	65.6	58.6	33.2	38.9	32.4	19.7	382.2	382.2	1
14	58.5	51.6	24.6	33.6	33.9	18.0	454.9	454.9	1
15	55.9	43.7	21.6	28.3	34.4	15.3	481.6	481.6	1
16	53.4	47.6	18.6	30.1	34.8	17.5	508.9	508.9	1
17	51.7	37.8	16.6	23.0	35.1	14.8	527.8	527.8	1
18	49.8	39.3	14.5	23.2	35.3	16.0	549.6	549.6	1
19	43.6	31.6	8.4	15.9	35.2	15.7	623.7	623.7	1
20	41.7	28.7	6.9	13.7	34.8	14.9	647.4	647.4	1
21	39.6	25.7	5.4	10.2	34.1	15.5	675.4	675.4	1
22	38.2	26.7	4.7	11.8	33.5	14.9	694.1	694.1	1
23	36.3	23.6	3.8	8.7	32.5	14.9	719.0	719.0	1
24	31.3	17.6	2.3	4.9	29.0	12.7	790.7	790.7	1
25	29.5	17.6	1.9	4.9	27.6	12.8	816.6	816.6	1
26	27.6	15.7	1.6	4.0	26.0	11.7	845.0	845.0	1
27	26.0	12.3	1.4	2.5	24.6	9.8	869.2	869.2	1
28	24.6	13.0	1.2	2.4	23.4	10.5	890.9	890.9	1
29	20.2	10.3	0.8	1.6	19.4	8.7	960.7	960.7	1

A= 19
N = 20
IN = 20000
DT =0.05000H
COUNT = 29
M = 29
TOCK = 960.7H
TOTAL TIME = 960.7H
AV SQ DVN TOTS4 = 97.6
AV SQ DVN FRS4 = 36.9
AV SQ DVN RBS4 = 185.3
RNMIX = 1

FILE: SULFRN11 TABLGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 0.4M [GLYCINE] = 0.5M [S(IV)] = 0.0365M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.8	99.9	56.0	56.0	43.9	43.9	5.9	5.9	2
2	99.3	97.1	55.5	55.6	43.8	41.5	20.7	20.7	2
3	98.1	97.4	54.4	56.0	43.7	41.4	44.0	44.0	2
4	92.2	92.9	48.7	50.4	43.5	42.6	118.3	118.3	2
5	89.5	88.6	46.0	46.7	43.5	41.9	144.1	144.1	2
6	87.3	85.8	43.8	44.0	43.5	41.8	164.1	164.1	2
7	83.9	89.1	40.4	46.1	43.5	43.1	192.7	192.7	2
8	81.6	87.1	38.0	44.6	43.5	42.5	212.0	212.0	2
9	71.2	82.5	27.6	39.7	43.6	42.8	290.5	290.5	2
10	68.3	77.0	24.7	34.7	43.6	42.3	312.0	312.0	2
11	64.5	71.3	21.0	30.1	43.5	41.1	339.5	339.5	2
12	61.4	72.0	18.0	29.0	43.4	43.0	361.5	361.5	2
13	58.7	75.6	15.5	32.6	43.2	43.0	381.2	381.2	2
14	48.9	67.5	7.4	25.3	41.5	42.2	453.9	453.9	2
15	45.4	64.7	5.4	22.8	40.1	42.0	480.6	480.6	2
16	41.9	62.9	3.8	21.7	33.1	41.2	507.9	507.9	2
17	39.5	60.3	3.0	19.0	36.4	41.2	526.8	526.8	2
18	36.7	56.7	2.4	16.6	34.4	40.1	548.5	548.5	2
19	27.7	48.4	1.1	9.7	26.6	38.7	622.5	622.5	2
20	24.8	44.0	0.9	7.9	24.0	36.1	646.5	646.5	2
21	21.5	41.5	0.7	6.0	20.8	35.5	674.7	674.7	2
22	19.4	39.3	0.6	5.2	18.8	34.1	693.1	693.1	2
23	16.5	35.0	0.4	4.2	16.0	30.8	718.1	718.1	2
24	8.4	23.2	0.2	2.4	8.2	20.9	789.8	789.8	2
25	5.5	19.2	0.1	1.6	5.4	17.6	815.6	815.6	2
26	2.3	14.3	0.0	1.5	2.3	12.8	844.0	844.0	2
27	-0.3	10.9	0.0	1.3	-0.3	9.6	868.3	868.3	2
28	-2.7	9.1	0.0	1.3	-2.7	7.8	889.9	889.9	2

A= 17
N = 18
IN = 18000
DT =0.05000H
COUNT = 28
M = 28
TOCK = 889.9H
TOTAL TIME = 889.9H
AV SQ DVN TOTS4 = 194.9
AV SQ DVN FRS4 = 86.6
AV SQ DVN RBS4 = 60.2
RNMIX = 2
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 0.8M [GLYCINE] = 0.5M [S(IV)] = 0.0398M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.9	100.0	45.8	45.9	54.1	54.2	4.4	4.4	3
2	99.4	99.0	45.5	46.5	54.0	52.5	19.2	19.2	3

FILE: SULFRN11 TABLGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

3	98.2	98.7	44.5	46.4	53.8	52.2	42.5	42.5	3
4	91.5	98.5	38.8	40.5	52.7	58.0	116.8	116.8	3
5	88.3	90.5	36.1	37.6	52.2	52.9	142.7	142.7	3
6	85.7	87.8	33.8	35.7	51.8	52.1	162.6	162.6	3
7	81.5	86.9	30.3	34.1	51.2	52.8	191.3	191.3	3
8	78.6	87.7	27.8	35.3	50.7	52.4	210.5	210.5	3
9	65.6	78.7	17.2	28.3	43.5	50.5	289.0	289.0	3
10	61.9	73.2	14.3	23.3	47.7	49.9	310.5	310.5	3
11	57.2	63.1	10.7	16.3	46.4	46.7	338.0	338.0	3
12	53.3	65.0	8.2	17.2	45.2	47.8	360.0	360.0	3
13	49.9	64.4	6.2	17.4	43.7	47.0	379.8	379.8	3
14	37.4	52.3	2.1	9.5	35.3	42.8	452.4	452.4	3
15	32.9	46.4	1.4	7.4	31.4	39.0	479.1	479.1	3
16	28.3	41.4	1.0	5.4	27.3	36.0	506.4	506.4	3
17	25.1	36.7	0.8	3.7	24.3	33.0	525.4	525.4	3
18	21.5	32.3	0.6	3.1	20.9	29.2	547.0	547.0	3
19	9.5	16.3	0.2	1.8	9.3	14.5	621.1	621.1	3
20	5.6	10.7	0.1	1.6	5.5	9.2	645.0	645.0	3
21	1.1	8.0	0.0	1.7	1.1	6.3	673.2	673.2	3
22	-1.8	6.4	0.0	1.9	-1.8	4.5	691.6	691.6	3

