The Effect of Bleaching Agents on Mineralised Tooth Tissues and Metallic Biomaterials

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In the memory of my late uncle

Professor Naji A R N Al-Salehi

who was always a source of inspiration to me.

Abstract

In recent years, bleaching has become very popular as an effective method of whitening teeth. Although bleaching is considered a conservative technique for improving aesthetics, it has the potential to cause adverse changes to dental tissues and biomaterials. The aim of this research was to assess the effect of varying concentrations of bleaching agents on (i) tooth demineralisation and microhardness, and (ii) metal ion release from dental amalgams and casting alloys. Freshly extracted bovine incisor teeth were investigated before and after bleaching with hydrogen peroxide (10% - 30% w/v). The results showed a significant decrease in mineral content of both enamel and dentine following bleaching. The enamel also exhibited an associated reduction in microhardness when bleached. Tests were carried out on amalgam discs bleached with hydrogen peroxide (0-30% w/v). Data showed a significant increase in Hg, Cu, Ag and Sn ion release with increasing hydrogen peroxide concentrations. Similar tests were carried out on two typical dental casting alloys, Ni–Cr and Pd-Cu-Ga. The data again indicated an increase in ion release with increasing hydrogen peroxide concentrations. The elevated ion release from dental amalgams, casting alloys and mineralised tooth tissues suggested that caution should be exercised when applying bleaching agents. Moreover, there is a case for not applying hydrogen peroxide at relatively high concentrations. The data reported here reinforces the view that bleaching agents may have deleterious effects, especially if bleaching agents are applied at high concentrations and/or long periods.

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1. Introduction

The ultimate objective of aesthetic dentistry is to create a perfect smile. As well as a healthy mouth, patients increasingly demand a "beautiful smile". The teeth must be in harmony with the gingiva, lips and face of the patient. The aesthetics of any restoration are influenced by several factors including surface form, translucency and colour (1). Tooth discolouration is an increasingly common problem, particularly as people are keeping their teeth for longer. Discoloration may, however, affect both primary and secondary teeth. Additionally, many patients dislike the darkening process that follows the deposition of secondary dentine.

The aetiology of dental discolouration is multifactorial with different parts of the tooth taking up different stains (2). Many researchers classify staining as either extrinsic or intrinsic. The colour of the teeth is determined by the combined effects of extrinsic and intrinsic colorations. Extrinsic discoloration occurs when an agent stains or damages the enamel surface of the teeth and usually increases with increasing age and is more common in men than women (3). Intrinsic staining can be defined as endogenous staining that has been incorporated into the tooth matrix and cannot be removed by prophylaxis (4).

Intrinsic tooth colour is associated with the optical properties of the enamel and dentine in terms of light scatter and absorption. Teeth are polychromatic and the colour varies among the gingival, incisal and cervical areas according to the thickness, reflectance of different colours and translucency in the enamel and dentine. The colour of healthy teeth is primarily determined by the dentine (5).

Bleaching can significantly enhance the whiteness of teeth and it is also considered to be a relatively conservative aesthetic procedure. A number of bleaching techniques are now routinely used such as Nightguard® vital bleaching or surgery-based bleaching carried out by a dentist. All such techniques are effective in improving the patient's appearance. Tooth bleaching may, however, have adverse effects on dental soft and hard tissues as well as dental biomaterials. Active hydrogen peroxide (HP) concentrations vary enormously and can be as high as 35% (w/v) even though current UK law limits the peroxide content to only 0.1% (6, 7). Despite widespread debate, there is currently a trend towards employing greater concentrations of HP as the active agent in tooth bleaching preparations. The debate is understandably complex, and includes concern over the effects

of bleaching agents on tooth tissues and, to a lesser extent, dental materials.

In recent years a large number of studies have been carried out to assess the effects of bleaching agents on tooth tissue (8-10) but relatively little attention has been devoted to the effects of bleaching agents on dental biomaterials. It is recognised that bleaching can cause demineralisation, perhaps even adversely affecting the structure of the tooth (11, 12). While it is also believed that HP causes ion release such as mercury from dental amalgam (13, 14) and nickel from dental casting alloys (15), available data are limited and contradictory. Understanding the interaction of bleaching agents and substrates is important, as the released ions may have local and/or systemic toxic effects. This thesis therefore describes a thorough and detailed study of ion release from tooth tissues and metallic dental materials. The data presented is of value to researchers, clinicians and regulatory bodies. As well as quantifying ion release from dental amalgams and casting alloys, the effects of varying HP concentrations on tooth demineralisation and microhardness were also investigated. The specific aims and objectives of the work are set out in Chapter 3.

2. Literature Survey

Before reporting the results of this study, it is important to consider background research published in this field and related areas. Relevant aspects include clinical use, mechanism of bleaching and effects on oral tissues. In addition, the effects of bleaching agents on dental materials have also been reviewed, as such papers have particular relevance to this research.

2.1 Oral Tissues

2.1.1 Enamel

Enamel is the hardest mineralised tissue known, consisting of 96% mineral and 4% organic material and water. The inorganic content of enamel consists of a crystalline calcium phosphate known as hydroxyapatite, which is also found in bone, calcified cartilage, dentine and cementum. Although nearly the entire volume of enamel is occupied by the densely packed hydroxyapatite crystals, a fine, lacey network of organic material appears between the crystals. The chemical nature of this material has not been completely determined, but it is largely proteinaceous and contains some polysaccharide material (16). Both bones and dentine are a relatively spongy assembly of the

apatite crystals interwoven with type I collagen and other proteins, producing a very tough mineral. Tooth enamel is much more heavily mineralised than bone, making it much harder, and enamel does not contain collagen, though it does, when mature, contain small amounts of specialised matrix proteins (17).

Tooth surface loss is becoming an increasingly common problem as more patients retain their natural teeth for longer. The aetiology is usually multifactorial, encompassing attrition, abrasion and erosion (18, 19). Physiological attrition increases with age with enamel wear facets becoming more evident. Other characteristics of aging in enamel include changes in colour, permeability and the nature of the surface layer. Teeth darken with age. This could be due to the addition of organic material to enamel from the environment, it could also be due to the darker dentine colour seen through the thinner more translucent enamel layer (16).

2.1.2 Dentine

Vertebrate tooth dentine is a complex tissue formed in an organised fashion by a layer of odontoblasts initially in opposition to the layer of ameloblasts that are involved in enamel formation. The secretory odontoblasts are elongated polarized cells that secrete collagen at the end facing the dentino–enamel junction (DEJ). The main body of each odontoblast retracts from the DEJ as the secreted dentine collagen thickens and mineralises, forming a channel which becomes a dentinal tubule. The tubules penetrate the entire thickness of the dentine and predentine. The majority of the dentine collagen fibril network is deposited between the tubules, with each fibril axis approximately perpendicular to the direction of the tubule and nearly parallel to the DEJ. The network becomes mineralised forming the intertubular dentine (ITD). However, an elongated odontoblastic process remains within each tubule for a substantial portion of the total tubule length. The tubules become surrounded by annular collars of a differently structured mineralised material, the peritubular dentine (PTD) (20-22). In the coronal dentine the PTD is more heavily mineralised than the ITD, with the difference in the mineral content ranging from less then 9% (23) to 40% (22) depending on location. The

differences between the PTD and ITD mineral content control the mechanical properties of the dentine. Thus, the dentine microhardness varies in different parts of a given tooth (24). Marshall *et al*. (25), found that PTD hardness was not dependent upon location and had a uniform Young's modulus, concluding that the changes in dentine microhardness with location could be attributed to changes in the hardness of ITD and not to a local increase in number of tubules. By comparing the microhardness of the tubular mantle dentine just below the DEJ with the bulk of the dentine, Wang and Weiner (26) showed that the presence of PTD enhances dentine stiffness. Clearly, although the function of the PTD is not well established, the dentinal tubules and surrounding PTD have an important biomechanical function in dentine. The evident microheterogeneity in mechanical properties, mineral content, and matrix organisation of dentine raises fundamental questions regarding the control over formation, growth, and composition relevant not only to the dentine layer but to mineralised tissues in general.

2.1.3 Oral Mucosa

The oral cavity is lined by a mucous membrane that consists of two layers, an epithelium layer and a connective tissue layer known as the lamina propria. The oral mucosa can be classified as masticatory, lining and specialised mucosa. Masticatory mucosa covers the gingiva and the hard palate. It is tightly bound down by the underlying connective tissue to alveolar bone and the covering epithelium is keratinized. The lining mucosa, on the other hand, is very flexible to perform its function of protection and its epithelium is not keratinized. Specialised mucosa refers to the mucosa covering the tongue, this tissue contains papillae and taste buds that serve special functions. A unique feature of the oral mucosa is that it is perforated by teeth. The mucosa immediately surrounding the erupted tooth is known as the gingiva. The gingiva consists of two parts, that facing the oral cavity which is masticatory mucosa and that facing the tooth which is involved in attaching the gingiva to the tooth and also forms part of the periodontium (16).

2.2 Discolouration of Tooth Tissue

Discolouration (staining) of teeth is often classified as either extrinsic or intrinsic (27, 28). The aetiology is summarised in Table 2.1 below. A number of restorative techniques can be used in the treatment of discoloured teeth. These include tooth coloured direct restorations and resin/ceramic veneers and crowns. Tooth bleaching, however, can be a cheaper and more conservative alternative in appropriately selected clinical cases. Haywood (29) described Nightguard® vital bleaching to be probably the safest, most cost-effective, patient pleasing method to improve the appearance of a smile.

Plaque, chromogenic bacteria, surface protein denaturation Mouthwashes eg. chlorhexidine Beverages (tea, coffee, red wine, cola) Foods (curry, cooking oils, dried foods, food colourings, berries, beetroot) Dietry precipitates Illness Antibiotics (erythromycin, penicillin) Iron supplements	(i) Pre-eruptive Disease • Haematological disease • Liver disease • Diseases of enamel and dentine Medication • Tetracycline stain • Fluorosis stain (ii) Post-eruptive Trauma Primary and secondary caries Dental restorative materials Aging Smoking Chemicals Some food stuffs (long term use causes deeper intrinsic stains) Minocycline Functional and parafunctional changes

Table 2.1 Aetiology of tooth discoloration (adapted from ref. 2)

2.3 Bleaching Systems - Clinical Use

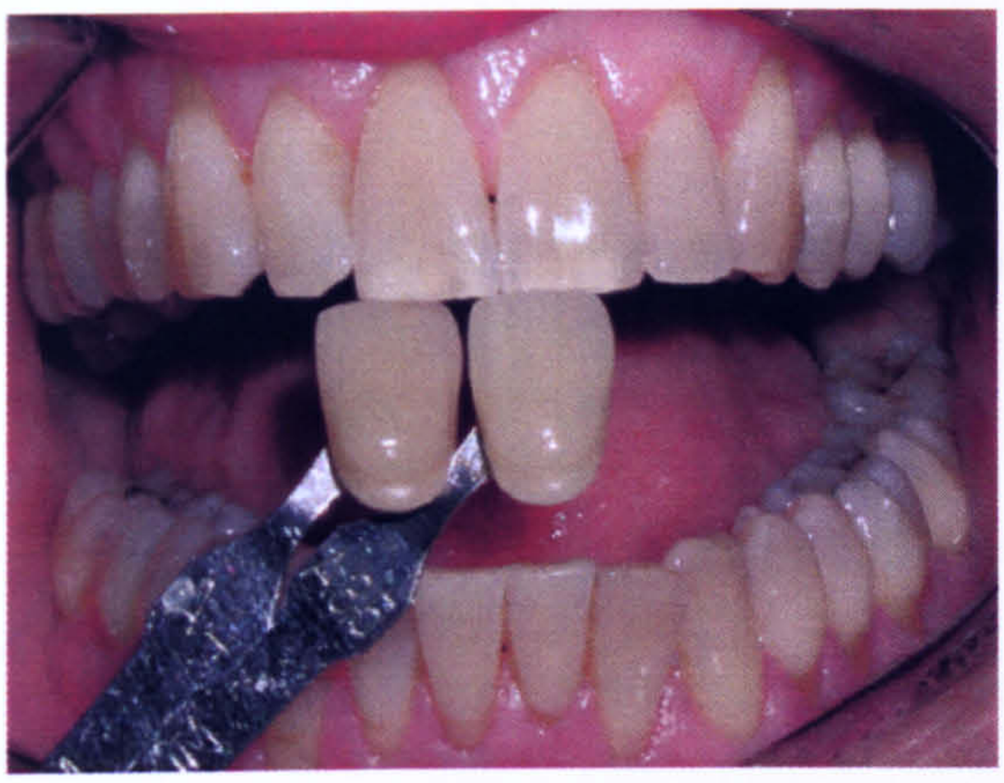
Several bleaching systems have been introduced in recent years as a direct response to an increase in demand for aesthetic dentistry. The active ingredient in bleaching agents is hydrogen peroxide (HP) which was originally introduced as an irrigant for disinfecting alveolar abscesses (30). At the time, it was also recommended that it might be used in bleaching of discoloured teeth. HP is applied either directly or via its generation in a carbamide peroxide (CP) gel. CP has been used as a bleaching agent since Haywood and Heymann published a report on the technique in 1989 (31). CP decomposes to produce HP which is the active ingredient in bleaching because of its low molecular weight and its ability to denature proteins (32).

A number of bleaching procedures are routinely used, these include in-surgery (power bleaching) and matrix (tray) home applied procedures (Figure 2.1). Key factors in determining the efficacy of bleaching agents are the concentration of the peroxide, duration of application and the rate of chemical reaction (2). A number of agents such 10% CP and HP solutions in varying concentrations up to 35% are now routinely used for

dental bleaching. A summary of commonly used bleaching agents is given in Table 2.2.

Hydrogen Peroxide	Carbamide Peroxide	Sodium Perborate
Pola Day 3%, 7.5% Pola Office 35% (SDI) Brite Smile 15% (Brite Smile)	Pola Night 10%, 16%, 22% (SDI) Opalescence 10%, 15% (Ultradent)	Opalescence SP (Ultradent)

Table 2.2 Bleaching agents in common clinical use (application times depend upon half-life and activation methods for chair side use).



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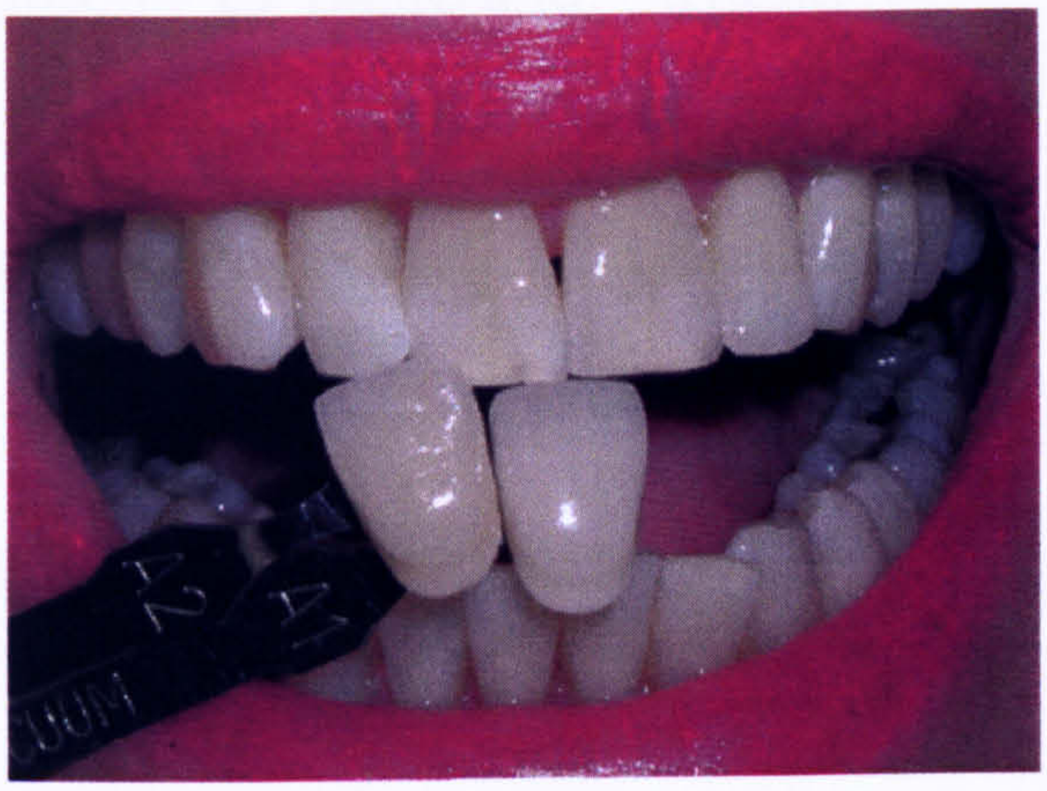

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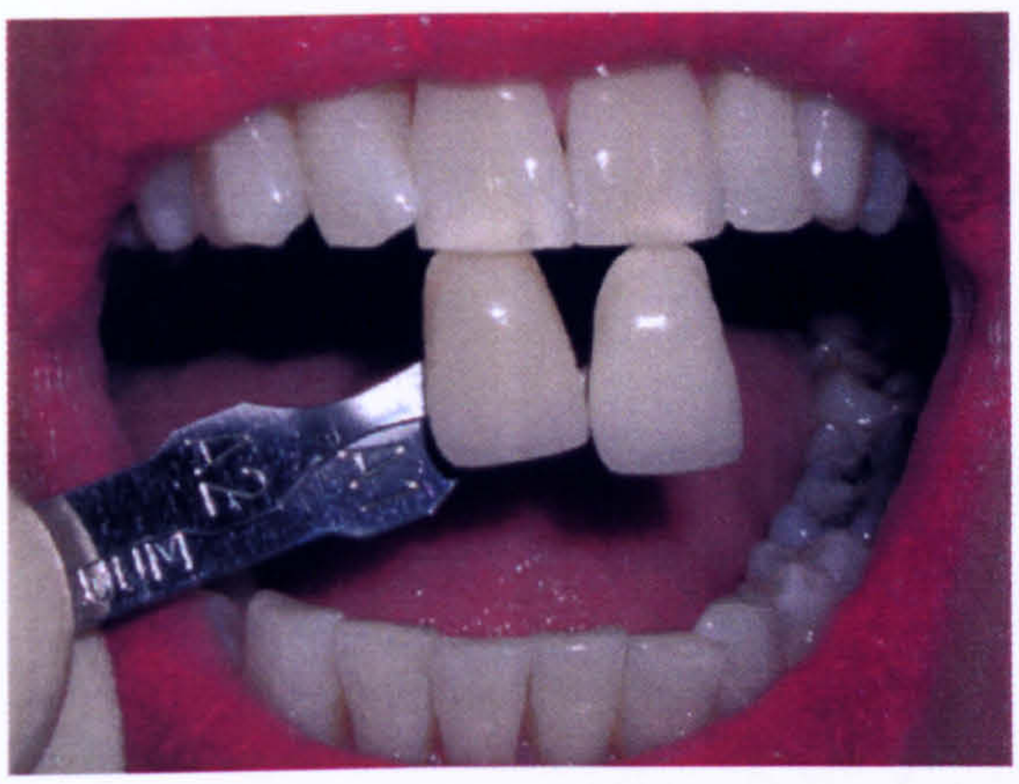

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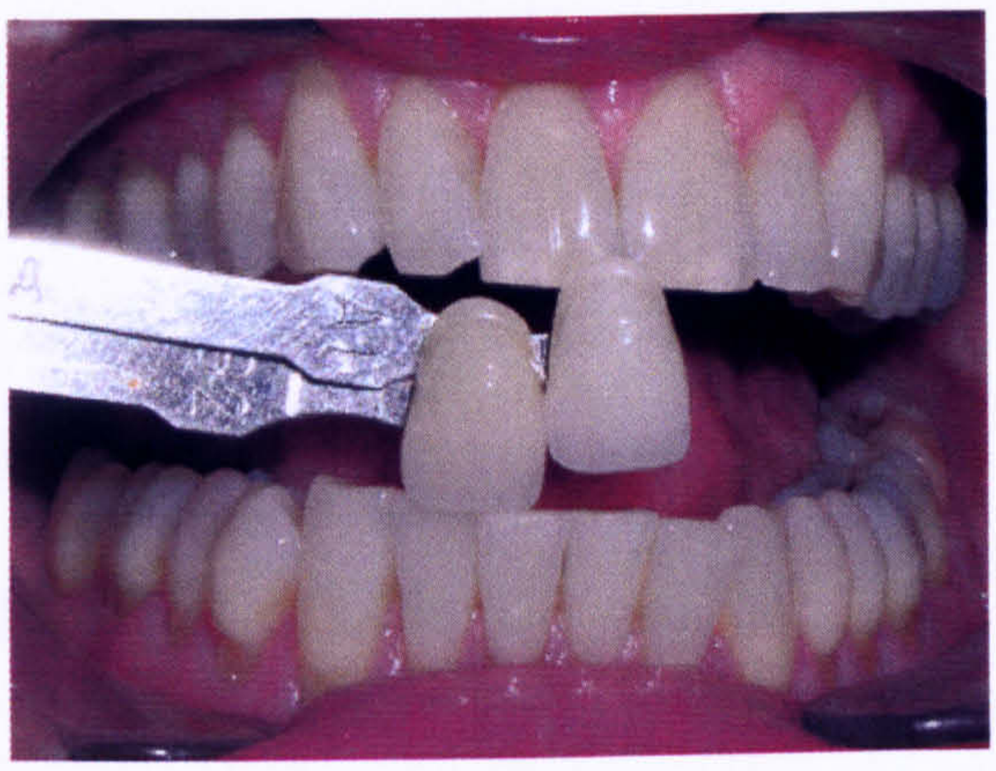

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Figure 2.1 Opalescence (Ultradent) tray bleaching.
(a) initial presentation (b) one week bleaching
(c) two weeks bleaching (d) 12 month review
(clinical photographs provided by Dr.S.Northeast from his private practice)

2.3.1 Contra-indications to Bleaching

There are a number of contra-indications to bleaching. These include, periodontal disease, caries, failing restorations (gross disease). Bleaching may not be effective in severe or ongoing staining (2). Multiple anterior restorations often result in the need for replacement after bleaching, this may be unacceptable. Root surface exposure and enamel loss can lead to increased sensitivity following bleaching. The incidence of minor thermal sensitivity following bleaching has been reported to be approximately 50% and major sensitivity to be about 10%. The duration of sensitivity is 24 h to 48 h after bleaching applications and the sensitivity decreases with prolonged use (33). The use of potassium nitrate and fluoride added to a 10% CP gel can reduce dental sensitivity (34).

2.4 Factors Influencing Colour and Tooth Whitening

Three factors can influence the perception of colour. These are the light source, the object being viewed and the observer viewing the object. The Commission Internationale de L'Eclairage (CIE) defined a colour space, CIE Lab, that supports the accepted theory of colour perception based on three separate colour receptors (red, green and blue) in the eye and is currently one of the most popular colour spaces. The CIE Lab colour space represents a uniform colour space, with equal distances corresponding to equal perceived colour differences. The advantage of the CIE Lab system is that colour differences can be expressed in units that can be related to visual perception and clinical significance. Instrumental objective measurements using spectrophotometers, colourimeters and image analysis techniques can be used to measure tooth colour as well as subjective visual examination (1).

In the CIE Lab system, three parameters L*, a* and b* are used to represent the degree of lightness within a sample ranging from 0 (black) to 100 (white), the degree of green/red colour, and the degree of blue/yellow colour in the sample, respectively.

A change in colour ΔE can be calculated in terms of changes in L*, a* and b* by the equation,

$$\Delta E = \sqrt{(L^{*2}+a^{*2}+b^{*2})}.$$

With regard to whiteness, two of the key factors in determining overall tooth whitening efficacy from peroxide containing products are the concentration of the peroxide and duration of application (35). Sulieman *et al.* (36) carried out an *in vitro* study examining the effect of various concentrations of HP (5-35%) on tooth whitening. Extracted third molar teeth were sectioned and stained using a standardised tea solution to Vita shade C4. The stained specimens were bleached with a series of gels containing 5, 10, 15, 25 or 35% w/w HP. The change in shade was assessed via three different techniques; visual assessment with a Vita shade guide, use of a shade vision system and using a chromometer (utilizing the L* a* b* system). It was concluded that the concentration of HP has a marked effect on the number of applications required to produce an optimal shade outcome (eg. a 35% HP required only one application whereas a 5% HP solution required 12 applications). Leonard *et al.* (37) carried out a similar study but, substituted CP for HP at varying concentrations. They reported that lower (5%) concentrations of CP take longer to whiten teeth but, eventually

achieve similar results as that of higher concentrations (10-16%). Similarly, Braun *et al.* (38) assessed the effects of varying concentrations of CP agents on tooth bleaching. A double blind study design was carried out on 30 subjects undertaking home bleaching with 10%, 17% or 0% (control) CP concentration for one week. Tooth shades were determined using a visual shade matching system and a spectrophotometer. The results showed that higher CP concentrations whiten teeth faster with major changes in lightness and chroma but similar effects can be obtained with lower concentrations by bleaching daily for one week. Luo *et al.* (39) investigated the suitability of different whiteness indices and colour parameters in assessing changes in tooth whiteness, following bleaching, using a digital-colour imaging system. Digital images of teeth were captured and the data obtained was used to calculate colour parameters and whiteness indices. The test group showed significant changes both in the colour parameters and whiteness indices. The whiteness was assessed via three different whiteness indices. Matis *et al.* (40) evaluated the degree of colour change with two different concentrations of CP. Twenty five subjects used 10% and 15% CP in trays for 14 days on different sides of their maxillary arches. The subjects returned in 3 days and at 1, 2, 3 and 6 weeks for assessment. The difference in tooth

whitening between 15% and 10% CP was statistically significant after 2 weeks. However, when the subjects were evaluated after 6 weeks, the differences were not statistically significant.

In addition to peroxide concentration and duration of its application, the rate of chemical reaction can be increased by increasing the temperature. Most in-office bleaching systems are based on HP using concentrations of up to 35% applied as a paste or gel to the tooth surface. A light source is then applied to the tooth surface to increase the reaction rate and accelerate the decomposition of HP to oxygen and perhydroxyl free radicals, which are thought to bleach the teeth. Many different types of lights are available for use in tooth bleaching, including halogen, plasma arc, light emitting diodes and diode lasers (41). There has been concern about using lights as part of the bleaching process as the heat generated could cause pulpal necrosis. Zach *et al.* (42) reported known temperature rises to histologically observable pulpal injury in Rhesus monkeys. They found that 15% of teeth heated by 5.5°C had irreversible damage. Other workers (43) have suggested that the threshold value should be higher as an intact pulpal blood flow will act as an efficient heat sink and dissipate some of the applied heat before pulpal cells are damaged. Sulieman *et al.* (44) measured the surface and

intra-pulpal temperature increases *in vitro* on upper and lower anterior teeth during tooth whitening procedures. An *in vitro* experimental technique was developed to measure temperature rises in extracted human anterior teeth. A thermocouple and associated equipment was used to record the data when four different curing lights were, in turn, applied to the tooth surface with and without a bleaching gel. The main conclusion of the work was that a bleaching gel acts as an insulating layer against excessive temperature increases and that the use of a laser diode (one of the four curing lights tested) on full power may cause pulpal necrosis. In power bleaching, the dentist must be aware of the likely intra-pulpal temperature rise. A strict protocol should be in place to eliminate the likelihood of applying the lamp without a gel.

The type of intrinsic stain and the initial tooth colour can play a significant part in the ultimate outcome of tooth bleaching (35). Ishikawa-Nagai *et al.* evaluated the tooth colour change of 80 subjects after using 10% CP in a gum shield over 14 days and found a strong correlation between total colour change and b* values (a measure of blue/yellow), demonstrating that bleaching works efficiently for teeth with a yellow hue (45). Joiner *et al.* (46) evaluated the effect of a 6% HP tooth

whitening gel on the colour change of human enamel *in vitro.* The enamel samples showed an increase in L* and a decrease in b*. The colour stability has been reported to be 75% at 18 months and 65% at 3 years after treatment with a 10% CP tray bleaching system (47). Although some reversal of the lightening effect occurs over time following the original bleaching treatment, the loss of the lighter colour appears to be gradual (48). Greater shade changes can be expected with darker teeth. Variable results are seen with fluorosis staining. Tetracycline belongs to a class of broad spectrum antibiotics all of which can stain teeth. The colour is derived from photo-oxidation of tetracycline molecules bound within the tooth structures (49). The mechanism by which peroxide affects the tetracycline stain is considered to be by chemical degradation of the unsaturated quinone type structures found in tetracycline leading to less coloured molecules (35, 50). Tetracycline staining is more challenging to treat and has been shown to respond to a longer bleaching regime (3-6 months). Leonard *et al.* (51) carried out a longitudinal whitening study to determine patient satisfaction 90 months post treatment and after 6 months active treatment of tetracycline stained teeth with 10% CP. Sixty percent of participants reported no obvious shade change 90 months post active bleaching treatment. The results indicated that

tetracycline stained teeth can be whitened successfully using extended treatment time.

2.5 Mechanism of Bleaching

Bleaching is an oxidation reaction which results in a decolourisation or whitening process that can occur in solution or on a surface. The colour-producing materials in solution or on a surface are typically organic compounds that possess extended conjugated chains of alternating single or double bonds and often include heteroatoms, carbonyl, and phenyl rings in the conjugated system and are referred to as a chromophore.

Bleaching and decolourisation of the chromophore can occur by destroying one or more of the double bonds in the conjugated chain, by cleaving the conjugated chain, or by oxidation of other chemical moieties in the conjugated chain (52). HP oxidises a wide variety of organic and inorganic compounds and can form a number of different active oxygen species depending on reaction conditions, including temperature, pH, light and presence of transition metals (35). Under alkaline conditions, HP bleaching generally proceeds via the perhydroxyl anion (HO_2^-). Other conditions can give rise to free radical formation, for example, by

homolytic cleavage of either an O–H bond or the O–O bond in HP to give $H^{\bullet}$ + $^{\bullet}OOH$ and $2^{\bullet}OH$ (hydroxyl radical), respectively (52). Under photochemically initiated reactions using light or lasers, the formation of hydroxyl radicals from HP has been shown to increase (53).