A= 13
N = 14
IN = 14000
DT =0.05000H
COUNT = 22
M = 22
TOCK = 691.6H
TOTAL TIME = 691.6H
AV SQ DVN TOTS4 = 84.8
AV SQ DVN FRS4 = 30.1
AV SQ DVN RBS4 = 23.0
RNMIX = 3
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.2M [GLYCINE] = 0.5M [S(IV)] = 0.0414M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.9	99.9	38.8	39.5	61.1	60.4	6.6	6.6	4
2	99.4	98.4	38.4	38.8	61.0	59.6	21.4	21.4	4
3	98.1	97.3	37.4	38.3	60.6	59.0	44.7	44.7	4
4	90.3	93.1	31.8	33.1	58.6	60.0	119.1	119.1	4
5	86.6	89.2	29.1	29.4	57.6	59.8	144.9	144.9	4
6	83.5	87.1	26.8	27.8	56.7	59.3	164.8	164.8	4
7	78.7	87.7	23.3	28.6	55.4	59.1	193.5	193.5	4
8	75.2	86.0	20.8	26.7	54.4	59.3	212.8	212.8	4
9	60.0	72.3	10.6	17.4	49.4	54.9	291.3	291.3	4
10	55.6	67.5	8.0	14.0	47.6	53.5	312.8	312.8	4
11	50.0	56.8	5.2	8.9	44.8	47.9	340.3	340.3	4
12	45.5	55.9	3.6	7.9	41.9	48.0	362.2	362.2	4
13	41.5	50.5	2.6	6.2	38.9	44.3	382.0	382.0	4
14	26.7	30.0	0.8	2.6	25.8	27.4	454.7	454.7	4
15	21.3	21.2	0.5	2.3	20.7	18.9	481.4	481.4	4
16	15.8	12.9	0.3	2.2	15.4	10.8	508.7	508.7	4

FILE: SULFRN11 TABLGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

17	12.0	9.5	0.2	2.0	11.7	7.4	527.6	527.6	4
18	7.6	7.4	0.1	1.9	7.5	5.5	549.2	549.2	4
19	-7.1	6.9	-0.1	2.0	-7.0	4.9	623.3	623.3	4
20	-11.8	8.2	-0.1	1.7	-11.7	6.6	647.3	647.3	4
21	-17.3	7.5	-0.2	1.7	-17.1	5.8	675.4	675.4	4

A= 13
N = 14
IN = 14000
DT =0.05000H
COUNT = 21
M = 21
TOCK = 675.4H
TOTAL TIME = 675.4H
AV SQ DVN TOTS4 = 94.9
AV SQ DVN FRS4 = 10.6
AV SQ DVN RBS4 = 59.4
RNMIX = 4
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.6M [GLYCINE] = 0.5M [S(IV)] = 0.0438M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.9	100.1	33.6	33.9	66.3	66.2	5.4	5.4	5
2	99.4	100.6	33.3	33.1	66.2	67.5	20.3	20.3	5
3	98.0	100.7	32.4	35.2	65.7	65.4	43.6	43.6	5
4	89.2	92.7	26.7	27.1	62.5	65.7	117.9	117.9	5
5	84.9	91.5	24.0	26.5	61.0	65.0	143.7	143.7	5
6	81.3	87.8	21.7	24.0	59.6	63.8	163.7	163.7	5
7	75.6	81.4	18.2	19.4	57.4	62.0	192.3	192.3	5
8	71.5	78.4	15.7	17.9	55.8	60.5	211.6	211.6	5
9	53.7	57.1	6.1	7.2	47.6	49.9	290.1	290.1	5
10	48.6	49.9	4.2	5.5	44.4	44.4	311.6	311.6	5
11	42.0	37.4	2.5	3.3	39.5	34.1	339.1	339.1	5
12	36.6	30.0	1.7	2.5	35.0	27.5	361.1	361.1	5
13	31.9	21.5	1.2	1.9	30.7	19.6	380.8	380.8	5
14	14.1	8.5	0.3	2.4	13.8	6.0	453.5	453.5	5
15	7.6	8.3	0.1	2.1	7.5	6.2	480.2	480.2	5

A= 9
N = 10
IN = 10000
DT =0.05000H
COUNT = 15
M = 15
TOCK = 480.2H
TOTAL TIME = 480.2H
AV SQ DVN TOTS4 = 27.0
AV SQ DVN FRS4 = 2.7
AV SQ DVN RBS4 = 24.3
RNMIX = 5
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 2.0M [GLYCINE] = 0.5M [S(IV)] = 0.0434M

FILE: SULFRN11 TABLGLC A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR TOTAL S(IV) = 99.8
THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR FREE S(IV) = 33.4
THE SUM OF THE MEANS FOR THE KINETIC RUN AS A WHOLE = 133.2

RK1=0.0001091140ME-1HE-1 RK2=0.0040525383ME-2HE-1 RK3=0.0014449998HE-1
RK4=0.0059499981ME-1HE-1 RK5= 15.0MHE-1
EK1= 1.000000M EK2= 0.280000M EK3= 0.000660M

FILE: SULFRN11 TABLGLY A LEEDS UNIVERSITY COMPUTING SERVICE VM/4PO 5.0 CMS

KEY FOR ABBREVIATIONS.

COUNT=THE DATA POINT NUMBER.TH=THEORETICAL VALUES. EX=EXPERIMENTAL VALUES.
TOT=TOTAL [S(IV)]. FR=FREE [S(IV)]. RB=REVERSIBLY BOUND [S(IV)].
EACH [S(IV)] IS AS A % OF THE ORIGINAL [S(IV)].
TIM=TIME. RNMIX=REACTION MIXTURE N.O. A=N-1.
N=N.O. OF TIME SEGMENTS OF 50H. IN=N.O. OF CYCLES OF LOOP 20.
DT=STEP TIME LENGTH. M=N.O. OF DATA POINTS. TOCK=TH TIME.
AV SQ DVN=AVERAGE SQUARED DEVIATION PER PAIR OF TH & EX VALUES.
TOT AV SQ DVN=TOTAL AV SQ DVN.
RK=RATE CONSTANT. EK=EQUILIBRIUM CONSTANT.

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.6	100.0	50.3	50.9	49.3	49.1	5.7	5.7	6
2	98.4	99.0	49.6	50.1	48.8	48.9	20.9	20.9	6
3	96.6	100.5	48.6	53.3	48.0	47.2	44.1	44.1	6
4	90.7	95.6	45.2	48.1	45.5	47.5	118.4	118.4	6
5	88.5	95.1	43.9	49.1	44.7	46.0	144.2	144.2	6
6	86.9	91.4	42.9	44.6	44.0	46.8	164.4	164.4	6
7	84.5	88.2	41.4	41.8	43.2	46.4	193.1	193.1	6
8	83.0	87.1	40.4	41.2	42.6	45.9	211.9	211.9	6
9	76.5	83.8	36.1	41.3	40.4	42.5	290.5	290.5	6
10	74.7	83.1	34.9	39.2	39.8	43.9	312.7	312.7	6
11	72.4	79.2	33.3	37.4	39.1	41.8	341.0	341.0	6
12	70.7	77.8	32.2	42.9	38.5	34.9	361.6	361.6	6
13	69.1	78.8	31.1	36.9	38.1	41.9	381.2	381.2	6
14	63.4	75.5	27.0	34.6	36.4	40.9	453.9	453.9	6
15	61.3	70.9	25.5	33.6	35.8	37.3	480.9	480.9	6
16	59.3	71.1	24.0	31.3	35.3	39.8	507.2	507.2	6
17	57.9	59.5	23.0	21.5	34.9	37.9	526.1	526.1	6
18	56.3	67.5	21.8	29.8	34.5	37.7	547.7	547.7	6
19	51.1	57.3	17.9	23.8	33.2	33.5	621.8	621.8	6
20	49.4	54.3	16.6	21.8	32.8	32.6	645.8	645.8	6
21	47.5	51.0	15.2	19.4	32.3	31.6	674.0	674.0	6
22	46.3	50.4	14.3	19.2	32.0	31.2	692.4	692.4	6
23	44.7	46.2	13.1	16.9	31.6	29.3	717.3	717.3	6
24	40.3	40.1	9.8	12.8	30.4	27.3	789.0	789.0	6
25	38.7	40.6	8.8	13.6	30.0	27.0	814.9	814.9	6
26	37.1	39.0	7.7	12.4	29.5	26.6	843.3	843.3	6
27	35.8	32.4	6.8	9.7	29.0	22.8	867.5	867.5	6
28	34.6	34.4	6.1	9.3	28.5	25.1	889.2	889.2	6
29	30.9	32.1	4.1	7.7	26.8	24.4	959.0	959.0	6
30	29.8	28.2	3.6	6.9	26.1	21.3	980.0	980.0	6
31	28.2	25.9	3.0	5.4	25.2	20.5	1010.4	1010.4	6
32	27.1	26.0	2.7	5.2	24.4	20.8	1032.6	1032.6	6
33	26.2	22.3	2.4	4.0	23.8	18.3	1052.2	1052.2	6
34	22.7	18.1	1.6	3.0	21.1	15.2	1124.7	1124.7	6
35	21.5	18.5	1.4	2.4	20.1	16.1	1148.7	1148.7	6
36	19.2	14.0	1.1	1.9	18.1	12.1	1198.7	1198.7	6
37	17.9	13.0	0.9	1.8	16.9	11.2	1227.4	1227.4	6
38	14.7	8.8	0.7	1.1	14.0	7.6	1296.3	1296.3	6