The mechanism by which teeth are bleached by oxidizing materials such as HP and CP are not completely understood (54). It is thought that peroxide diffuses through the enamel to reach the enamel dentine junction, dentine regions and the pulp. In an *in vitro* study, Joiner *et al.* (55) reported that the amount of HP in tooth pulp chambers to be 0.44 mM, which is over 3000 times below the concentration needed to cause pulpal enzyme damage. As peroxide diffuses into the tooth, it can react with organic coloured materials found within the tooth structures leading to a reduction in colour particularly in dentine (35). Sulieman *et al.* (54) quantified the penetration of 35% HP into enamel and dentine and related this to the resultant shade change of the tooth. Twenty four caries free teeth were sectioned and stained with a tea solution. The teeth were split into two groups, a control and experimental group. The test group was subjected to power bleaching with 35% HP. It was shown that significant bleaching occured within the dentine on the buccal surface.

2.6 Bleaching of Oral Tissues

2.6.1 Vital and Non-Vital Tooth Bleaching

Vital and non-vital bleaching of teeth is normally carried out using varying concentrations of HP and CP to improve the whiteness of teeth. The improvement in tooth colour may, however, be at the expense of tooth strength such as a reduction in ultimate tensile strength and a reduction in tooth microhardness (9, 56).

A number of vital bleaching procedures are now routinely used to obtain whiter teeth. In-office bleaching usually involves the application of 30% HP and a light source (power bleaching). Mouth guard bleaching allows the procedure to be carried out outside the dental office. It is commonly carried out using mouth trays custom made and fitted by the dentist using lower concentrations of HP (2-10%) and applied for several hours a day for several weeks. Haywood *et al.* (57) evaluated the effects of CP on tooth surfaces. Thirty-three extracted teeth were subjected to 10% CP for a period equivalent to 5 weeks night time wear. A control area on each tooth had been covered and sealed. Epoxy replicas of the teeth were examined under a

scanning electron microsocope (SEM). They reported no difference in surface texture between treated and control areas. Spalding *et al.* (58) carried out an *in vitro* study on the effect of bleaching agents on human enamel surface. The enamel was examined under SEM. Three experimental protocols were carried out. In experimental protocol one, specimens were treated with 35% HP. In protocol two, after treatment with 35% HP, specimens were immersed in natural saliva for one week. In protocol three, 35% HP was applied once and 10% CP for one week. These protocols all had in common a period of 20 minutes contact between enamel fragments and light activated 35% HP. It was concluded that despite the changes shown to have occurred in the enamel surface after bleaching, normal variation in tooth morphology may exceed the effects of 35% HP and 10% CP on the teeth. Accordingly, bleaching was considered safe for enamel. A number of other SEM studies (59-64) have also shown little or no topographic changes to bleached enamel. In contrast, Covington *et al.* (65) using SEM reported changes in surface morphology of bleached enamel but, with no major changes to the composition of the enamel. Several other studies (66, 67) have reported pittings, "waviness" and increased surface roughness to the enamel after bleaching.

While studies on the effects of bleaching on morphological changes to tooth tissue are contradictory, it is generally agreed that peroxides can alter tooth mineral contents. A number of studies (8, 56, 68, 69) have been carried out to investigate the effect of these alterations on the mechanical properties of teeth. Potocnik *et al.* (68) examined the microhardness of human enamel treated with 10% CP. They reported no significant difference between bleached and control sides. Lewinstein *et al.* (56) examined the effect of HP and sodium perborate on the microhardness on human enamel and dentine. It was shown that the use of high concentrations of HP (30%) for intracoronal bleaching should be limited as it caused a significant reduction in enamel microhardness after a 15 minute treatment. Sodium perborate appeared to be a less damaging agent. Sehgi and Denry (69) showed a change in the fracture toughness of enamel following bleaching. The apparent fracture toughness of enamel was reduced by 30% after bleaching for a period of 12 hours with no significant difference in surface hardness. Cavalli *et al.* (8) examined the effect of CP bleaching agents on the tensile strength of human enamel. Samples were divided into 6 groups and bleached in solutions of varying concentrations of CP (0% to 20%). Specimens subjected to the bleaching regimens presented significantly lower ultimate tensile strength than the

control group. Joiner *et al.* (46) evaluated the effect of a 6% HP tooth whitening gel on enamel and dentine microhardness *in vitro*. Polished human enamel and dentine specimens were exposed to 20 min cycles of water, 6% HP gel or Sprite Light® for up to 28 cycles. The 6% HP and water gave no statistically significant ($p>0.05$) changes in enamel and dentine microhardness after 28 treatments. However, after one 20 min treatment, Sprite Light® gave a significant ($p<0.00002$) reduction in enamel microhardness.

Studies of tooth mineral release due to exposure to extrinsic agents including HP and acidic carbonated beverages have been carried out. McCraken and Haywood (11) measured the amount of calcium (Ca) loss from enamel exposed to 10% CP. The data were compared to Ca loss when enamel exposed to a 16 oz Cola beverage with distilled water used as a control. Calcium losses of about 1.06 $\mu g/mm^2$, 1.25 $\mu g/mm^2$ and 0.27 $\mu g/mm^2$ were recorded when the enamel was exposed to 10% CP, cola beverage and distilled water respectively. Grobler *et al.* (70) reported a Ca loss of 1.9 $\mu g/mm^2$ from enamel after 2 minute of exposure to Pepsi Cola. In another paper, Grobler *et al.* (71) reported Ca loss from exposure to Pepsi Cola to be equal to Ca loss from exposure to orange juice but, greater than exposure to

apple juice which was in turn greater than Ca loss from exposure to Diet Pepsi Cola. Potocnik *et al.* (68) using electron probe microanalysis showed lowered concentrations of Ca and phosphorous (P) and with mean Ca/P value of all bleached samples decreasing after bleaching with 10% CP. Lee *et al.* (72) investigated mineral loss from bovine enamel by a 30% HP solution. They reported a decrease in Ca/P ratio of bleached enamel. They also measured the release of magnesium (Mg) and reported that Mg/Ca ratio to be 0.06 in the HP solution. Efeoglu *et al.* (12) used computerised tomography to evaluate the effect of 10% CP on enamel. The application of 10% CP was found to cause significant demineralisation ($p<0.05$) extending to a depth of 50 μm below the enamel surface. In recent years, however, at home tooth bleaching using peroxide agents has become well accepted. It is generally agreed that vital bleaching which involves the use of 10% CP is safe if applied properly, however, adverse effects may occur in inappropriate applications (73-77).

Non-vital bleaching is a common procedure for restoring the shade of discoloured endodontically treated teeth. The walking method is the preferred method for bleaching teeth which have been root treated. It can be performed at the time of treatment

or some years later. The bleaching material is placed in the pulp chamber and sealed temporarily for several days. A thermocatalytic method may also be used to activate the bleaching material inside the pulp chamber (78). A complication of the use of HP as the bleaching material is the development of external root resorption during non-vital bleaching (79). It has been reported that the penetration of HP is significantly greater in teeth that have cementum defects. Rotstein *et al.* (80) examined the influence of different cementum defects and their location on the radicular penetration ability of 30% HP during thermocatalytic bleaching. The teeth were divided into three groups; one group with no cementum defects at the cementoenamel junction (CEJ), one group with artificial cementum defects at the CEJ and another group with artificial cementum defects at the middle third of the root. The radicular penetration of 30% HP in the three groups was assessed directly and compared using an *in vitro* model. Statistical analysis revealed that teeth with artificial cementum defects at the CEJ were significantly more permeable than those without defects ($p<0.01$) and teeth with artificial mid-root defects were more permeable than those without defects ($p<0.025$). In a later study by Rotstein *et al.* (81) the effect of HP treatment on human dentine and cementum was examined. Intact teeth were

pulverized and separated into dentine and cementum powders. The pulvarized tissues were exposed to 30% HP, 3% HP, 2% sodium perborate in 30% HP, 2% sodium perborate in 3% HP and 2% sodium perborate in bidistilled water for periods of 15 min and 1, 24 and 72 h. The greatest increase in calcium ion loss occurred in 30% HP and 2% sodium perborate in 30% HP after 24 h and 72 h treatments. It was concluded that 30% HP caused morphological and structural changes in both dentine and cementum. The exact chemical changes in the hard dental tissues remain unestablished. Lewinstein *et al.* (56) examined the effect of HP and sodium perborate on the microhardness on human enamel and dentine. It was shown that the use of high concentrations of HP (30%) for intracoronal bleaching should be limited as it caused a significant reduction in enamel microhardness after a 15 minute treatment. However, sodium perborate appeared to be a less damaging agent. Tam *et al.* (82) examined the effect of bleaching agents on the flexural strength (FS) and modulus (FM) of bovine dentine. Direct *in vitro* application of CP bleaches caused significant ($p<0.05$) decreases in dentine FS and FM. Similarly, Chng *et al.* (83) evaluated the effect of 30% HP on the surface changes and non mechanical properties of the intertubular dentine of sectioned human premolar teeth. Exposure to 30% HP for 24 h caused

surface changes to intertubular dentine and significantly decreased the hardness (p=0.002) and Young's modulus (p=0.001) of intertubular dentine. Heller *et al.* (84) and Madison *et al.* (85) assessed cervical root resorption following non-vital bleaching in the anterior teeth of Beagle dogs. External root resorption was found in teeth treated with 30% HP although Madison *et al.* only found resorption when the 30% HP was heat treated.

2.6.2 Cytotoxicity Caused by Bleaching Agents

A number of studies have demonstrated the risk of tissue damage from contact of bleaching agents with the oral mucosa and living cells (86-91). Kinomoto *et al.* (86) examined the cytotoxicity of intracoronal bleaching agents on human periodontal ligament cells (PDL) *in vitro*. Three bleaching agents 30% HP, 2.0 g/ml sodium perborate (SP) solution and 2.0 g/ml SP in HP were diluted from 10^{-3} to 10^{-7} with Eagle's minimal essential medium and incubated with PDL cells isolated and cultured from extracted teeth. The rank order of the TD_{50} values after exposure for 24 h was SP in H_20_2 > H_2O_2 > SP solution. The TD_{50}s for 24 and 72 h incubations in the study were 0.47 mmol/l and 0.56 mmol/l, respectively. It was concluded that the

mixture of SP with HP was the most toxic to the PDL cells *in vitro*. Similarly, Hanks *et al.* (87) examined the cytotoxicity and dentine permeability of CP and HP *in vitro*. The TD_{50}s for 1 and 6 h incubations of HP with Balb/c 3T3 cells were reported to be 0.58 mmol/l and 0.44 mmol/l, respectively. Inhibition of succinyl dehydrogenase activity corresponded to the amount of HP that can rapidly diffuse through dentine *in vitro* and reach concentrations which are toxic to cultured cells in less than 1 h. Aren *et al.* (88) investigated the *in vitro* effects of bleaching agents on FM3A cell line. The cytotoxicicty of PowerGel® and Opalescence PF® were determined by evaluation of cell growth and viability in comparison to untreated controls. PowerGel® and Opalescence PF® showed a cytotoxic effect on cell growth in FM3A cell line. Asfora *et al.* (89) evaluated the biocompatibility of sodium perborate and 30% HP using the analysis of the adherence capacity and morphology of macrophages. Irreversible cellular damage as well as an elevated adherence index was found with 30% HP demonstrating the aggressive potential of 30% HP regardless of its dilution. Koulaouzidou *et al.* (90) investigated the *in vitro* cytotoxicity of Colgate Platinum® bleaching gel and compared the results with that of HP on cell cultures of L929 and BHK21/C13 cells. They found that although both bleaching agents were cytotoxic to

fibroblasts, Colgate Platinum® gel was less toxic than HP. Reibeiro *et al.* (32) assessed genetic damage induced by dental bleaching agents on mouse lymphoma cells by single gel assay. It was found that dental bleaching agents may be a factor that increases the level of DNA damage, with the most damage observed with higher dose peroxides such 35% HP. In contrast, Woolverton *et al.* (91) compared the cytoxicity of two CP products with seven widely used dental products and reported that the CP products were no more toxic to fibroblast cells (L929) than the control groups.

2.7 Bleaching Metallic Dental Materials

2.7.1 Dental Amalgam

Dental amalgam was first used in the early 19th century as a restorative material. The development of dental amalgam is due to G.V.Black who, in 1895, recognised the need to determine the properties of dental amalgam with some accuracy (92). He provided a formulation for dental amalgam that proved acceptable clinically (93). This formulation remained unchanged for about seventy years. In 1962, a spherical particle dental alloy was introduced (94). This was followed in 1963 by a high-

copper dispersion alloy system. This was found to be superior to its low-copper predecessors (95) because the additional copper combined with the tin resulted in a copper-tin phase that was more corrosion resistant than the tin-mercury (gamma 2) phase found in the low-copper alloys (96, 97).

Currently used dental amalgam alloys are, in general, composed of silver (40 to 70 percent), tin (12 to 30 percent) and copper (12 to 30 percent). Indium (0 to 4 percent), palladium (0.5 percent) and zinc (up to 1 percent) may also be present (98). Zinc may help to inhibit corrosion of amalgam, although its role in enhancing clinical performance is not fully understood (99). The alloy is mixed with mercury (43.0 to 50.0 percent by weight) to form the amalgam. The amalgam may be supplied as small spheres, lathe-cut or a combination of the two. Handling characteristics of the amalgam vary according to formulation, particle size and shape however, the various formulations do not differ significantly (98, 100).

Amalgam is particularly suitable for Class I and II restorations in teeth that encounter heavy chewing forces. Class II restorations tend to be large with extensive tooth-material interface areas. These present a potential for leakage of oral fluids around the

margins of the tooth-filling interface, increasing the risk for recurrent caries. However, amalgam has been reported to be capable of sealing the tooth-restoration margins with corrosion products that accumulate with time. Since it is metallic in composition, amalgam is unable to mimic the colour or translucency of natural teeth, and its silver-gray colour limits its use on anterior teeth (101).

The use of mercury for the production of silver amalgam restorations and the release of mercury from these restorations has been a cause for concern for many decades (102). In 1926, Alfred Stock, a German chemist published an article condemning amalgam restorations (103). The procedure used at the time to produce the dental amalgam, released significant amounts of mercury vapour. As a result of this publication, in 1930, a newer formulation of amalgam was produced which did not require heating. In 1985, Huggins (104) published a book on mercury toxicity. He stated that amalgam restorations released enough mercury to cause cardiovascular, immunological, neurological, collagen, emotional and allergic disorders. The resulting conditions include multiple sclerosis, depression tachycardia and arthritis. There are currently many anti amalgam groups who make similar claims. Some studies have not found these

concerns to be warranted (102, 103). Mercury in certain forms and at certain levels does produce toxic effects, however, the very small amounts of mercury vapour escaping from amalgam restorations have not been shown to produce detectable effects on the body (105). Mercury vapour (elemental mercury) is the major source of concern to dentists and patients. Mercury has a high vapour pressure (0.005 mg of mercury at 37 °C), and approximately 75 percent of inhaled inorganic mercury vapour will be absorbed through the lungs. Elemental mercury accumulates in the kidneys and brain and is excreted in the urine, secreted in bile and exhaled from the lungs (103). Amalgam corrosion is an oxidation-reduction reaction in which the metals in the amalgam react with nonmetallic elements in the environment to produce chemical compounds (106). Corrosion is a major factor in determining the amount of mercury that is released into the oral cavity. Amalgam corrosion is influenced by factors that disrupt the surface layer of the restoration such as tooth brushing and chewing, which can cause an increase in mercury release. Strong oxidizing agents such as bleaching agents will also disrupt the surface. The mercury released in this fashion can be in two forms: mercury vapour or mercuric ions. The mercury vapour can be inhaled or exhaled, depending on the subject's breathing pattern, while mercuric

ions can pass into the saliva and enter the gastrointestinal tract (107). The corrosion of amalgam restorations is complex and can effect the amounts of mercury release (107).