A= 25
N = 26
IN = 26000

FILE: SULFRN11 TABLGLY A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

DT =0.05000H
COUNT = 38
M = 38
TOCK = 1296.3H
TOTAL TIME = 1296.3H
AV SQ DVN TOTS4 = 31.8
AV SQ DVN FRS4 = 19.8
AV SQ DVN RBS4 = 12.9
RNMIX = 6
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.2M [S(IV)] = 0.0406M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.8	99.9	50.4	51.3	49.4	48.6	5.3	5.3	7
2	99.0	100.1	49.9	50.8	49.1	49.4	20.5	20.5	7
3	97.7	102.3	49.0	55.0	43.7	47.3	43.7	43.7	7
4	92.7	94.9	45.3	47.1	47.4	47.8	118.0	118.0	7
5	90.6	94.5	43.7	47.8	46.9	46.7	143.8	143.8	7
6	88.9	90.6	42.4	43.5	46.5	47.1	164.0	164.0	7
7	86.4	90.1	40.3	42.7	46.0	47.4	192.7	192.7	7
8	84.6	88.6	38.9	40.4	45.7	48.1	211.6	211.6	7
9	76.9	82.3	32.5	37.2	44.4	45.1	290.1	290.1	7
10	74.7	79.1	30.6	33.3	44.1	45.8	312.3	312.3	7
11	71.8	76.5	28.2	32.8	43.6	43.7	340.6	340.6	7
12	69.6	74.6	26.3	30.0	43.3	44.6	361.3	361.3	7
13	67.6	72.0	24.6	28.4	43.0	43.6	380.8	380.8	7
14	59.9	64.4	18.1	22.8	41.8	41.7	453.5	453.5	7
15	57.1	61.9	15.8	20.3	41.3	41.6	480.5	480.5	7
16	54.3	58.3	13.5	17.9	40.8	40.5	507.4	507.4	7
17	52.2	53.4	11.8	15.2	40.4	38.2	528.1	528.1	7
18	50.2	51.8	10.3	13.9	39.9	37.9	548.0	548.0	7
19	42.8	42.8	5.5	7.6	37.2	35.2	622.2	622.2	7
20	40.4	38.6	4.4	6.4	36.0	32.2	646.2	646.2	7
21	37.7	35.8	3.4	5.6	34.3	30.3	674.5	674.5	7
22	36.0	34.0	2.9	4.4	33.2	29.6	692.0	692.0	7
23	33.7	29.4	2.3	3.4	31.4	26.0	716.9	716.9	7
24	27.0	19.4	1.3	1.6	25.7	17.8	788.6	788.6	7
25	24.6	16.1	1.0	1.6	23.6	14.5	814.5	814.5	7
26	22.0	12.4	0.8	1.4	21.2	11.0	842.9	842.9	7
27	19.8	8.9	0.7	1.5	19.1	7.4	867.1	867.1	7
28	17.8	7.3	0.6	1.4	17.3	5.9	888.8	888.8	7

A= 17
N = 18
IN = 18000
DT =0.05000H
COUNT = 28
M = 28
TOCK = 888.8H
TOTAL TIME = 888.8H
AV SQ DVN TOTS4 = 26.2
AV SQ DVN FRS4 = 8.9
AV SQ DVN RBS4 = 22.1

FILE: SULFRN11 TABLGLY A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

RNMIX = 7
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.4M [S(IV)] = 0.0403M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.8	100.1	50.4	52.4	49.4	47.7	4.8	4.8	8
2	99.2	98.7	49.9	50.6	49.3	48.2	20.0	20.0	8
3	97.9	98.0	48.9	50.4	49.0	47.5	43.3	43.3	8
4	91.9	93.9	43.8	45.7	48.1	48.2	117.5	117.5	8
5	89.2	95.0	41.5	46.3	47.7	48.7	143.4	143.4	8
6	86.9	93.1	39.5	45.1	47.5	48.0	163.6	163.6	8
7	83.5	89.6	36.5	41.0	47.1	48.6	192.2	192.2	8
8	81.2	88.5	34.4	40.0	46.8	48.4	211.1	211.1	8
9	70.6	79.5	24.9	31.0	45.7	48.5	289.6	289.6	8
10	67.5	76.0	22.2	27.8	45.3	48.2	311.8	311.8	8
11	63.5	68.9	18.7	22.6	44.8	46.3	340.1	340.1	8
12	60.5	67.4	16.1	20.8	44.4	46.6	360.8	360.8	8
13	57.7	65.0	13.8	18.7	43.9	46.3	380.3	380.3	8
14	47.4	53.4	6.4	10.8	41.0	42.6	453.0	453.0	8
15	43.6	47.1	4.5	7.5	39.1	39.6	480.1	480.1	8
16	39.8	41.1	3.1	5.6	36.7	35.5	506.9	506.9	8
17	37.0	35.4	2.4	4.1	34.6	31.3	527.6	527.6	8
18	34.3	31.4	1.9	3.4	32.4	28.0	547.5	547.5	8
19	24.3	15.9	0.8	2.2	23.4	13.6	621.7	621.7	8
20	21.1	11.5	0.6	2.1	20.4	9.4	645.8	645.8	8
21	17.4	7.9	0.5	2.3	16.9	5.6	673.7	673.7	8

A= 13
N = 14
IN = 14000
DT =0.05000H
COUNT = 21
M = 21
TOCK = 673.7H
TOTAL TIME = 673.7H
AV SQ DVN TOTS4 = 36.2
AV SQ DVN FRS4 = 14.0
AV SQ DVN RBS4 = 20.1
RNMIX = 8
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.6M [S(IV)] = 0.0414M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.9	100.1	50.4	50.9	49.5	49.2	4.6	4.6	9
2	99.2	101.2	49.9	52.6	49.3	48.6	19.8	19.8	9
3	97.7	99.2	48.6	50.7	49.2	48.5	43.0	43.0	9
4	89.9	94.4	41.5	44.4	48.4	50.0	117.2	117.2	9
5	86.3	94.4	38.1	43.7	48.2	50.7	143.1	143.1	9
6	83.2	90.3	35.3	40.3	47.9	50.0	163.3	163.3	9
7	78.6	86.6	31.0	36.7	47.5	49.9	192.0	192.0	9
8	75.4	84.5	28.1	34.1	47.2	50.4	210.8	210.8	9

FILE: SULFRN11 TABLGLY A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

9	61.2	69.2	15.5	21.1	45.7	48.1	289.3	289.3	9
10	57.0	64.0	12.1	16.7	44.9	47.3	311.5	311.5	9
11	51.7	54.9	8.2	11.2	43.5	43.7	339.9	339.9	9
12	47.9	51.9	5.9	9.1	42.0	42.7	360.5	360.5	9
13	44.3	46.0	4.2	6.6	40.0	39.5	380.1	380.1	9
14	31.0	23.9	1.3	3.2	29.7	20.8	452.7	452.7	9
15	26.2	15.8	0.9	3.0	25.2	12.8	479.8	479.8	9
16	21.4	9.7	0.6	2.9	20.8	6.8	506.6	506.6	9
17	17.7	8.0	0.5	2.6	17.3	5.4	527.3	527.3	9
18	14.3	7.2	0.3	2.5	13.9	4.7	547.2	547.2	9
19	1.4	7.7	0.0	1.9	1.4	5.7	621.5	621.5	9
20	-2.7	7.7	0.0	1.8	-2.6	5.9	645.5	645.5	9
21	-7.4	6.0	-0.1	1.4	-7.3	4.6	673.4	673.4	9

A= 13
N = 14
IN = 14000
DT =0.05000H
COUNT = 21
M = 21
TOCK = 673.4H
TOTAL TIME = 673.4H
AV SQ DVN TOTS4 = 57.1
AV SQ DVN FRS4 = 12.1
AV SQ DVN RBS4 = 44.3
RNMIX = 9
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.8M [S(IV)] = 0.0410M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	99.9	100.1	50.4	52.3	49.5	47.8	4.8	4.8	10
2	99.2	100.5	49.8	52.2	49.4	48.3	20.0	20.0	10
3	97.4	98.3	48.1	49.6	49.3	48.7	43.2	43.2	10
4	87.4	94.5	38.7	44.0	48.7	50.6	117.5	117.5	10
5	82.7	91.5	34.4	40.7	48.4	50.7	143.3	143.3	10
6	78.8	86.5	30.7	36.2	48.1	50.3	163.5	163.5	10
7	72.8	81.3	25.2	31.4	47.6	49.9	192.2	192.2	10
8	68.8	75.4	21.5	25.9	47.3	49.5	211.0	211.0	10
9	51.2	52.1	7.3	9.6	43.9	42.6	289.6	289.6	10
10	46.2	45.2	4.7	6.5	41.5	38.6	311.8	311.8	10
11	39.8	32.6	2.6	4.0	37.2	28.6	340.1	340.1	10
12	35.2	24.7	1.8	3.1	33.5	21.7	360.8	360.8	10
13	31.0	17.7	1.2	3.4	29.7	14.3	380.3	380.3	10
14	15.3	9.7	0.3	3.8	14.9	6.0	453.0	453.0	10
15	9.6	9.1	0.2	3.4	9.4	5.7	480.0	480.0	10

A= 9
N = 10
IN = 10000
DT =0.05000H
COUNT = 15
M = 15
TOCK = 480.0H

FILE: SULFRN11 TABLGLY A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

TOTAL TIME = 480.0H
AV SQ DVN TOTS4 = 45.2
AV SQ DVN FRS4 = 13.8
AV SQ DVN RBS4 = 38.9
RNMIX = 10
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 1.0M [S(IV)] = 0.0423M

THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR TOTAL S(IV) = 39.3
THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR FREE S(IV) = 13.7
THE SUM OF THE MEANS FOR THE KINETIC RUN AS A WHOLE = 53.0

RK1=0.0000872912ME-1HE-1 RK2=0.0032420313ME-2HE-1 RK3=0.0010199999HE-1
RK4=0.0041999985ME-1HE-1 RK5= 25.0MHE-1
EK1= 1.000000M EK2= 0.280000M EK3= 0.000660M

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

KEY FOR ABBREVIATIONS.

COUNT=THE DATA POINT NUMBER.TH=THEORETICAL VALUES. EX=EXPERIMENTAL VALUES.
TOT=TOTAL [S(IV)]. FR=FREE [S(IV)]. RB=REVERSIBLY BOUND [S(IV)].
EACH [S(IV)] IS AS A % OF THE ORIGINAL [S(IV)].
TIM=TIME. RNMIX=REACTION MIXTURE N.O. A=N-1.
N=N.O. OF TIME SEGMENTS OF 50H. IN=N.O. OF CYCLES OF LOOP 20.
DT=STEP TIME LENGTH. M=N.O. OF DATA POINTS. TOCK=TH TIME.
AV SQ DVN=AVERAGE SQUARED DEVIATION PER PAIR OF TH & EX VALUES.
TOT AV SQ DVN=TOTAL AV SQ DVN.
RK=RATE CONSTANT. EK=EQUILIBRIUM CONSTANT.

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	99.8	50.0	51.2	49.9	48.6	5.8	5.8	2
2	99.3	100.6	49.2	52.4	50.0	48.2	26.2	26.2	2
3	97.6	90.8	47.6	45.0	50.0	45.7	48.0	48.0	2
4	86.0	83.2	36.8	38.5	49.2	44.7	118.1	118.1	2
5	79.2	81.2	30.9	33.2	48.2	48.0	145.3	145.3	2
6	72.3	76.0	25.3	29.9	47.0	46.1	168.8	168.8	2
7	64.1	67.8	19.2	22.8	44.9	45.0	193.5	193.5	2
8	57.2	63.9	14.7	19.9	42.5	44.0	212.6	212.6	2
9	24.4	38.5	2.8	8.5	21.6	30.0	289.8	289.8	2
10	14.1	31.0	1.3	6.5	12.8	24.6	311.1	311.1	2
11	-0.3	18.6	0.0	5.1	-0.2	13.5	339.3	339.3	2
12	-9.8	13.9	-0.6	5.1	-9.2	8.8	357.2	357.2	2
13	-23.6	12.1	-1.2	5.2	-22.3	6.9	381.9	381.9	2
14	-66.7	11.1	-2.3	4.0	-64.4	7.1	453.8	453.8	2

A= 9
N = 10
IN = 10000
DT =0.05000H
COUNT = 14
M = 14
TOCK = 453.8H
TOTAL TIME = 453.8H
AV SQ DVN TOTS4 = 632.9
AV SQ DVN FRS4 = 20.5
AV SQ DVN RBS4 = 481.3
RNMIX = 2
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0078M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.3	50.1	51.2	49.8	49.1	5.9	5.9	3
2	99.6	101.5	49.6	52.4	50.0	49.1	26.2	26.2	3
3	98.7	95.6	48.6	47.3	50.1	48.2	48.0	48.0	3
4	92.4	96.4	42.2	46.8	50.2	49.6	118.1	118.1	3
5	88.6	91.9	38.6	42.8	50.1	49.1	145.3	145.3	3
6	84.9	85.0	35.0	37.3	49.8	47.8	168.8	168.8	3
7	80.4	80.8	30.9	33.2	49.5	47.5	193.6	193.6	3
8	76.7	78.0	27.6	30.9	49.1	47.1	212.7	212.7	3
9	58.8	62.2	13.3	18.2	45.5	44.0	289.8	289.8	3

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

10	53.2	57.9	9.9	15.0	43.4	42.9	311.1	311.1	3
11	45.4	50.6	6.2	11.3	39.3	39.4	339.3	339.3	3
12	40.3	46.5	4.5	8.8	35.8	37.7	357.3	357.3	3
13	33.0	39.6	2.8	8.8	30.2	30.8	381.7	381.7	3
14	10.0	17.2	0.5	2.1	9.5	15.1	453.9	453.9	3
15	1.4	11.6	0.1	2.7	1.3	8.9	479.3	479.3	3
16	-8.6	7.7	-0.3	2.6	-3.3	5.0	508.0	508.0	3