2.7.2 Effect of Bleaching on Dental Amalgam

Various reports have described the effects of bleaching agents on dental materials including glass-ionomer cements, ceramics and gold (108-112). These reports generally concluded that there was little evidence for the bleaching systems causing significant changes to the materials. In the case of dental amalgam, however, several *in vitro* studies have reported a significant increase in mercury release as a result of treatment with peroxides compared to control treatments (14, 113-115). Prior to the early 1980s it was believed that mercury in dental amalgam restorations combined with tin and silver during the amalgamation reaction to take the form of stable intermetallic compounds. Therefore, there would be no free mercury present in the final restoration (116). It has since been established that mercury does escape from dental amalgam and finds its way into many organs of the body (117). There has been much concern in recent years to the exposure to mercury from amalgam restorations (118). A number of ions as well as mercury are

released from dental amalgam following exposure to strong oxidising agents such as HP. Robertello *et al.* (113) compared the effects of three peroxide-containing commercial tooth whitening products and saline control on a zinc-free, palladium-enriched high copper amalgam. After 80 h of bleaching, 0.98 $mg.m^{-3}$ (µg/l) of mercury was detected for one of the products. Hummert *et al.* (114) studied the effects of two tooth whitening products and saline on mercury release from four different amalgams. After 8 h treatment, the level of mercury released was between 109 and 158 $ng.ml^{-1}$ (109 and 158 µg/l) for the tooth whitening products and 5 $ng.ml^{-1}$ (5 µg/l) for saline. Mackert and Berglund (115) reported similar levels of mercury release in their tests. Rotstein *et al.* (14) studied the effects of 10% CP and phosphate buffer (both at pH 6.5) on four different amalgams. After 48 h, the level of mercury released was reported to be in the range 23-161 $\mu g.ml^{-1}$ (23000-161000 µg/l). In another study (119), the effect of Copalite coating on mercury release from dental amalgam following treatment with 10%, 20%, 30% and 40% CP *in vitro* was assessed. Exposure of amalgam restorations to 10%-40% CP based bleaching agents increased the mercury release. Pre-coating of the external amalgam surfaces with Copalite significantly ($p<0.01$) reduced the release of mercury. The mercury release from amalgam also

appears to be modified by the presence of biofilm (containing saliva, bacteria and polysacchrides) on the amalgam surface (120). Steinberg *et al.* (121) showed that an experimentally induced biofilm coating on amalgam reduced mercury release into the surrounding environment.

Schemehorn *et al.* (110) evaluated a 6% HP tooth whitening gel on a high copper amalgam, a hybrid resin composite, feldspathic porcelain and type III gold specimens. One half of each specimen surface was covered with nail varnish to serve as the control side, leaving the other half exposed to the bleaching gel. There were no observable differences under SEM between the control and peroxide treated side of any of the materials tested. Al-Salehi *et al.* (122) investigated the effect of 10% CP on metal ion release from dental amalgam. Amalgam discs were treated with either 0% CP gel, 10% CP gel or Sprite Light® for 24 h. The discs were then removed and immersed in water for 24 h. Following immersion, eluents were taken for metal ion release determination (Hg, Ag, Cu, Sn) using inductively coupled plasma mass spectrometry (ICP-MS). It was found that metal ions released after treatment with the 10% CP gel and a placebo gel treatment were not statistically significant ($p>0.05$). In a more recent study (13) the effect of HP concentration on metal ion

release from dental amalgam was investigated. Amalgam discs were treated with either 0%, 1%, 3%, 10% or 30% HP solution. The actual HP solutions rather than the eluents were analysed using ICP-MS to determine metal ion release from amalgam discs. There was a statistically significant ($p<0.05$) increase in metal ion release for the elements (Hg, Ag, Sn and Cu) between control (0% HP) and each of 1, 3, 10 and 30% HP solutions. Metal ion release increased with increasing HP concentration confirming the results of a number of other investigators (14, 114, 119).

2.7.3 Casting Alloys

An alloy is a metallic material formed by the combination of two or more metals or one or more metals with a non metal (123). In dentistry alloys usually contain at least 4 metals and often six or more. Thus, dental alloys are complex metallurgically (124). The selection of an alloy for a cast dental restoration is the legal responsibility of the clinician. Selecting alloys with the best physical properties, biological and chemical priorities should always be the first priority. Several properties of alloys are critical to their performance these include: grain size, phase

structure, yield strength, hardness and elastic modulus, colour, corrosion and porcelain bonding properties (92, 125).

Casting alloys are categorised several ways, but the classification most used is the American Dental Association (ADA) compositional classification system. The ADA divides casting alloys into three groups on the basis of wt % composition. The high noble alloys are those with a noble metal content (sum of gold, palladium and platinum) of at least 60-wt % and a gold content of at least 40-wt %. Most gold-based alloys before 1975 fell in this category. The noble alloys must contain at least 25-wt % noble metal but have no specific requirement for gold content. The predominantly base metal alloys contain less than 25-wt % noble metal, with no other specification on composition. The ADA also classifies alloys on physical properties of yield strength and elongation. Each casting alloy is, therefore, defined by two ADA classification systems one for composition and one for physical strength (126).

There is a further way of describing alloys that is important to their biocompatibility. This is by describing their phase structure. Single-phase alloys have a fairly homogenous structure. Multiple phase alloys contain areas that differ in

composition to others. The phase structure of an alloy is critical to its corrosion properties and its biocompatibility (127).

Manufacturers of alloys must measure corrosion by specific standards to gain ISO or ADA certification of their alloys, and this information is usually available from manufacturers, although it is not commonly reported in brochures (128). Corrosion generally results in the release of mass from the alloy into the oral environment and is related in complex ways to alloy biocompatibility (125). Lack of biocompatibility of dental alloys may lead to different adverse effects for a patient. Some patients can develop allergic or hypersensitivity reactions to very small quantities of metals such as nickel, that may be released due to corrosion (92, 129-131). However, nickel allergy as a result of intra oral devices is rare (92). Beryllium is a known carcinogen. It has been advised that alloys containing beryllium are not used for dental applications (132). Other elements with mutagenic/carcinogenic status include cadmium, chromium, cobalt, copper, iron, nickel, palladium and tin. No carcinogenic effects from dental alloys have been demonstrated (124).

2.7.4 Effect of Bleaching on Dental Casting Alloys

Casting alloys are known to corrode on contact with a strong oxidising agent and metal ions are released into the oral cavity (133-137). Corrosion may compromise the strength of a dental restoration leading to catastrophic failure or the release of oxidised components which may discolour natural teeth, porcelain veneers or even soft tissues in severe cases (124). Elemental release from dental alloys is related to known biocompatibility issues due to the interaction of these elements with surrounding or systemic tissues (124, 125, 127, 138, 139). Scott *et al.* (140) reported on adverse reactions to dental materials. This study was UK based and involved pooling of incidents reported in general dental practice. It showed that the most common dental materials that produced adverse reaction reports in patients were metals. Amalgam gave rise to the most reports, with three reports for every one for base metals (40% of which were nickel). Reactions to precious metals accounted for about 5% of the metal reactions. The total numbers of reports were approximately 500 per year for all materials, for the whole of the UK; therefore, reactions to materials are relatively uncommon. This may also be due to failure of detection and possibly under-reporting. Reactions to metals by other groups

(technicians and dental staff) were minimal. This suggests that the metal may need to be in the right environment for a long time to allow corrosion to occur (as it is when a crown is fitted in the mouth). It may also be indicative of a delayed hypersensitivity reaction.

The phase structure of an alloy is critical to its corrosion properties and therefore it is also related to biocompatibility (125, 139, 141). However, an alloy does not necessarily release elements in proportion to its composition. Multiple phase alloys tend to release more elements than single phase alloys (125). Certain metallic elements or ions have a tendency to be released (lability) regardless of the alloy composition. Copper, nickel and gallium, for example, are labile elements whereas gold, palladium and platinum have low labilities. In a high gold alloy only 2% of the total mass released is actually gold, whilst the lesser constituent, copper, accounts for 85% of the mass released (124). An element's lability may be altered by other elements in the alloy. Palladium has been shown to reduce the lability of copper from gold based dental alloys (142).

Environments around the alloy will influence corrosion of an alloy (124, 127, 138, 143), with acidic environments increasing

corrosion in some systems (144, 145). This is more relevant to Ni-based alloys but, not the high noble alloys (146, 147). The corrosion of alloys may be investigated using a variety of techniques including visual observation of the alloy surface, electrochemical tests that measure elemental release indirectly through the flow of released electrons, or by direct measurements of the released elements (124, 148). For direct measurements, Tufekci *et al.* used ICP-MS to measure the *in vitro* elemental release from two high palladium alloys into a corrosion medium having a pH value of 2.24 (149). They reported a significant increase in ion release with increased immersion time. It was also reported that there were significantly more ions released into the solution from the Pd-Cu-Ga alloy compared to the Pd-Ga alloy. The mean measured ion release data for Pd-Cu-Ga alloy at 7 h immersion time was reported to be 0.74 μg/cm² for Pd, 0.57 μg/cm² for Ga, 1.50 μg/cm² for Cu and 0.06 μg/cm² for Sn. Wataha *et al.* (143) also used ICP-MS to investigate the effect of pH on element release from dental casting alloys. High noble, noble and base metal casting alloys were treated in different solutions with pH values of 1, 4 and 7. High noble and noble alloys were resistant to acidic environments, whereas, Ni based alloys released large amounts of Ni in solutions of pH values of 1 and 4. For the Pd-

Cu-Ga alloy they reported ion releases of 0.02 µg/cm² for Pd and 0.01 µg/cm² for Cu at pH value of 4. For the base metal alloy they reported 1.25 µg/cm² Ni release. Syverud *et al.* (133) examined corrosion and biocompatibility of a Pd-Cu-Ga alloy and a palladium alloy without copper. Specimens were cast, rubbed and ultrasonically cleaned. All specimens were tested after pre-oxidation, removal of 0.1 mm thick surface and after removal of an additional 0.1 mm by grinding. Immersion tests were carried out in pH solutions of 2.3 and 7 for 7 days. Both alloys with oxide films from pre-oxidation released considerably more ions than the ground specimens (28 ug/cm^2 compared to 1.4 ug/cm^2 for Pd-Cu-Ga alloy). Larger amounts of ions were released from the copper containing palladium alloy (1.4 ug/cm^2 for ground Pd-Cu-Ga alloy as compared to 0.4 ug/cm^2 for the ground non copper containing Palladium alloy).

2.8 Measurement of Ion Release

In the work presented in this thesis, ion release for metallic restorations and tooth demineralisation following bleaching was measured using an ICP-MS instrument (13, 122, 148). This is an analytical technique, which provides high sensitivity as well as high sample throughput. The instrument records all elements in the periodic table present in a particular sample as opposed to recording mercury only when using a Gold Film Mercury Analyser, for example. ICP-MS not only offers extremely low detection limits in the sub parts per trillion (ppt) range, but also enables quantitation at the high parts per million (ppm) level. This unique capability makes the technique very attractive compared to other trace metal techniques (150). The original ICP-MS introduction systems was designed for solutions but now, through the addition of a laser to the system, solids can be analysed (151-153). The laser ablation ICP-MS, however, is difficult to calibrate and problematic when heterogeneous materials and samples of differing matrices are to be analysed (151, 154).

The survey presented on bleaching of oral tissues (section 2.6) and metallic dental materials (section 2.7) showed that

relatively few research papers have reported direct measurements of ion release data following bleaching (11, 13, 14, 68, 71, 113, 114, 122, 143, 148, 149, 155). Relevant data from these papers are shown in Tables 2.3 to 2.5.

Reference	Amalgam	Bleaching System	Ion Release Values	Comments
Hummert *et al.* [114]	Sybraloy®, Tytin®, 4 x 8 mm cylinders *in vitro study*	Rembrandt® (10% CP) White and Brite® (10% CP) Saline (0.9% w/v)	Sybraloy® – Hg release: Saline = 5 ng/ml Rem = 109 ng/ml W and B =158 ng/ml Tytin® – Hg release: Saline = 9 ng/ml Rem = 47 ng/ml W and B = 36 ng/ml	Gold film Hg analyser 50/50 mix of agent with sterile saline.
Robertello *et al.* [113]	Valiant Ph.D® 12.5 x 2.5 mm discs *in vitro study*	CP products; Nite White® (NW) Opalescence® (O) Colgate Platinum® (P) And saline (S)	NW=0.58 mg/m^3 O =0.98 mg/m^3 P =0.47 mg/m^3 S = 0.52 mg/m^3	Insufficient information to convert data to units of mass/surface area. Specimens not polished.
Rotstein *et al.* [14]	Megalloy®, Meg$^+$®, Non gamma 2, Valiant Ph.D. ® 10 x 5 x 3 mm *in vitro study*	10% CP	After 48 h Hg release ranged from 23-161 µg/ml	MAS-50 D Hg analyser system. Specimens not polished.

Table 2.3 Ion release data from amalgam test samples

Al-Salehi *et al.* [122] (Appendix I)	Amalgam cylindrical discs (Sybraloy®) 10 x 2 mm *in vitro study*	10% CP	1.80 µg/cm^2 Hg 0.31 µg/cm^2 Ag 1.15 µg/cm^2 Sn 0.65 µg/cm^2 Cu	ICP-MS instrument One surface of disc polished Eluents analysed for ion release
Al-Salehi *et al.* [13] (Appendix II)	Amalgam cylindrical discs (Tytin®) 10 mm dia x 2 mm height	10% HP 30% HP	7.11 µg/cm^2 Hg 6.10 µg/cm^2 Ag 2.10 µg/cm^2 Sn 0.75 µg/cm^2 Cu 12.98 µg/cm^2 Hg 11.76 µg/cm^2 Ag 6.84 µg/cm^2 Sn 1.76 µg/cm^2 Cu	ICP-MS instrument One surface of disc polished Samples from bleaching solutions analysed for ion release

Table 2.3 Continued

Reference	Casting Alloy	HP/pH agents	Ion Release Values	Comments
Tufekci *et al.* [149]	Pd-Cu-Ga alloy *in vitro study*	Solution pH 2.24	After 7 h Pd = 0.74 μg/cm^2 Cu = 1.5 μg/cm^2 Ga = 0.57 μg/cm^2 Sn = 0.06 μg/cm^2 In = 0.04 μg/cm^2	ICP-MS instrument Ion release data measured at 7 h, 70 h and 700 h
Wataha *et al.* [143]	Pd-Cu-Ga alloy *in vitro study*	Solution pH 4.	Pd = 0.002 μg/cm^2 Cu = 0.01 μg/cm^2	Atomic absorption spectrometry. Data measured over 30 min period.
Al-Salehi *et al.* [148] (Appendix III)	Ni-Cr alloy cylindrical discs 10 x 2 mm Pd-Cu-Ga alloy cylindrical discs 5 x 1 mm *in vitro study*	10% HP 30% HP 10% HP 30% HP	5.05 μg/cm^2 Ni 3.63 μg/cm^2 Cr 4.11 μg/cm^2 Mo 8.23 μg/cm^2 Ni 6.65 μg/cm^2 Cr 4.62 μg/cm^2 Mo 0.12 μg/cm^2 Pd 0.04 μg/cm^2 Cu 0.01 μg/cm^2 Ga 0.01 μg/cm^2 In 0.28 μg/cm^2 Pd 0.09 μg/cm^2 Cu 0.04 μg/cm^2 Ga 0.03 μg/cm^2 In	ICP-MS instrument Release data measured at 3%, 10% and 30% HP over 24 h period

Table 2.4 Ion release data from dental casting alloys

Reference	Mineralised Tooth Tissue	HP/pH Agents	Ion Release Values	Comments
McCraken and Haywood [11]	Human enamel *ex-vivo study*	10% CP Cola beverage Distilled water	Ca = 1.06 µg/mm^2 Ca = 1.25 µg/mm^2 Ca = 0.27 µg/mm^2	Atomic Absorption Spectrophotometer. Ca data only reported.
Grobler *et al.* [71]	Human enamel *ex-vivo study*	Pepsi Cola®	Ca = 1.9 µg/mm^2	Atomic Absorption Spectrophotometer. Ca data only reported.