A= 10
N = 11
IN = 11000
DT =0.05000H
COUNT = 16
M = 16
TOCK = 508.0H
TOTAL TIME = 508.0H
AV SQ DVN TOTS4 = 37.9
AV SQ DVN FRS4 = 13.7
AV SQ DVN RBS4 = 18.2
RNMIX = 3
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0159M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	50.2	50.9	49.7	49.2	5.8	5.8	4
2	99.7	98.8	49.8	49.5	49.9	49.4	26.2	26.2	4
3	99.1	92.6	49.0	44.7	50.1	47.9	48.0	48.0	4
4	94.5	101.0	44.1	50.4	50.4	50.7	118.0	118.0	4
5	91.8	92.0	41.4	42.8	50.4	49.2	145.3	145.3	4
6	89.1	90.2	38.8	39.9	50.4	50.3	168.8	168.8	4
7	85.9	91.6	35.6	41.4	50.3	50.2	193.6	193.6	4
8	83.2	89.7	33.1	38.9	50.1	50.7	212.7	212.7	4
9	70.3	78.7	21.3	29.0	49.0	49.7	289.8	289.8	4
10	66.3	73.4	17.9	24.3	48.4	49.1	311.1	311.1	4
11	60.7	69.6	13.5	21.8	47.2	47.8	339.3	339.3	4
12	57.0	66.1	10.9	18.7	46.1	47.4	357.2	357.2	4
13	51.7	61.2	7.7	21.1	44.0	40.1	381.6	381.6	4
14	35.3	45.4	2.4	7.4	32.9	38.0	453.8	453.8	4
15	29.1	40.0	1.6	5.5	27.6	34.6	479.2	479.2	4
16	22.1	32.4	0.9	3.7	21.1	28.7	507.9	507.9	4
17	16.1	23.0	0.6	2.6	15.5	20.5	531.7	531.7	4
18	10.5	20.5	0.3	2.5	10.2	18.0	553.6	553.6	4
19	-7.8	9.5	-0.2	3.2	-7.7	6.3	623.3	623.3	4
20	-13.3	7.9	-0.3	2.7	-13.1	5.2	643.6	643.6	4
21	-21.6	8.2	-0.4	3.2	-21.2	5.0	673.9	673.9	4

A= 13
N = 14
IN = 14000
DT =0.05000H
COUNT = 21
M = 21
TOCK = 673.9H

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/370 5.0 CMS

TOTAL TIME = 673.9H
AV SQ DVN TOTS4 = 126.2
AV SQ DVN FRS4 = 29.8
AV SQ DVN RBS4 = 69.6
RNMIX = 4
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0243M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	99.9	50.3	49.4	49.6	50.5	5.8	5.8	5
2	99.8	100.7	49.9	50.6	49.8	50.2	26.2	26.2	5
3	99.2	96.6	49.2	47.2	50.0	49.4	48.0	48.0	5
4	95.5	101.2	45.1	50.9	50.4	50.3	118.1	118.1	5
5	93.3	95.4	42.8	46.0	50.5	49.4	145.3	145.3	5
6	91.1	90.9	40.6	41.0	50.5	49.9	168.8	168.8	5
7	88.5	91.6	38.0	42.6	50.5	49.0	193.6	193.6	5
8	86.3	87.2	35.8	37.6	50.5	49.6	212.7	212.7	5
9	75.8	77.7	25.9	30.0	49.9	47.7	289.8	289.8	5
10	72.5	73.4	22.9	26.7	49.7	46.7	311.1	311.1	5
11	68.0	68.7	18.8	23.1	49.1	45.6	339.3	339.3	5
12	64.9	66.5	16.3	20.7	48.7	45.8	357.3	357.3	5
13	60.7	60.8	12.8	22.6	47.8	38.2	381.6	381.6	5
14	47.2	48.2	4.8	9.6	42.4	38.7	453.8	453.8	5
15	42.2	44.4	3.2	7.9	39.0	36.5	479.2	479.2	5
16	36.5	38.0	2.1	5.2	34.4	32.7	508.0	508.0	5
17	31.7	29.2	1.5	3.6	30.2	25.6	531.7	531.7	5
18	27.2	27.4	1.1	2.6	26.1	24.8	553.7	553.7	5
19	12.5	14.6	0.3	2.2	12.1	12.4	623.4	623.4	5
20	8.1	10.0	0.2	2.1	7.9	8.0	643.6	643.6	5
21	1.5	7.0	0.0	2.4	1.5	4.6	673.9	673.9	5

A= 13
N = 14
IN = 14000
DT =0.05000H
COUNT = 21
M = 21
TOCK = 673.9H
TOTAL TIME = 673.9H
AV SQ DVN TOTS4 = 5.4
AV SQ DVN FRS4 = 14.8
AV SQ DVN RBS4 = 9.0
RNMIX = 5
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0324M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	99.9	50.5	50.7	49.5	49.2	5.8	5.8	6
2	99.8	99.3	50.1	51.4	49.7	48.0	26.2	26.2	6
3	99.4	95.8	49.4	48.3	49.9	47.5	48.0	48.0	6
4	96.3	99.7	45.8	50.6	50.4	49.1	118.1	118.1	6

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/4PO 5.0 CMS

5	94.4	96.0	43.9	47.4	50.6	48.6	145.3	145.3	6
6	92.6	92.6	41.9	43.4	50.6	49.2	168.8	168.8	6
7	90.4	90.6	39.7	41.9	50.7	48.7	193.6	193.6	6
8	88.5	88.1	37.8	38.8	50.7	49.4	212.7	212.7	6
9	79.7	80.4	29.3	32.2	50.5	48.2	289.8	289.8	6
10	77.0	78.3	26.7	30.7	50.3	47.6	311.1	311.1	6
11	73.1	73.3	23.1	26.3	50.0	47.0	339.3	339.3	6
12	70.6	73.2	20.8	26.4	49.8	46.9	357.3	357.3	6
13	67.0	66.5	17.6	28.1	49.4	38.4	381.7	381.7	6
14	55.7	57.4	8.7	14.4	47.0	43.0	453.9	453.9	6
15	51.5	54.9	6.1	13.4	45.4	41.6	479.3	479.3	6
16	46.7	48.1	4.0	8.6	42.7	39.5	508.0	508.0	6
17	42.6	39.0	2.8	6.4	39.9	32.6	531.8	531.8	6
18	38.9	38.8	2.0	5.6	36.8	33.3	553.7	553.7	6
19	26.6	29.9	0.8	2.9	25.8	27.0	623.4	623.4	6
20	23.0	25.0	0.6	2.6	22.4	22.5	643.7	643.7	6
21	17.6	18.9	0.4	2.0	17.2	16.8	673.9	673.9	6
22	13.6	15.4	0.3	2.2	13.3	13.2	696.1	696.1	6
23	10.1	10.0	0.2	1.6	9.9	8.5	715.7	715.7	6
24	-3.2	5.2	0.0	1.6	-3.2	3.6	788.2	788.2	6

A= 15
N = 16
IN = 16000
DT =0.05000H
COUNT = 24
M = 24
TOCK = 788.2H
TOTAL TIME = 788.2H
AV SQ DVN TOTS4 = 6.5
AV SQ DVN FRS4 = 15.6
AV SQ DVN RBS4 = 13.7
RNMIX = 6
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0423M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	50.5	48.4	49.5	51.6	6.5	6.5	7
2	99.8	103.8	50.1	53.2	49.7	50.6	26.8	26.8	7
3	99.4	100.1	49.5	52.6	49.9	47.5	48.9	48.9	7
4	96.4	101.0	46.0	52.1	50.4	48.8	120.1	120.1	7
5	94.8	95.5	44.2	47.6	50.6	47.9	146.0	146.0	7
6	93.1	92.0	42.4	41.7	50.7	50.3	169.4	169.4	7
7	91.0	95.4	40.3	44.6	50.7	50.8	194.8	194.8	7
8	89.4	93.2	38.6	44.1	50.8	49.1	213.3	213.3	7
9	81.2	84.7	30.6	35.1	50.6	49.6	290.4	290.4	7
10	78.6	85.6	28.1	36.2	50.5	49.4	312.0	312.0	7
11	75.0	79.3	24.7	29.5	50.3	49.8	340.6	340.6	7
12	72.7	80.6	22.6	30.4	50.1	50.2	358.1	358.1	7
13	69.1	74.0	19.3	25.2	49.8	48.8	384.4	384.4	7
14	58.9	66.4	10.8	19.7	48.2	46.7	454.6	454.6	7
15	54.9	63.0	7.9	16.1	47.0	46.9	481.1	481.1	7
16	50.6	58.0	5.4	12.9	45.2	45.1	508.6	508.6	7