Table 2.5 Ion release data from mineralised tooth tissue

2.9 Legal Position for Use of Bleaching in the UK

In 1995 Opalescence (a 10% CP gel) was granted a CE mark as a medical device under the Medical Devices Directive (MDD). This meant that the product could be marketed in all countries throughout the EC without any further authorisation. However, the Medical Devices Agency (MDA), the Departments of Trade and Industry (DTI) and of Health (DOH) decided that bleaching agents intended to be placed in contact with the teeth are cosmetic products within the meaning of the Cosmetics Products Regulations 1993. Therefore, products intended for bleaching teeth containing more than 0.1% HP were prohibited from sale in the UK. The manufacturers of Opalescence, Ultradent Inc. and the UK distributors Optident Ltd. sued the DTI and DOH in the High Court claiming that they had placed obstacles to the marketing in the UK of a product which had been granted a CE mark in Germany (6). The case ended in August 1998 and it was concluded that Opalescence was a medical device, the CE mark was appropriate and that the DTI and DOH had placed unlawful obstacles to the marketing in the UK of this bleaching product (156). The DTI and DOH appealed against the decision. In July 1999 the original judgment was overturned by the Court of Appeal (157). The matter was referred to the House of Lords and the manufacturers of Opalescence appealed against the

decision that the product was a cosmetic preparation as opposed to a medical device. This appeal was dismissed in July 2001 (158). Although bleaching agents with peroxide content as high as 30% are often used (in office bleaching), current UK law limits the effective peroxide content to 0.1% (6, 7, 156-158).

3. Aims and Objectives

From the survey of the literature presented here, it is apparent that there are relatively little data available on ion release related to tooth bleaching. The aim of this work, therefore, was to investigate ion release from mineralised tooth tissue and metallic dental materials at different CP/HP concentrations *in vitro*. Specific objectives of the work were to investigate:

1. Mineral loss from bovine teeth and its effect on enamel and dentine microhardness following exposure to HP (0-30% w/v). The whitening effect of 30% HP solution on enamel and dentine was also calculated from measurements of reflectance of enamel and dentine tissues.

2. Metal ion release (Hg, Ag, Cu and Sn) from two typical amalgams; Sybraloy treated with 10% CP and Tytin treated with 0-30% HP.

3. Metal ion release from two typical casting alloys Ni-Cr, a base metal alloy (Wiron 99) and Pd-Cu-Ga, a noble alloy (Cerapall II).

On completion, this study will add significantly to knowledge of the potential for deleterious changes in dental tissue and materials following exposure to bleaching agents. This timely research, therefore, has great relevance to the development of regulations governing the formulation, distribution and use of commercial tooth bleaching preparations for clinical uses.

Hypothesis: To test the null hypothesis that increasing concentrations of HP is no more effective than water at increasing ion release from tooth tissue, dental amalgam and dental casting alloys during bleaching.

4. Experimental Procedures

A number of experimental procedures together with related material, equipment and instrumentation were used in this work to investigate the effects of bleaching agents on dental restorations and tooth tissue. The work was an *in vitro* study. Cylindrical test samples (amalgam and casting alloys) were prepared and tested to investigate the effect of bleaching agents on dental restorations. A number of tests on bovine teeth were also carried out to determine the effect of bleaching on microhardness, demineralisation and colour change. The principal features of the techniques, sample preparation, equipment and instrumentation used are described in more detail below.

4.1 Design and Manufacture of Jigs for Amalgam Packing

Two types of jigs, almost identical, were designed and manufactured; one type for packing and the other for polishing amalgam test samples.

Perspex was used in the manufacture of the jigs to avoid possible contamination of the amalgam when metal jigs are used. Each jig consists of three components (Fig 4.1). The main component is a cylindrical body 24 mm diameter x 40 mm height. A small central hole was drilled all the way through this component. A recess of 10 mm diameter x 3.5 mm depth was next milled at one of the flat faces of the cylinder. The second component of the jig is a plunger consisting of a thin cylindrical platform 10 mm diameter x 1 mm thickness with a relatively long thin shank. The plunger fits inside the main body of the jig with its cylindrical platform resting against the base of the recess and the shank running through the main cylinder and protruding out at the other end. The third component of the jig is a disc of 24 mm diameter x 10 mm height with a central hole drilled through it similar to that drilled through the main cylinder. The height of this disc is made longer than the length of the protruding part of the shank. The jig can be assembled simply by inserting the plunger into the main body of the jig such that the platform is seated at the base of the recess at one end of the cylinder with its shank running through the central hole and protruding at the other end of the cylinder. The disc (the third component of the jig) is then placed against the main cylinder base with the protruding part of the plunger shank locating

centrally inside it. All the three parts of the jig fit to close tolerances. During packing of the amalgam, the assembled jig is placed vertically on a horizontal work surface. The amalgam is then packed by pressing against the plunger platform inside the recess of the cylinder and left to set. Once the amalgam is set, the disc is first removed from the base of the cylinder. The amalgam disc is next removed out of the recess by gently pushing the protruding end of the plunger shank with a fingertip.

The second jig is almost identical to the first except the depth of the recess is made about 0.5 mm smaller. The set amalgam was placed into this second jig so that the protruding part of the amalgam can be polished flush with the main cylinder surface.

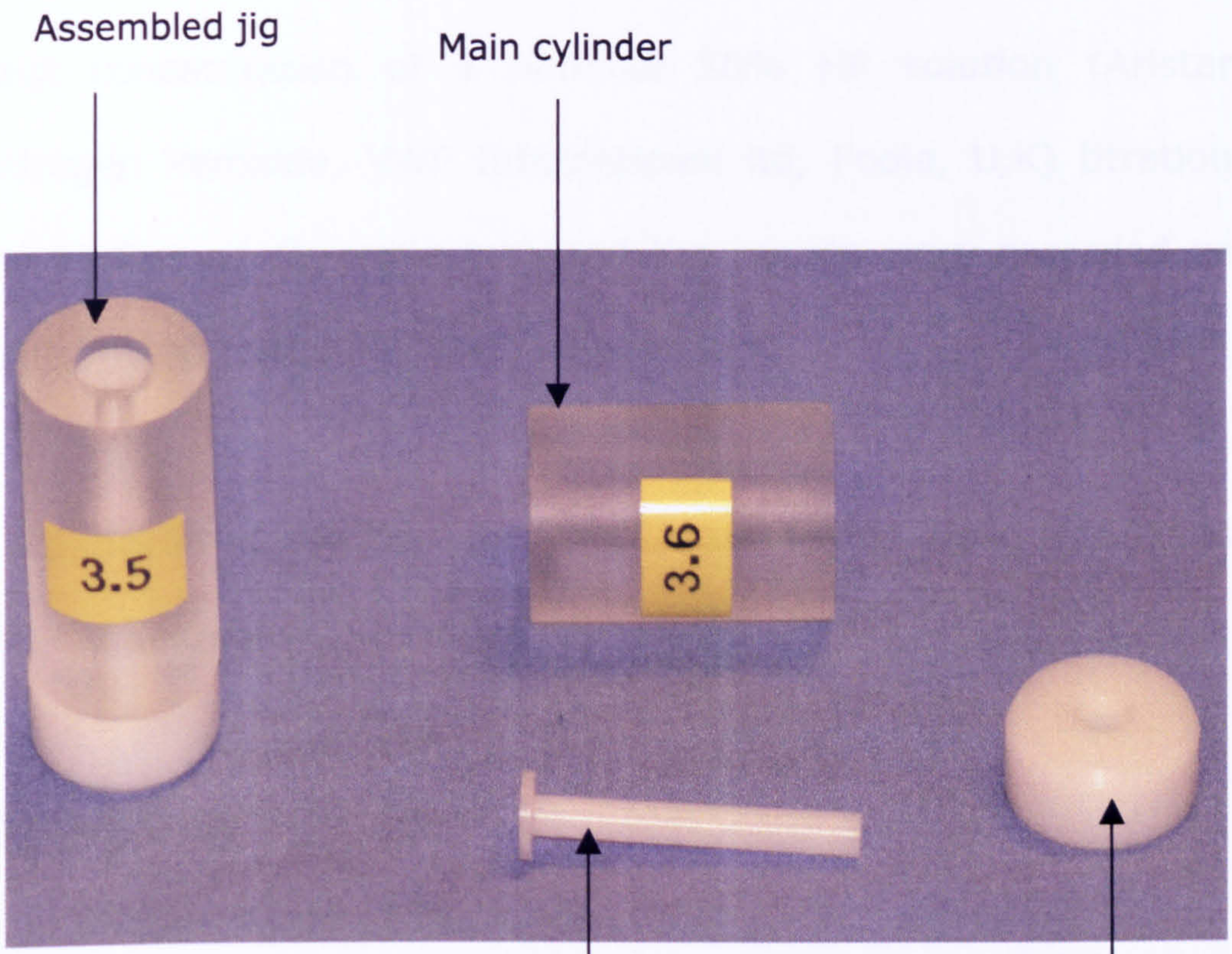


Figure 4.1 Jigs for amalgam packing and polishing

4.2 Chemical Measurements

4.2.1 Volumetric Titration

Hydrogen peroxide solutions of different concentrations (0-30% w/v) were used throughout this work. In order to determine the exact concentration of a nominal 30% HP solution (Aristar Hydrogen Peroxide, VWP International ltd, Poole, U.K) titration was performed (5 replicates) and the results were recorded as mean and standard deviation values (159).

A standardised method for the titration was used (160) as detailed below:

(i) Reagents:

1) Sulphuric acid (20% w/w)
2) Sodium thiosulphate (0.1M)
3) Ammonium molybdate solution (produced by dissolving ammonium molybdate in 10 mls of NH_4OH (6M). Adding 24g NH_4NO_3 (24g) and diluting to 100ml. This solution acts as a catalyst for the reaction.
4) High purity Milli-Q water
5) KI solution (1% v/v)
6) Starch indicator

(ii) Method:

1) 50 ml of water was placed in a clean conical flask. 10 ml of sulphuric acid, 10 ml of KI and 3-4 drops of ammonium molybdate were next added and the solution stirred.
2) 0.1-0.2 g of the nominal 30% hydrogen peroxide solution (oxidizing agent) was weighed and poured into a clean beaker.
3) The peroxide solution was diluted by adding 1 ml of Milli-Q water and the contents were completely transferred to the conical flask (Figure 4.2a).
4) The liberated I_2 was titrated with the 0.1 M sodium thiosulphate in the burette (initial reading = A, final reading = B).
5) As soon as the solution turned pale yellow, due to the presence of iodine (Figure 4.2b), a few drops of starch were added forming a coloured product with iodine (Figure 4.2c), which disappeared at the end point to a clear solution (iodide in excess) (Figure 4.2d).

The equations describing the reactions are as follows:

$H_2O_2 + 2KI + H_2SO_4 \longrightarrow I_2 + K_2SO_4 + 2H_2O$ (solution goes yellow due to I_2 in solution)

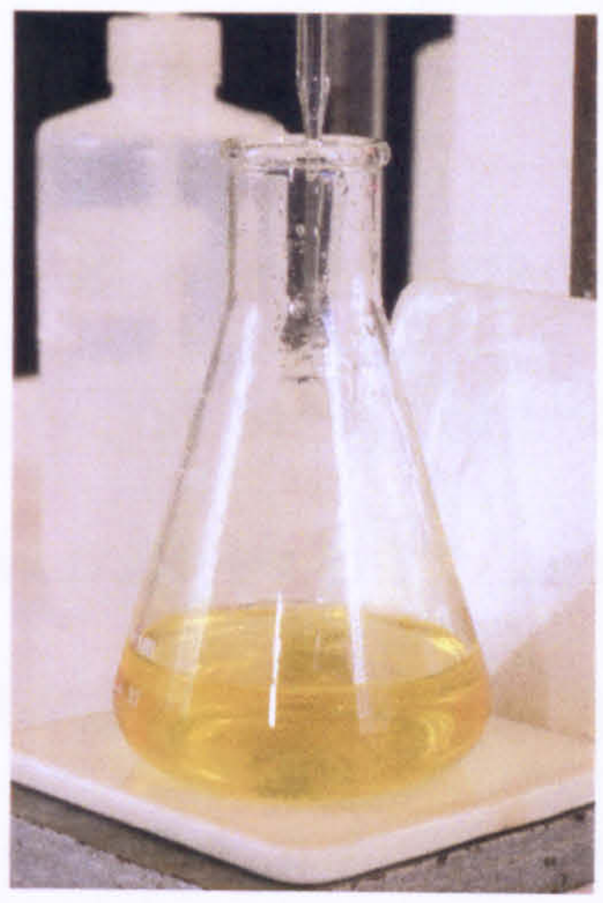
$I_2 + 2Na_2S_2O_3 \longrightarrow Na2S_4O_6 + 2NaI$ (solution goes colourless as I_2 is consumed by thiosulphate)

It was important not to analyse more than 0.2 g of HP solution to avoid the real risk of liberating I_2 vapour which escapes from the flask and thus leads to a lower % of peroxide analysed. On occasions black deposits precipitated in the conical flask (solid I_2). Such samples were ignored and the test repeated using a smaller amount of the sample.