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

17	47.0	48.4	3.8	9.6	43.1	38.8	531.8	531.8	7
18	43.5	49.1	2.8	7.9	40.7	41.2	553.7	553.7	7
19	32.2	42.8	1.1	4.5	31.1	38.3	623.5	623.5	7
20	28.7	38.4	0.8	3.5	27.8	35.0	644.7	644.7	7
21	24.0	30.4	0.6	2.6	23.4	27.8	672.9	672.9	7
22	20.2	27.7	0.4	2.2	19.8	25.5	696.1	696.1	7
23	16.9	21.8	0.3	2.1	16.6	19.7	715.7	715.7	7
24	4.8	9.7	0.1	1.7	4.7	8.0	788.3	788.3	7
25	0.7	8.1	0.0	2.0	0.7	6.0	812.4	812.4	7
26	-7.6	6.5	-0.1	2.0	-7.5	4.5	861.7	861.7	7

A= 17
N = 18
IN = 18000
DT =0.05000H
COUNT = 26
M = 26
TOCK = 861.7H
TOTAL TIME = 861.7H
AV SQ DVN TOTS4 = 40.5
AV SQ DVN FRS4 = 24.2
AV SQ DVN RBS4 = 15.2
RNMIX = 7
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0480M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	50.7	53.5	49.3	46.5	6.5	6.5	8
2	99.8	94.2	50.3	48.1	49.6	46.1	26.8	26.8	8
3	99.5	91.5	49.7	47.1	49.8	44.5	48.9	48.9	8
4	96.9	93.2	46.5	47.6	50.4	45.5	120.2	120.2	8
5	95.5	92.2	44.9	47.0	50.6	45.1	146.0	146.0	8
6	94.0	89.1	43.3	42.6	50.7	46.5	169.4	169.4	8
7	92.2	80.9	41.4	32.6	50.8	48.3	194.8	194.8	8
8	90.7	86.7	39.8	39.5	50.9	47.1	213.4	213.4	8
9	83.6	83.3	32.7	34.5	50.9	48.8	290.5	290.5	8
10	81.4	75.1	30.5	26.5	50.8	48.6	311.8	311.8	8
11	78.2	76.5	27.5	27.9	50.7	48.6	340.6	340.6	8
12	76.2	76.8	25.6	28.2	50.6	48.6	358.1	358.1	8
13	73.0	73.0	22.6	25.1	50.4	47.9	384.5	384.5	8
14	64.1	62.9	14.6	16.3	49.5	46.6	454.6	454.6	8
15	60.5	63.7	11.6	17.4	48.9	46.2	481.2	481.2	8
16	56.8	59.6	8.7	14.6	48.0	45.0	508.5	508.6	8
17	53.5	50.1	6.5	11.2	47.0	38.9	531.8	531.8	8
18	50.5	51.0	4.8	9.6	45.7	41.4	553.5	553.5	8
19	40.5	48.1	1.8	6.4	38.8	41.6	623.5	623.5	8
20	37.5	43.3	1.4	4.6	36.1	38.7	644.7	644.7	8
21	33.4	36.5	1.0	3.3	32.5	33.2	673.0	673.0	8
22	29.9	32.6	0.7	2.9	29.1	29.6	697.5	697.6	8
23	26.5	28.7	0.6	2.5	26.0	26.1	720.9	720.9	8
24	16.7	17.5	0.3	1.8	16.4	15.7	789.2	789.2	8
25	13.4	15.0	0.2	1.7	13.2	13.3	812.4	812.4	8
26	6.3	9.3	0.1	2.1	6.2	7.2	861.7	861.7	8

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

27	2.0	7.4	0.0	2.0	2.0	5.4	891.0	891.0	8

A= 17
N = 18
IN = 18000
DT =0.05000H
COUNT = 27
M = 27
TOCK = 891.0H
TOTAL TIME = 891.0H
AV SQ DVN TOTS4 = 19.0
AV SQ DVN FRS4 = 11.4
AV SQ DVN RBS4 = 11.2
RNMIX = 8
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0602M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	50.8	51.7	49.2	48.3	5.4	5.4	9
2	99.8	99.2	50.4	50.7	49.5	48.5	25.7	25.7	9
3	99.5	92.7	49.8	46.9	49.7	45.9	48.9	48.9	9
4	97.1	94.9	46.7	49.2	50.4	45.8	120.1	120.1	9
5	95.8	94.1	45.2	49.2	50.6	44.9	145.9	145.9	9
6	94.4	91.7	43.7	45.0	50.7	46.7	169.4	169.4	9
7	92.7	90.9	41.8	42.6	50.8	48.3	194.7	194.7	9
8	91.3	85.8	40.4	40.2	50.9	45.6	213.3	213.3	9
9	84.7	81.0	33.7	32.8	51.0	48.2	290.4	290.4	9
10	82.6	80.8	31.6	33.6	51.0	47.2	311.7	311.7	9
11	79.6	79.9	28.7	32.4	50.9	47.5	340.5	340.5	9
12	77.7	78.5	26.9	31.0	50.8	47.5	358.0	358.0	9
13	74.8	72.8	24.1	25.8	50.6	47.0	384.4	384.4	9
14	66.4	67.6	16.5	21.6	49.9	46.0	454.5	454.5	9
15	63.0	65.3	13.6	19.5	49.5	45.8	481.1	481.1	9
16	59.5	61.4	10.6	16.3	48.9	45.1	508.5	508.6	9
17	56.5	51.6	8.3	12.3	48.2	39.3	531.7	531.7	9
18	53.5	53.3	6.3	11.7	47.2	41.6	554.4	554.4	9
19	44.2	49.9	2.3	7.1	41.9	42.7	624.0	624.0	9
20	41.4	46.0	1.8	5.6	39.7	40.4	644.5	644.6	9
21	37.6	39.2	1.2	4.0	36.4	35.2	672.9	672.9	9
22	34.3	49.7	0.9	3.2	33.3	46.5	697.5	697.6	9
23	31.1	31.6	0.7	2.2	30.4	29.5	720.8	720.8	9
24	22.0	21.3	0.4	1.5	21.6	19.8	789.1	789.1	9
25	18.8	18.3	0.3	1.5	18.5	16.8	812.8	812.8	9
26	12.2	12.3	0.2	1.5	12.0	10.8	862.5	862.5	9
27	8.4	8.8	0.1	1.6	8.3	7.3	890.9	890.9	9
28	-0.7	5.2	0.0	1.6	-0.7	3.6	959.8	959.8	9
29	-4.4	7.0	0.0	1.7	-4.4	5.3	988.1	988.1	9
30	-6.5	4.9	-0.1	1.5	-6.4	3.4	1003.4	1003.4	9