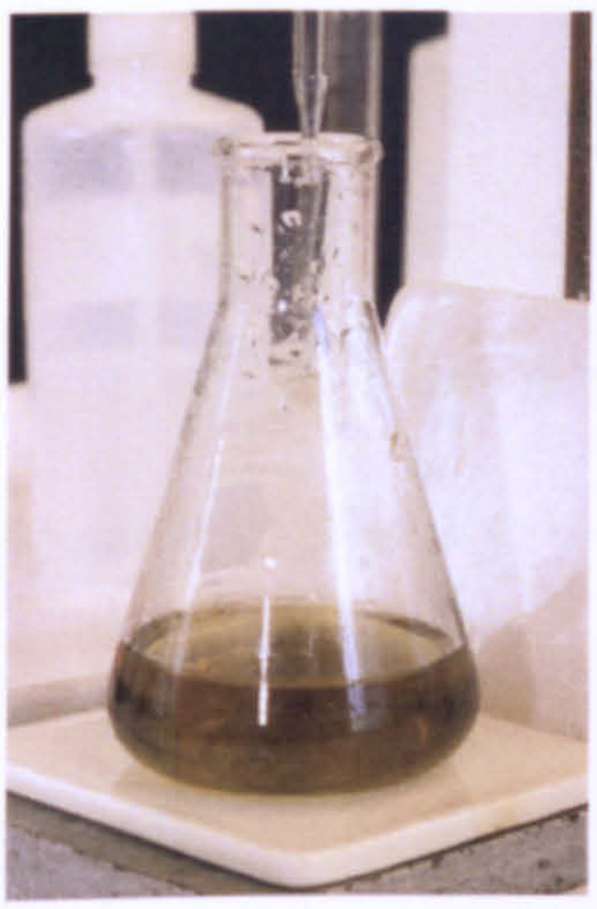
The % HP concentration was calculated from the following equation:

$$\% \text{ HP} = \frac{\text{(B-A) x molarity of thiosulphate (0.1) x 1.7}}{\text{Sample weight in grams}}$$

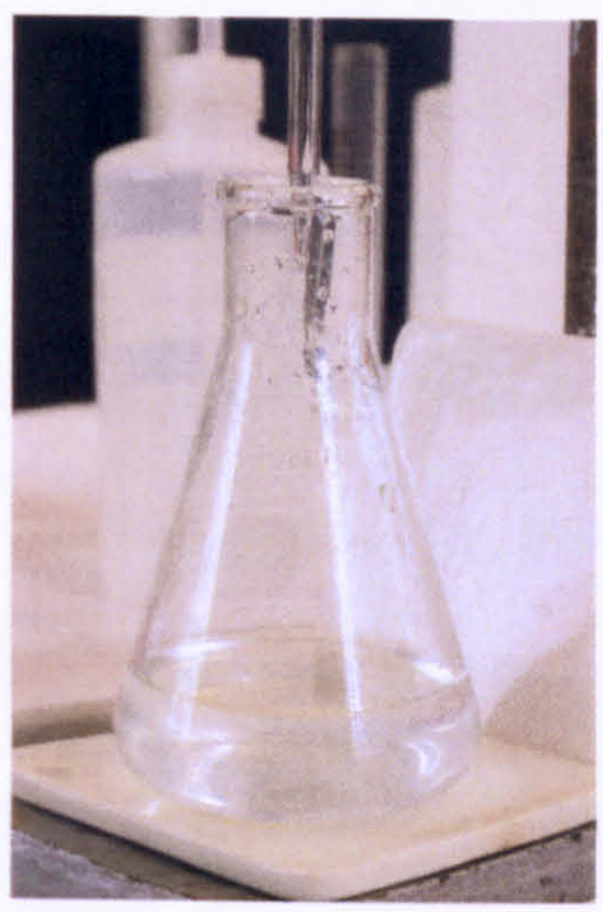
a



b



c



d

Figure 4.2 Stages of iodine/thiosulphate titration. The bleaching gel (oxidising agent) was added to an excess of acidified potassium iodide solution, liberating iodine (a). Iodine was then quantified by titration against a standard sodium thiosulphate solution. The colour changed from a pale yellow (b) solution of iodine to a clear solution of I^- ions at the end point. Starch formed a coloured product with iodine (c) which disappeared at the end point (d).

4.2.2 Atomic Spectroscopy

Atomic spectrometry encompasses atomic absorption, atomic emission and atomic fluorescence. Of these, atomic absorption and atomic emission are the most widely used. Atomic absorption is the process that occurs when a ground state absorbs energy in the form of light of a specific wavelength and is elevated to an excited state. The amount of light energy absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases. Atomic emission spectroscopy is a process in which the light emitted by excited atoms or ions is measured. The emission occurs when sufficient thermal or electrical energy is available to excite a free atom or ion to an unstable energy state. Light is emitted when the atom or ion returns to a more stable configuration or the ground state. The wavelengths of light emitted are specific to the elements, which are present in the sample. Inductively coupled plasma mass spectrometry (ICP-MS) is an affiliated technique used for the present work and described in more detail below.

4.2.3 Inductively Coupled Plasma Mass Spectrometry

ICP-MS is an analytical technique that performs elemental analysis with excellent sensitivity and high sample throughput. The ICP-MS instrument employs a plasma (ICP) as the ionisation source and a mass spectrometer (MS) analyser to detect the ions produced. It can simultaneously measure most elements in the periodic table and determine analyte concentration down to the subnanogram-per-litre or part per trillion (ppt) level.

Liquid samples are introduced by a peristatic pump to the nebulizer where a sample aerosol is formed. A double-pass spray chamber ensures that a consistent aerosol is introduced to the plasma. Argon (Ar) gas is introduced through a series of concentric quartz tubes, known as the ICP torch. The torch is located in the centre of a radio frequency (RF) coil, through which 27.12 MHz RF energy is passed. The intense RF field causes collisions between the Ar atoms, generating a high energy plasma. The sample aerosol is instantaneously decomposed in the plasma (plasma temperature is in the order of 6,000 to 10,000K) to form analyte atoms, which are simultaneously ionised. The ions produced are extracted from the plasma into the mass spectrometer region, which is held at

high vacuum. The vacuum is maintained by differential pumping.

The analyte ions are extracted through a pair of orifices approximately 1 mm in diameter known as the sampling cone. The analyte ions are then focussed by a series of ion lenses into a quadrapole mass analyser which separates the ions based on their mass/charge ratio. The term quadrapole is used because the mass analyser is essentially four parallel molybdenum rods to which a combination of RF and dc voltages is applied. The combination of these voltages allows the analyser to transmit only ions of a specific mass/charge ratio. Finally the ions are measured using an electron multiplier and data at all masses is collected by a counter. The mass spectrum generated is extremely simple. Each elemental isotope appears at a different mass with a peak intensity directly proportional to the initial concentration of that isotope. The system also provides isotopic ratio information. The HP 4500 ICP-MS, used here, offers high-throughput multi element analysis with ng/l (ppt) or better detection limits, very small sample volume requirements, robustness and ease of use. A schematic of the instrument is shown in Figure 4.3.

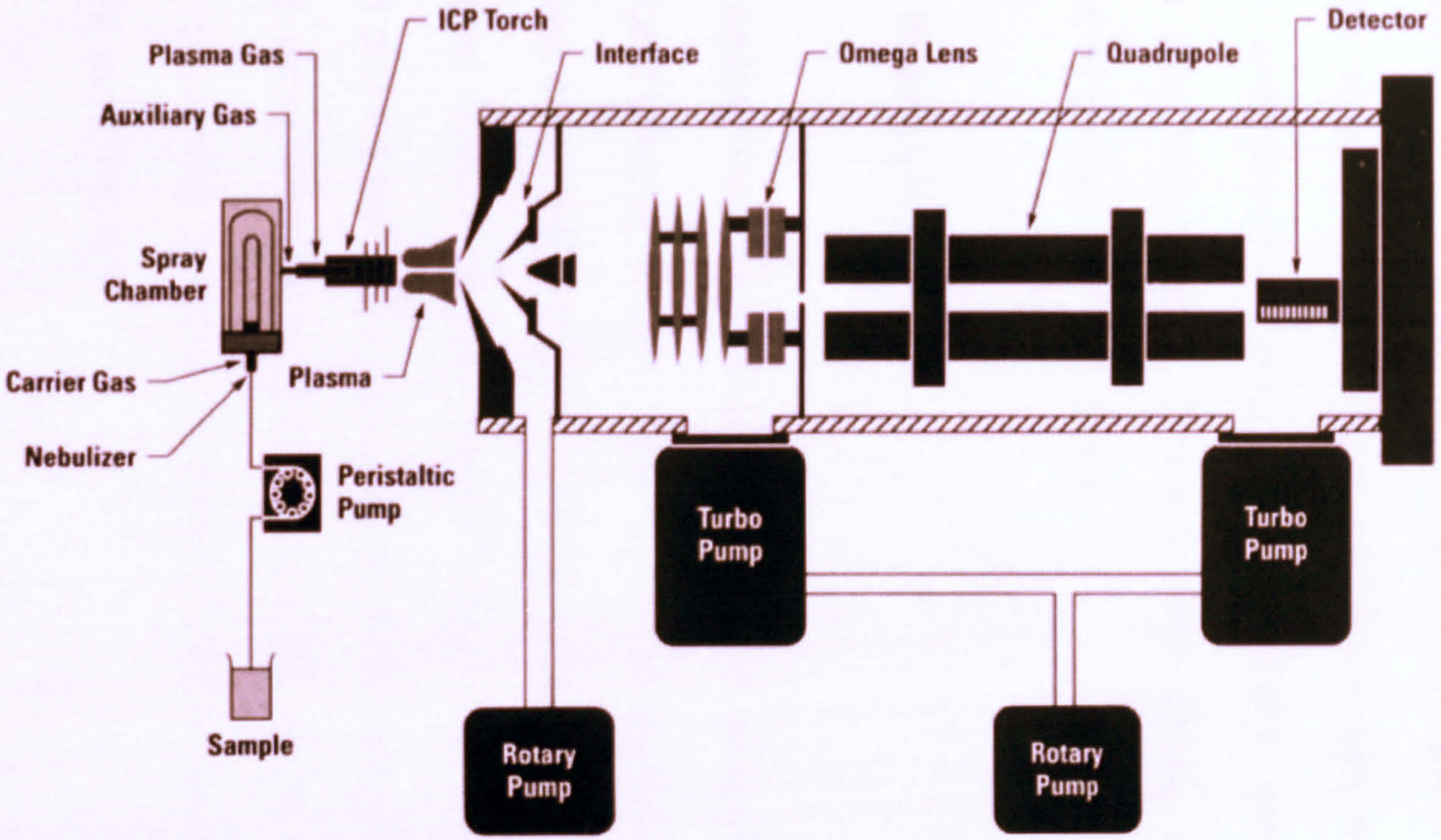


Figure 4.3 Schematic of ICP-MS instrument
Hewlett-Packard Journal, August 1997

4.2.4 Blank Samples

The determination of elements at the low ppt (pg/ml) level is dependent on the ability to monitor and control the magnitude of the blank signal. ICP-MS as a technique can be prone to memory and carryover effects; consequently, blank reagent samples are interspersed between each study sample and analysed, to check for these effects.

4.2.5 Detection Limits

The detection limits achievable for individual elements represent a significant criterion of the usefulness of an analytical technique for a given analytical problem. Without adequate detection limit capabilities, lengthy analyte concentration procedures may be required prior to analysis. Generally the best detection limits are attained using ICP-MS or graphite furnace atomic absorption. Table 4.1 provides a listing of detection limits by elements for ICP mass spectrometry.

Element	Detection Limit (ng/l)
^{31}P	1000
^{43}Ca	10
^{51}V	1
^{53}Cr	3
^{59}Co	1
^{60}Ni	1
^{65}Cu	3
^{71}Ga	1
^{95}Mo	1
^{105}Pd	1
^{107}Ag	3
^{115}In	3
^{118}Sn	1
^{197}Au	6
^{202}Hg	3

Table 4.1 ICP-MS detection limits for elements
Hewlett-Packard Journal, August 1997

4.2.6 Laser Ablation

Solutions and liquids are the normal sample types measured by ICP-MS. Solid samples are normally digested using mineral acids and analysed as solutions. However, solid samples can be analysed directly by using a laser ablation system (Figure 4.4).

The laser is used to vaporise the surface of the solid sample and it is this vapour, and any particulates, which is then transported by the carrier gas flow into the ICP-MS. This has clear advantages as it eliminates the need for sample dissolution procedures which are not only time consuming, but are susceptible to contamination and involve the use of acids which may in themselves cause problems in determining the concentration of certain isotopes.

The use of LA-ICP-MS, however, is not without problems, particularly when heterogeneous materials and samples of differing matrices are to be analysed. Elements present within the samples such as these are particularly hard to quantify, as calibration of the system is only possible if the ablation process removes the same mass of material and transports this with a constant efficacy to the ICP-MS system.

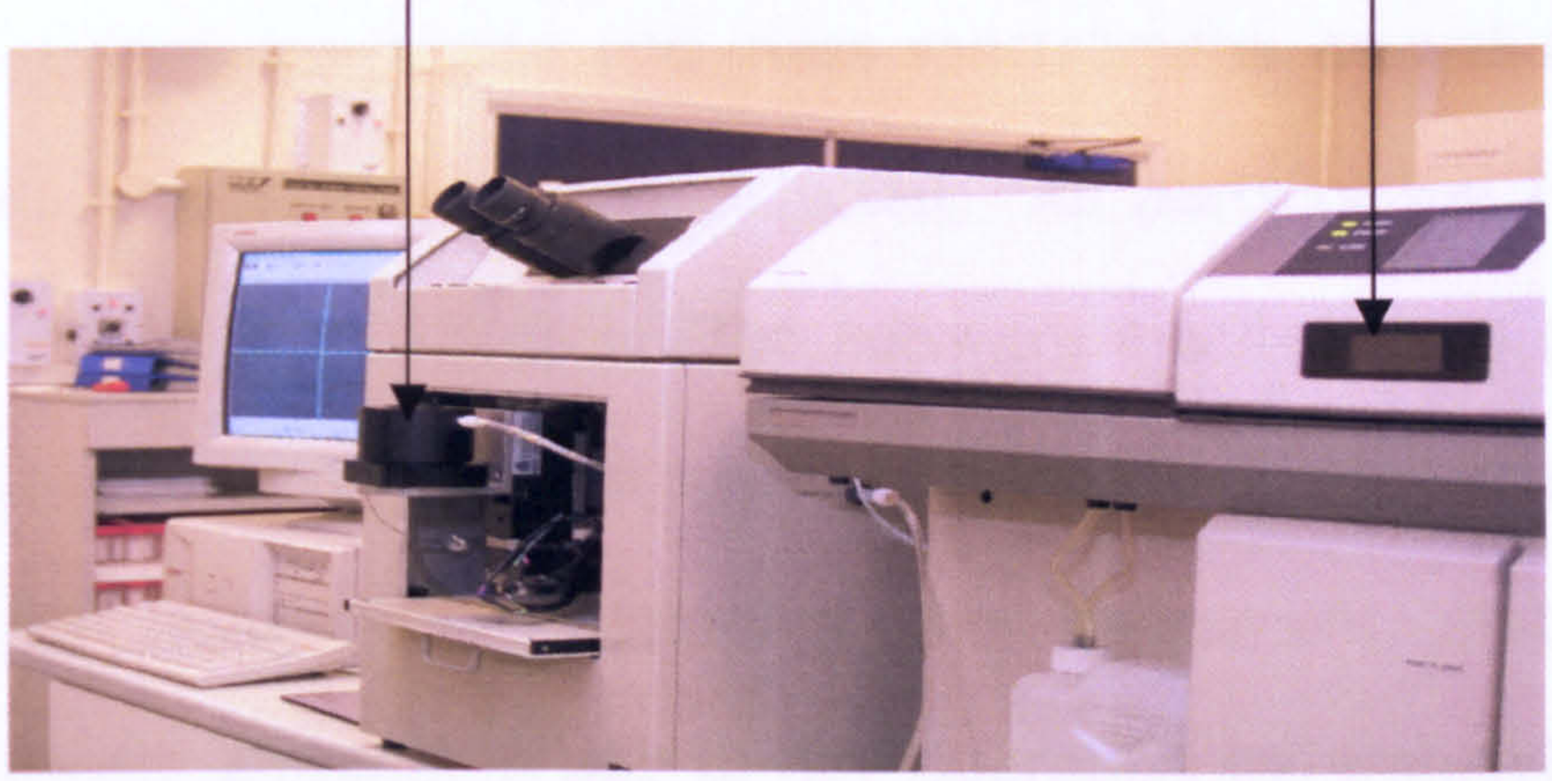


Figure 4.4 LA-ICP-MS machine (Cetax LSX200) used for ablating amalgam discs.

4.2.7 Data Handling and System Controller

All ICP instruments require computers and sophisticated software to control the plasma and mass spectrometer as well as perform calculations on the data collected. All parts of the ICP-MS are under software control. The software ensures each part of the instrument is working properly and can provide the operator with useful information regarding the instrument status. The software automates day-to-day operation by employing a suite of autotuning routines. Autotuning automatically optimises the sensitivity, background level and mass resolution and performs mass calibration. The ion counts measured by the detector are translated into useful information.

The ICP-MS instrument can provide data in one of four ways: semi quantitative analysis, quantitative analysis, isotope dilution analysis and isotope ratio analysis. Quantitative analysis was used here. The ICP-MS accurately determines how much of a specific element is in the material analysed. In a typical quantitative analysis, the concentration of each element is determined by comparing the counts measured for a selected isotope to an external calibration curve that was generated for that element. Liquid calibration standards are prepared. These

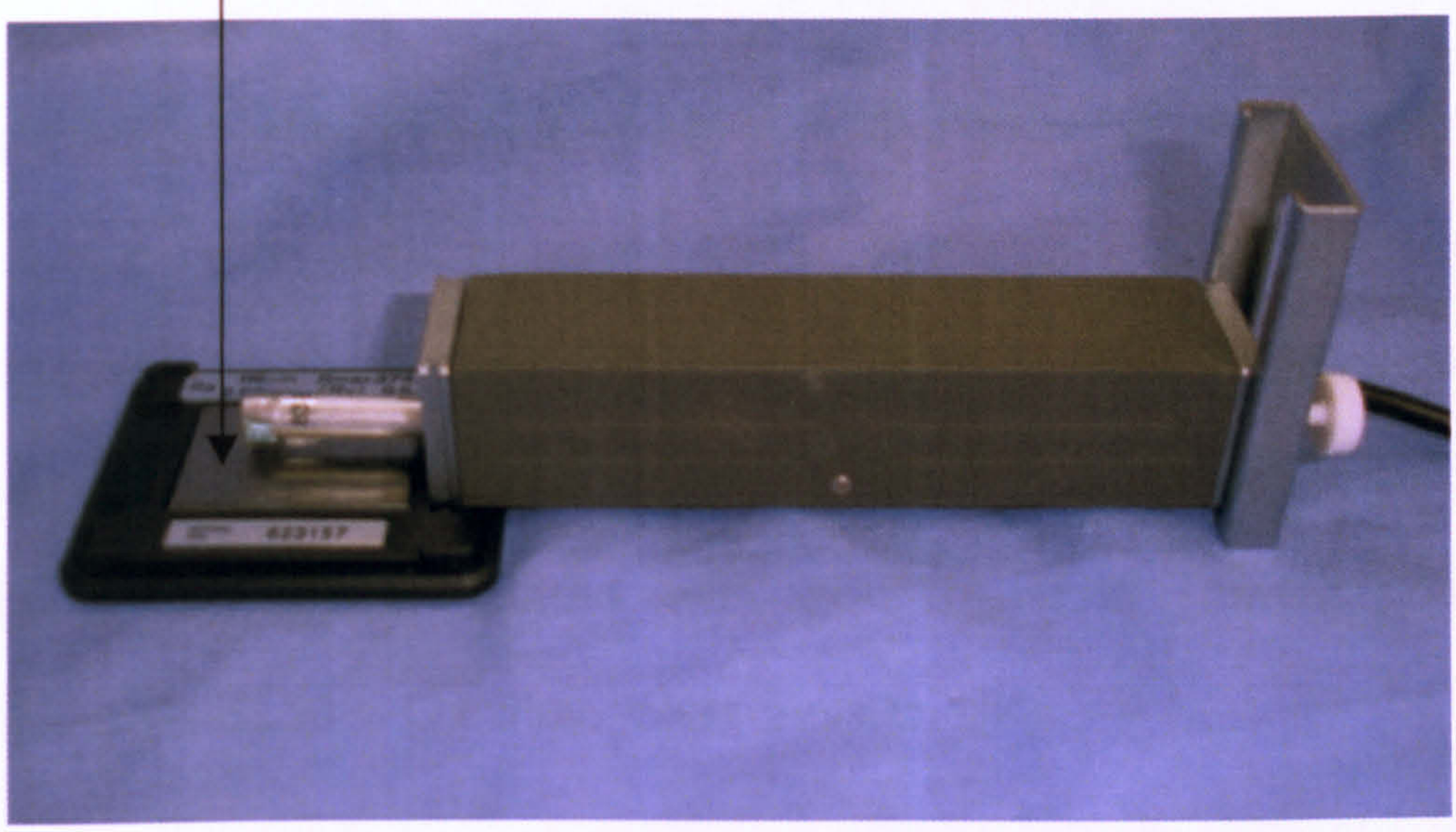
standards (Table 4.2) were analysed to establish the calibration. The unknown samples were then run and signal intensities were compared to the calibration curve to determine the concentration of the unknown.

Element	Standard 1 Concentration (µg/l)	Standard 2 Concentration (µg/l)
^{31}P	250	1250
^{43}Ca	250	1250
^{51}V	10	50
^{53}Cr	10	50
^{59}Co	10	50
^{60}Ni	10	50
^{65}Cu	10	50
^{71}Ga	10	50
^{95}Mo	10	50
^{105}Pd	10	50
^{107}Ag	10	50
^{115}In	10	50
^{118}Sn	10	50
^{197}Au	10	50
^{202}Hg	10	50

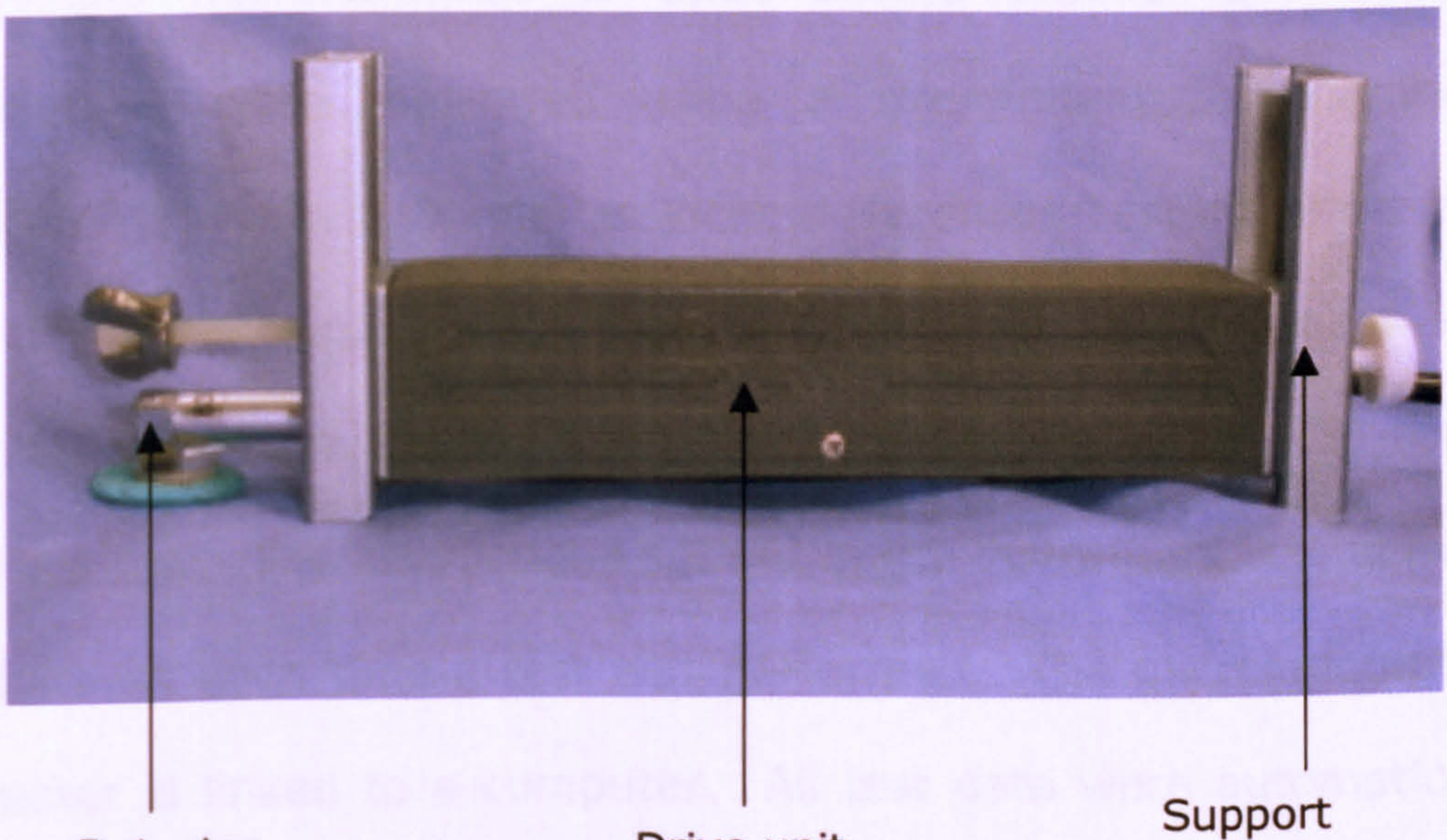
Table 4.2 Standard solutions used for element calibration

4.3 Surface Roughness

The surface roughness-measuring instrument used for the test samples was a Talysurf contact profilometer (Mitutoyo Corporation, Kawasaki, Japan). It consists of a drive unit with guide grooves at each end. Two support feet are inserted into the guide grooves and fastened to the drive unit to keep it in a parallel position with the bench surface onto which the Talysurf is placed (Figure 4.4). A detector unit in the form of a thin rod is inserted into the drive unit with its other end placed onto the specimen for roughness measurements. The specimen is held in position using a small amount of blue tac. Prior to sample roughness measurement, the instrument is calibrated by removing the support foot at the detector unit end. The second support foot is then adjusted so that the drive unit is level and the detector unit comes down properly onto the supplied roughness specimen (Figure 4.5). The start button is pressed and the displayed Ra value matched to the known roughness specimen by adjusting the detector's gain.



Instrument calibration



Roughness measurement

Figure 4.5 Talysurf contact profilometer used for surface roughness measurement

4.4 Preparation of Samples for Scanning Electron Microscopy

A number of amalgam and casting alloy discs as well as bovine tooth samples were prepared for SEM (Philips XL 20, Eindhoven, Holland) images before and after bleaching. The samples were dehydrated using a series of graded ethanol, mounted on carbon discs and sputter coated with gold using a sputter coater (Edwards S150B, Crawley, West Sussex, UK).

4.5 Hardness Testing

Vickers microhardness of each bovine enamel and dentine samples were measured using a microindentation hardness tester (Foundrex VX7 series Vickers Hardness Tester) before and after bleaching. The polished surface of each sample was placed, in turn, on the anvil facing upwards (Figure 4.5) ready for a test. The instructions set out in the instrument manual was followed each time a test was performed. The microindentation tester is linked to a computer. All test data were automatically stored and later retrieved for analysis.

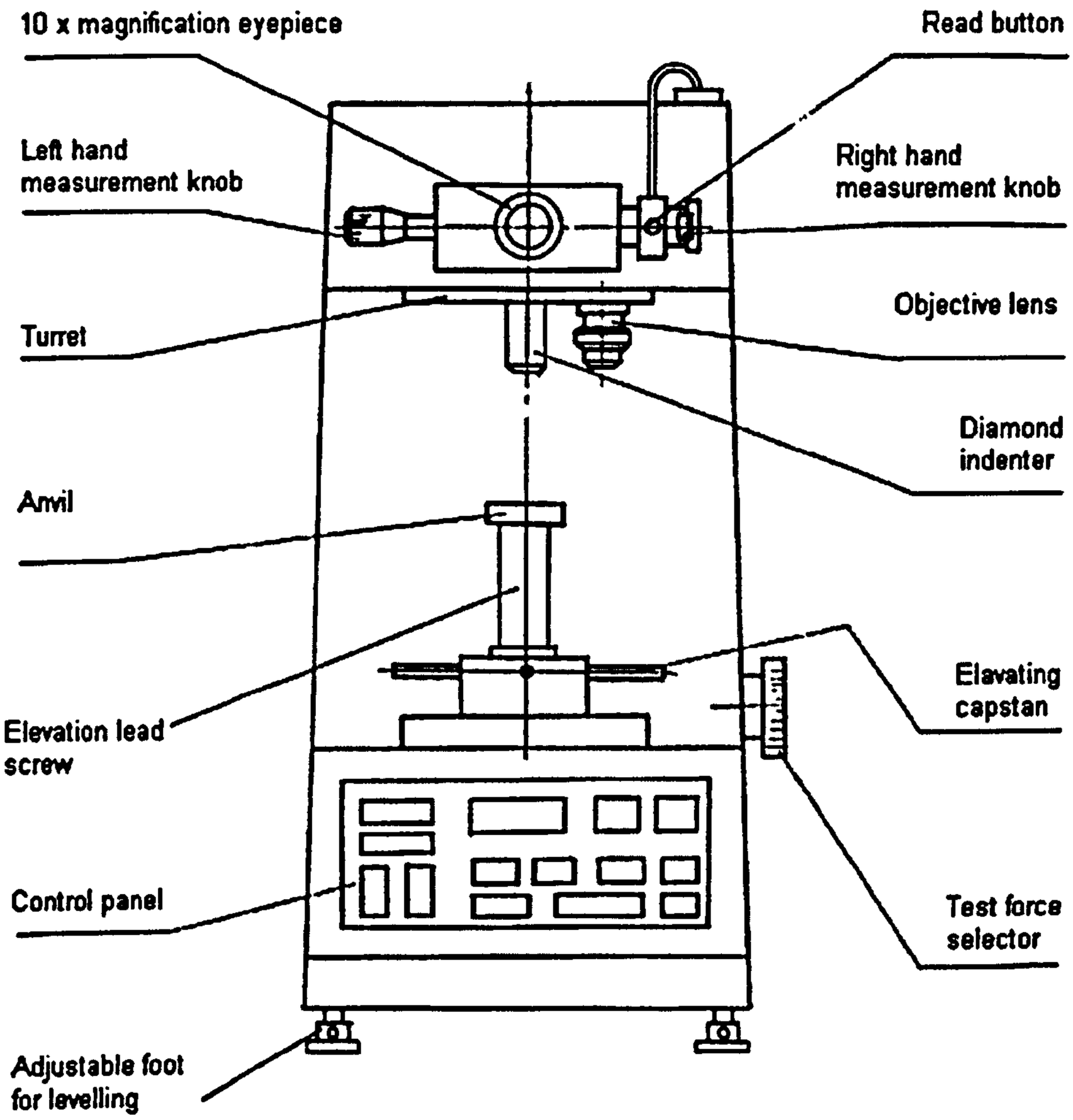


Figure 4.6 Schematic diagram of Vickers hardness tester (Foundrex VX7 series handbook)

4.6 Preparation of Bovine Enamel and Dentine

4.6.1 Materials and Methods

A number of intact bovine incisor teeth (n=27) were extracted washed and cleaned prior to storage in distilled water for up to three months. Five teeth were randomly selected for ion release measurements. Each of these five teeth was sectioned and the enamel and dentine separated using a dental diamond bur. From each tooth, four enamel and four dentine samples (each sample measuring approximately 2 mm x 2 mm x 1.5 mm) were cut. The total of 20 enamel samples were divided into four equal groups. Each group contained one sample from each of the five teeth making a total of five samples in a group. The 20-dentine samples were also divided in the same manner.