A= 20
N = 21
IN = 21000
DT =0.05000H

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

COUNT = 30
M = 30
TOCK = 1003.4H
TOTAL TIME = 1003.4H
AV SQ DVN TOTS4 = 24.7
AV SQ DVN FRS4 = 9.3
AV SQ DVN RBS4 = 24.2
RNMIX = 9
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0677M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	50.9	51.5	49.1	48.5	5.5	6.5	10
2	99.9	99.6	50.5	52.6	49.4	47.0	26.3	26.8	10
3	99.5	95.7	49.9	49.8	49.6	46.0	43.9	48.9	10
4	97.3	92.6	46.9	46.0	50.4	46.6	120.1	120.1	10
5	96.1	93.3	45.5	46.3	50.6	47.0	145.9	145.9	10
6	94.8	94.2	44.1	47.6	50.7	46.6	169.4	169.4	10
7	93.2	93.0	42.3	45.3	50.9	47.7	194.8	194.8	10
8	91.9	89.5	41.0	41.7	51.0	47.9	213.3	213.3	10
9	85.8	81.6	34.7	32.3	51.1	49.3	290.4	290.4	10
10	83.8	78.1	32.7	29.2	51.1	48.9	311.7	311.7	10
11	81.1	82.8	30.0	33.0	51.0	49.9	340.5	340.5	10
12	79.3	80.2	28.3	30.8	51.0	49.4	358.0	358.0	10
13	76.5	75.4	25.7	26.9	50.9	48.5	384.4	384.4	10
14	68.7	69.1	18.4	21.2	50.3	47.9	454.5	454.5	10
15	65.6	68.0	15.6	20.1	50.0	47.9	481.1	481.1	10
16	62.3	64.0	12.8	16.5	49.5	47.4	508.6	508.6	10
17	59.2	53.3	10.2	12.9	49.0	40.4	534.1	534.1	10
18	56.7	55.2	8.2	11.6	48.5	43.6	554.3	554.3	10
19	48.0	53.6	3.2	7.6	44.7	46.1	624.0	624.0	10
20	45.4	49.5	2.4	6.1	43.0	43.4	644.7	644.7	10
21	41.8	42.5	1.6	4.2	40.1	38.3	672.9	672.9	10
22	38.7	38.8	1.2	3.4	37.5	35.4	697.6	697.6	10
23	35.8	35.2	0.9	3.1	34.8	32.1	720.8	720.8	10
24	27.3	25.2	0.5	1.7	26.8	23.4	789.1	789.1	10
25	24.3	22.8	0.4	1.6	23.9	21.2	812.9	812.9	10
26	18.2	16.5	0.2	1.6	18.0	14.9	862.5	862.5	10
27	14.7	13.1	0.2	1.8	14.5	11.3	890.9	890.9	10
28	6.4	6.4	0.1	1.6	6.3	4.8	959.8	959.8	10
29	2.9	7.3	0.0	1.6	2.9	5.7	983.2	988.2	10
30	1.1	5.3	0.0	1.6	1.1	3.6	1003.4	1003.4	10

A= 20
N = 21
IN = 21000
DT =0.05000H
COUNT = 30
M = 30
TOCK = 1003.4H
TOTAL TIME = 1003.4H
AV SQ DVN TOTS4 = 8.3
AV SQ DVN FRS4 = 6.2

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

AV SQ DVN RBS4 = 9.5
RNMIX = 10
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0773M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	50.9	50.8	49.0	49.2	6.5	6.5	11
2	99.9	100.5	50.6	52.0	49.3	48.6	26.8	26.8	11
3	99.6	99.5	50.0	52.1	49.6	47.4	48.9	48.9	11
4	97.4	97.2	47.1	49.9	50.3	47.3	120.1	120.1	11
5	96.2	97.3	45.7	49.8	50.6	47.5	146.0	146.0	11
6	95.0	90.1	44.3	42.2	50.7	47.9	169.4	169.4	11
7	93.5	95.9	42.6	47.4	50.9	48.5	194.8	194.8	11
8	92.3	91.4	41.3	43.3	51.0	48.0	213.3	213.3	11
9	86.3	82.5	35.2	33.3	51.2	49.2	290.4	290.4	11
10	84.4	75.4	33.3	26.0	51.2	49.4	311.8	311.8	11
11	81.8	83.5	30.6	33.5	51.1	50.0	340.6	340.6	11
12	80.1	80.3	29.0	30.9	51.1	49.3	358.1	358.1	11
13	77.4	77.1	26.4	28.5	51.0	48.7	384.5	384.5	11
14	69.9	70.8	19.4	22.5	50.5	48.3	454.6	454.6	11
15	66.9	69.9	16.7	22.2	50.2	47.7	481.1	481.1	11
16	63.7	65.3	13.9	18.3	49.8	47.0	508.6	508.6	11
17	60.7	54.8	11.3	14.7	49.4	40.1	534.1	534.1	11
18	58.3	57.2	9.3	13.3	48.9	43.8	554.3	554.3	11
19	49.8	56.5	3.9	9.0	46.0	47.4	624.0	624.0	11
20	47.3	51.8	2.9	7.3	44.4	44.5	644.7	644.7	11
21	43.9	45.0	1.9	5.1	41.9	39.9	672.9	672.9	11
22	40.9	42.5	1.4	4.1	39.4	38.4	697.6	697.6	11
23	38.0	38.3	1.1	3.3	37.0	35.0	720.8	720.8	11
24	29.8	28.1	0.5	1.8	29.3	26.3	789.2	789.2	11
25	27.0	25.6	0.4	1.6	26.6	24.0	812.9	812.9	11
26	21.1	19.8	0.3	1.4	20.8	18.3	862.6	862.6	11
27	17.8	16.0	0.2	1.5	17.6	14.5	891.0	891.0	11
28	9.8	8.5	0.1	1.5	9.7	7.0	959.9	959.9	11
29	6.5	7.7	0.1	1.5	6.5	6.2	988.2	988.2	11
30	4.8	5.7	0.0	1.5	4.7	4.2	1003.5	1003.5	11

A= 20
N = 21
IN = 21000
DT =0.05000H
COUNT = 30
M = 30
TOCK = 1003.5H
TOTAL TIME = 1003.5H
AV SQ DVN TOTS4 = 8.7
AV SQ DVN FRS4 = 10.1
AV SQ DVN RBS4 = 8.1
RNMIX = 11
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0830M

FILE: SULFRN12 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR TOTAL S(IV) = 91.0
THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR FREE S(IV) = 15.6
THE SUM OF THE MEANS FOR THE KINETIC RUN AS A WHOLE = 106.6

RK1=0.0001909495ME-1HE-1 RK2=0.0070919421ME-2HE-1 RK3=0.0005099999HE-1
RK4=0.0020999993ME-1HE-1 RK5= 0.0MHE-1
EK1= 1.000000M EK2= 0.280000M EK3= 0.000660M

FILE: SULFRN19 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/4PO 5.0 CMS

KEY FOR ABBREVIATIONS.

COUNT=THE DATA POINT NUMBER.TH=THEORETICAL VALUES. EX=EXPERIMENTAL VALUES.
TOT=TOTAL [S(IV)]. FR=FREE [S(IV)]. RB=REVERSIBLY BOUND [S(IV)].
EACH [S(IV)] IS AS A % OF THE ORIGINAL [S(IV)].
TIM=TIME. RNMIX=REACTION MIXTURE N.O. A=N-1.
N=N.O. OF TIME SEGMENTS OF 50H. IN=N.O. OF CYCLES OF LOOP 20.
DT=STEP TIME LENGTH. M=N.O. OF DATA POINTS. TOCK=TH TIME.
AV SQ DVN=AVERAGE SQUARED DEVIATION PER PAIR OF TH & EX VALUES.
TOT AV SQ DVN=TOTAL AV SQ DVN.
RK=RATE CONSTANT. EK=EQUILIBRIUM CONSTANT.

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.2	57.1	60.6	42.9	39.6	3.6	3.6	2
2	99.1	101.8	55.5	57.6	43.5	44.2	23.2	23.2	2
3	96.4	101.4	52.1	54.7	44.4	46.7	46.1	46.1	2
4	77.5	86.1	31.3	34.0	46.2	52.1	119.3	119.3	2
5	67.9	75.1	22.0	24.6	45.9	50.4	144.0	144.0	2
6	57.6	63.6	13.6	16.0	44.1	47.5	167.0	167.0	2
7	43.1	44.2	5.7	8.4	37.4	35.8	196.1	196.1	2
8	32.1	30.2	2.8	5.0	29.3	25.1	216.2	216.2	2
9	-12.2	10.3	-0.4	3.8	-11.8	6.5	287.6	287.6	2

A= 5
N = 6
IN = 6000
DT =0.05000H
COUNT = 9
M = 9
TOCK = 287.6H
TOTAL TIME = 287.6H
AV SQ DVN TOTS4 = 78.5
AV SQ DVN FRS4 = 8.1
AV SQ DVN RBS4 = 48.9
RNMIX = 2
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0153M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	57.3	57.5	42.7	42.5	3.7	3.7	3
2	99.5	98.8	56.0	56.3	43.5	42.6	23.2	23.2	3
3	98.0	98.0	53.6	54.1	44.4	44.0	46.1	46.1	3
4	87.3	86.8	40.4	39.2	46.9	47.6	119.3	119.3	3
5	81.8	82.0	34.4	34.0	47.5	48.0	144.1	144.1	3
6	76.1	77.9	28.3	29.5	47.8	48.4	167.1	167.1	3
7	67.9	67.4	20.2	21.3	47.7	46.1	196.1	196.1	3
8	61.7	62.5	14.5	16.2	47.2	46.3	216.2	216.2	3
9	37.0	32.5	2.1	3.8	34.8	28.7	287.7	287.7	3
10	27.8	20.6	1.1	3.1	26.7	17.4	311.8	311.8	3
11	17.9	10.5	0.5	2.5	17.4	7.9	337.2	337.2	3
12	10.7	7.7	0.3	2.2	10.5	5.5	354.9	354.9	3

A= 7

FILE: SULFRN19 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

N = 8
IN = 8000
DT =0.05000H
COUNT = 12
M = 12
TOCK = 354.9H
TOTAL TIME = 354.9H
AV SQ DVN TOTS4 = 11.7
AV SQ DVN FRS4 = 1.8
AV SQ DVN RBS4 = 20.4
RNMIX = 3
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0332M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	99.9	57.4	55.7	42.6	44.2	3.7	3.7	4
2	99.6	98.2	56.2	53.8	43.4	44.4	23.2	23.2	4
3	98.4	96.2	54.0	51.9	44.4	44.3	46.2	46.2	4
4	90.3	87.0	43.1	40.7	47.2	46.3	119.3	119.3	4
5	86.1	83.4	38.3	36.7	47.8	46.7	144.1	144.1	4
6	81.7	80.2	33.4	33.2	48.3	47.0	167.1	167.1	4
7	75.4	71.9	26.7	27.3	48.6	44.7	196.2	196.2	4
8	70.7	69.8	22.0	23.1	48.7	46.8	216.2	216.2	4
9	51.6	50.5	6.0	8.9	45.6	41.6	287.7	287.7	4
10	44.5	40.4	3.0	5.6	41.5	34.7	311.9	311.9	4
11	37.0	31.2	1.5	3.3	35.4	27.8	337.2	337.2	4
12	31.5	24.6	1.0	2.5	30.5	22.1	354.9	354.9	4
13	23.5	13.2	0.5	2.2	22.9	11.0	380.8	380.8	4

A= 7
N = 8
IN = 8000
DT =0.05000H
COUNT = 13
M = 13
TOCK = 380.8H
TOTAL TIME = 380.8H
AV SQ DVN TOTS4 = 18.8
AV SQ DVN FRS4 = 3.6
AV SQ DVN RBS4 = 27.8
RNMIX = 4
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0503M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	57.6	56.7	42.4	43.4	3.7	3.7	5
2	99.7	100.0	56.3	56.6	43.4	43.4	23.3	23.3	5
3	98.7	97.7	54.2	55.4	44.5	42.3	46.2	46.2	5
4	91.6	91.8	44.2	44.9	47.4	46.9	119.3	119.3	5
5	88.0	87.5	39.9	39.6	48.1	47.9	144.1	144.1	5
6	84.2	87.7	35.6	38.8	48.6	48.9	167.1	167.1	5

FILE: SULFRN19 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

7	78.8	81.0	29.8	32.9	49.0	48.0	196.2	196.2	5
8	74.7	79.2	25.5	28.5	49.2	50.6	216.3	216.3	5
9	58.2	65.5	10.0	16.0	48.2	49.4	287.8	287.8	5
10	52.1	57.9	5.7	12.0	46.4	46.0	311.9	311.9	5
11	45.5	50.3	2.8	7.3	42.7	43.0	337.3	337.3	5
12	40.8	44.4	1.7	4.9	39.1	39.5	355.0	355.0	5
13	33.9	35.0	1.0	3.1	32.9	31.9	380.8	380.8	5
14	12.2	8.6	0.2	1.8	12.0	6.7	459.4	459.4	5
15	5.4	6.6	0.1	2.0	5.3	4.6	483.5	483.5	5

A= 9
N = 10
IN = 10000
DT =0.05000H
COUNT = 15
M = 15
TOCK = 483.5H
TOTAL TIME = 483.5H
AV SQ DVN TOTS4 = 11.8
AV SQ DVN FRS4 = 9.9
AV SQ DVN RBS4 = 2.7
RNMIX = 5
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0648M

COUNT	TOT TH	TOT EX	FR TH	FR EX	RB TH	RB EX	TIM TH	TIM EX	RNMIX
1	100.0	100.0	57.7	54.9	42.3	45.1	3.7	3.7	6
2	99.7	93.8	56.4	51.4	43.3	42.4	23.3	23.3	6
3	98.8	93.8	54.3	51.2	44.5	42.6	46.2	46.2	6
4	92.6	92.7	45.0	46.1	47.6	46.6	119.4	119.4	6
5	89.5	87.5	41.1	39.9	48.3	47.6	144.1	144.1	6
6	86.1	88.4	37.2	39.5	48.9	48.9	167.2	167.2	6
7	81.4	81.7	32.0	34.6	49.4	47.1	196.2	196.2	6
8	77.6	80.5	28.0	29.1	49.6	51.4	217.1	217.1	6
9	63.3	67.7	14.0	18.2	49.3	49.6	287.8	287.8	6
10	57.9	61.7	9.3	13.7	43.6	47.9	312.0	312.0	6
11	52.0	54.0	5.1	9.2	46.9	44.8	337.3	337.3	6
12	47.9	48.9	3.1	6.4	44.7	42.5	355.0	355.0	6
13	41.7	39.9	1.6	3.5	40.1	36.4	380.9	380.9	6
14	22.6	15.0	0.3	1.7	22.3	13.2	459.4	459.4	6
15	16.7	9.6	0.2	1.8	16.5	7.8	483.6	483.6	6
16	13.0	7.1	0.1	1.9	12.9	5.2	498.5	498.5	6

A= 9
N = 10
IN = 10000
DT =0.05000H
COUNT = 16
M = 16
TOCK = 498.5H
TOTAL TIME = 498.5H
AV SQ DVN TOTS4 = 16.5
AV SQ DVN FRS4 = 8.4

FILE: SULFRN19 TABLSIV A LEEDS UNIVERSITY COMPUTING SERVICE VM/HPO 5.0 CMS

AV SQ DVN RBS4 = 16.5
RNMIX = 6
FOR THE MIXTURE OF COMPOSITION [GLUCOSE] = 1.0M [GLYCINE] = 0.5M [S(IV)] = 0.0819M

THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR TOTAL S(IV) = 27.5
THE MEAN OF THE AVERAGE SQUARED DEVIATIONS FOR FREE S(IV) = 6.4
THE SUM OF THE MEANS FOR THE KINETIC RUN AS A WHOLE = 33.8

RK1=0.0005342963ME-1HE-1 RK2=0.0192120990ME-2HE-1 RK3=0.0005099999HE-1
RK4=0.0020999993ME-1HE-1 RK5= 0.0MHE-1
EK1= 1.330000M EK2= 0.280000M EK3= 0.000660M