Twenty-one teeth were used for microhardness measurements. For this purpose, 21 small cylindrical plastic containers with detachable bases were used. All the internal surfaces of the containers were smeared with a thin layer of petroleum jelly. Each tooth was positioned at the centre of a container using a small amount of wax and then fully embedded in epoxy resin and left to set. Once the resin was set, the base of each container

was first removed before each embedded tooth was pressed out of the remaining outer plastic ring. Both faces of the resulting cylindrical blocks of tooth embedded in resin were ground parallel to each other ensuring that the enamel and dentine on the top surface was exposed for testing. The exposed tooth tissue was polished with P240, P600 and P1200 SiC abrasive paper followed by final polishing on a felt wheel.

The remaining tooth, a single bovine central incisor, was sectioned using a Buhler diamond disc and a sample of enamel 0.7 mm thick and a similar sample of dentine were obtained. Each sample, prepared for spectrophotometry study, was ground using P600 followed by P1200 SiC abrasive paper to obtain parallel sided specimens.

The bleaching solution used was HP (nominal concentration 30% w/v, Aristar, VWP International ltd, Poole,U.K). Volumetric analysis based on iodine thiosulphate titration (described earlier) was performed to establish the active concentration of HP in test solutions. The HP solution was diluted to obtain 3% (w/v), 10% (w/v) as well as 30% (w/v) HP solutions with high purity distilled water as the control (0% HP).

4.6.2 Ion Release

The five samples in a group referred to above were each immersed in 10 ml of either 0% (control), 3%, 10% or 30% HP (w/v) for 24 h at 37 ^{o}C in a labelled, tapered centrifuge tube, with all surfaces exposed. All the solution samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 4500). For each analysis, the instrument performed five measurements and calculated the mean and relative standard deviation (%) for each element. Thus with the 5 samples tested in each group, the total number of measurements recorded per element was 25 for enamel and 25 for dentine samples.

4.6.3 Microhardness Measurements

The 21 specimens prepared for hardness measurements were divided randomly into three equal groups. The Vickers microhardness of each sample was measured using the microindentation hardness tester, described earlier, and readings were recorded at three locations at close proximity to each other, on the enamel and dentine of each sample. At the end of testing, the specimens were again stored in distilled water inside

individually labelled polythene bags. One group was subsequently treated with 3% HP (w/v), the second with 10% (w/v) HP and the third with 30% (w/v) HP for 24 h at 37 ºC. Vickers microhardness measurements were repeated for all the bleached specimens at locations parallel to the previous series of indentations.

4.6.4 Statistical Analysis

Calcium and Phosphorous ion release at different HP concentrations were analysed using non-parametric Kruskal–Wallis and Post-Hoc Mann-Whitney tests to determine significant differences ($p<0.05$). The microhardness measurements were analysed using a paired t-test.

4.6.5 Translucency Measurements

The optical properties of bovine enamel and dentine were measured before and after bleaching with HP (30% w/v) using computer controlled spectrophotometer (Perkin Elmer Lambda 2 with integrating sphere accessory). Spectral reflectance and transmittance data in the wavelength ranging from 380 to 700

nm under standard illuminant D65 were recorded at 1 nm intervals.

For the transmittance measurements, a background correction (auto zero) was carried out. The total transmittance was then measured, before inserting a sample in the transmittance port, using white reference material in the reflectance port (Figure 4.6). The unbleached enamel specimen was inserted into the transmittance port and the total transmittance T(tot) was recorded using a white reference material in the reflectance port. Diffuse transmittance T(dif) was recorded using a black background (light trap) in the reflectance port. The direct transmittance T(dir) was then obtained simply by subtracting the diffuse transmittance from the total transmittance. This procedure was repeated after bleaching the sample for 24 h at 37°C with HP (30% w/v).

The total reflectance was also measured without a specimen and then by placing the sample in the reflectance port using the 8° sample holder (measuring all the reflected light). The diffuse reflectance was measured by reflecting the incident beam straight back out of the entry port using a 0° sample holder.

The Perkin Elmer Lambda 2 spectrophotometer is equipped with software which can be used to measure colour parameters L*, a*, b* from the reflectance recordings (Figure 4.7). These parameters are used to calculate the change in colour ΔE from the equation:

$$\Delta E=\sqrt{[(\Delta L^*)^2 +(\Delta a^*)^2+(\Delta b^*)^2]}$$

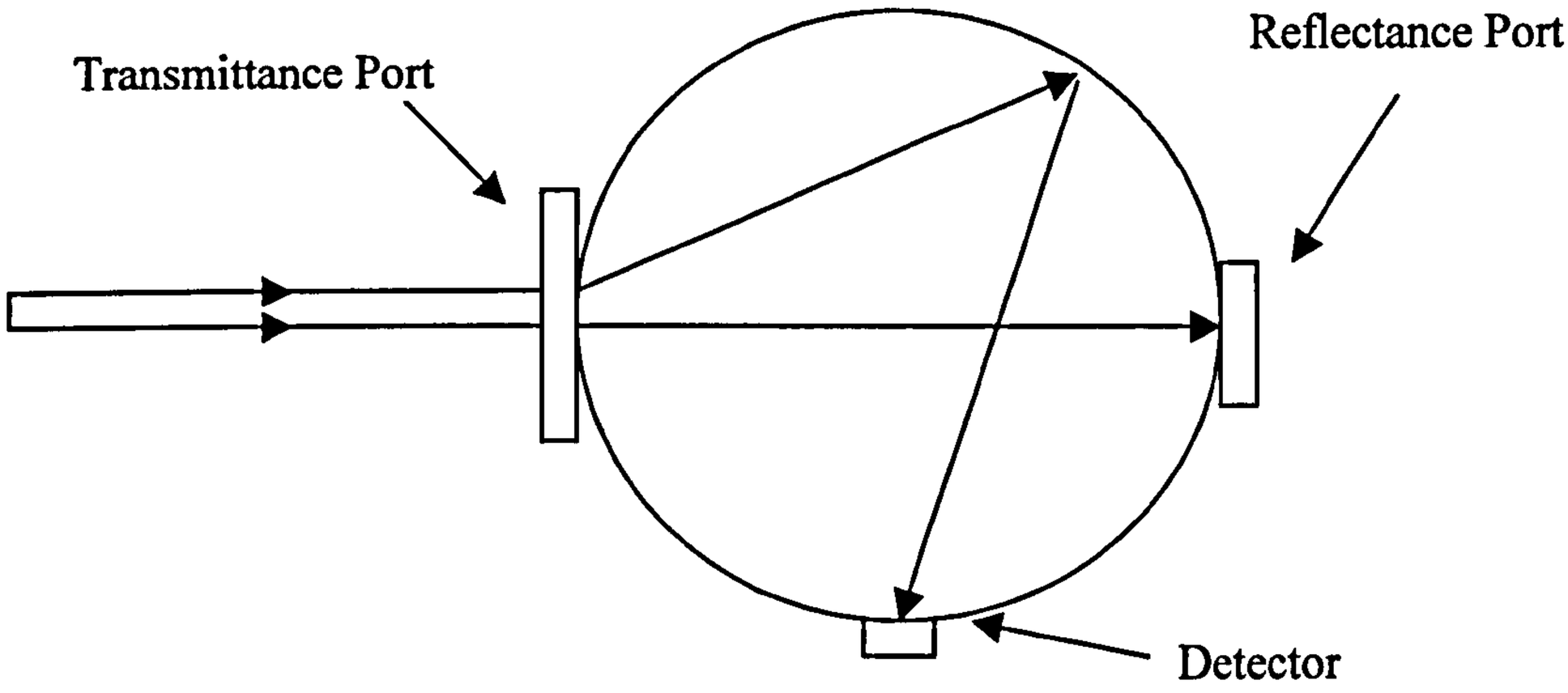


Figure 4.7 Integrating sphere used with a spectrophotometer

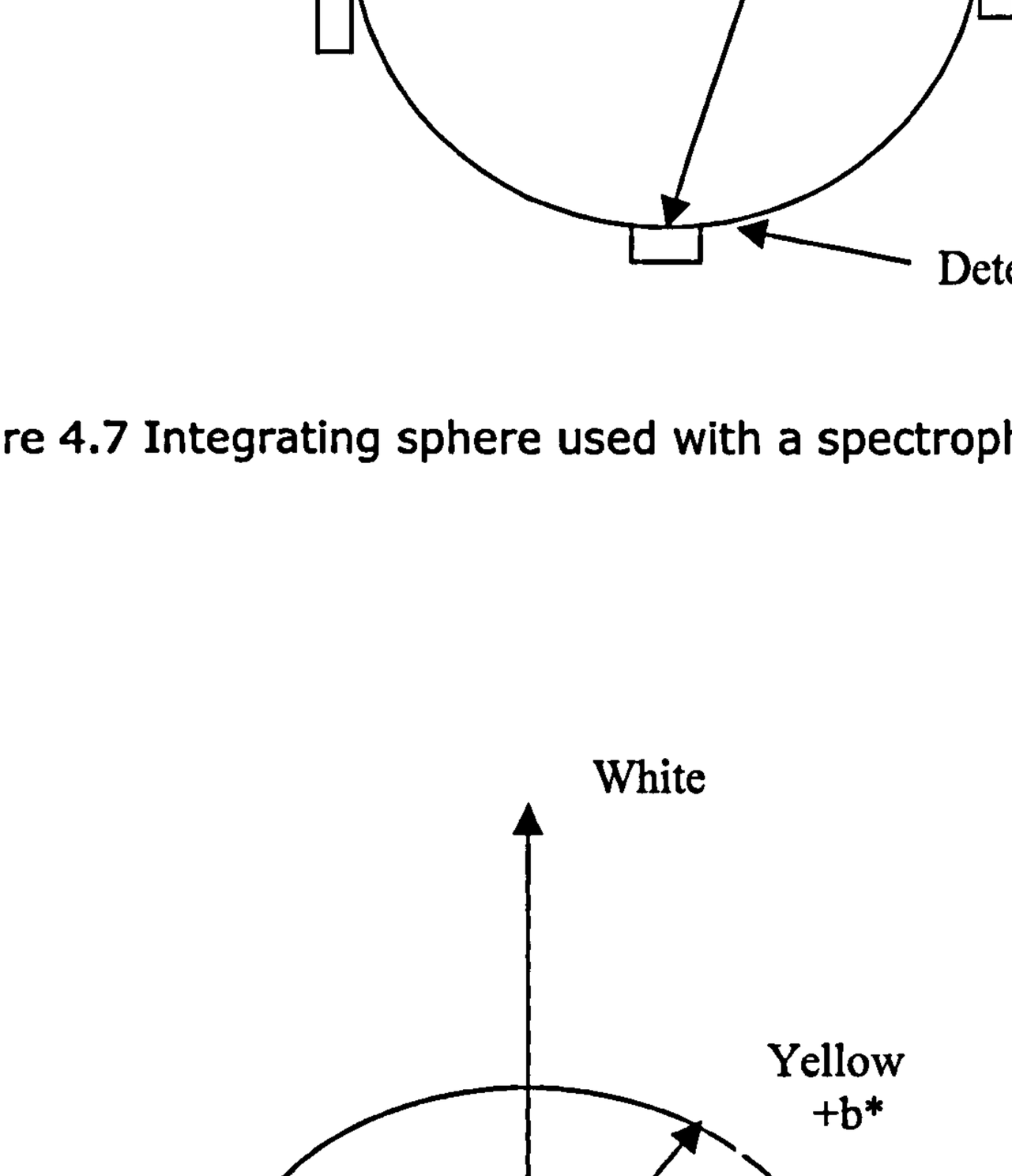


Figure 4.8 Representation of CIE Lab colour space (from ref.1)

4.7 Preparation of Amalgam Samples for Bleaching

4.7.1 Samples for Bleaching with 10% CP

4.7.1.1 Materials and Methods

The amalgam tested in this study was Sybraloy® (Kerr UK Ltd., Peterborough, Lot 71062). This is a typical restorative material based on a high copper, unicompositional spherical alloy. The Kerr data sheet gives the composition (%, w/w) for Sybraloy® as 41.8% Ag, 29.3% Sn, 28.2% Cu and 0.03% Zn; the final mercury concentration of the amalgams was 44.5% (w/w). Cylindrical discs (10 mm diameter x 2 mm height) were prepared, using the jigs described earlier, in accordance with the manufacturer's instructions and aged for 7 days at 37 °C in air. The packed amalgam discs were polished using standard dental equipment (silicone polishers) and left overnight in air at 37 °C. The disc specimens (n=20) were randomly divided into four equal groups of 5 discs. Each of the 5 discs in a group was treated with either a 10% carbamide peroxide (CP) gel, the 0% CP control gel (2.0g), a control gel based on lutrol polymer containing no peroxide (2.0g) or a carbonated beverage (Sprite Light®, The Coca Cola Co., Uxbridge, UK. Containing citric and

carbonic acids, pH = 2.84.) (20 ml) for 24 h. After treatment the specimens were wiped clean with cotton wool and placed in distilled water (20 ml) for 24 h at 37 °C.

4.7.1.2 Scanning Electron Microscopy

The amalgam samples were evaluated using SEM to study any changes in surface morphology. SEM images were taken of the surface of amalgam discs following the experimental treatments.

4.7.1.3 Ion Release

Each disc was immersed in 20 ml of water. Samples of this water (eluents) were taken for analysis of ion release by ICP-MS. All ion release samples were acidified with 200 µl of nitric acid (for Ag determination) or hydrochloric acid (for all other ions).

4.7.1.4 Surface Roughness

The surface roughness of the discs was determined using a Talysurf that was duly calibrated. The roughness of the uppermost surface was then measured by moving the stylus

across its diameter. This procedure was repeated (8 replicates) for each disc and the results averaged.

4.7.1.5 Statistical Analysis

The data were analysed using One-Way ANOVA followed by Bonferroni Post-Hoc analyses.

4.7.2 Preparation of Samples for Bleaching with 10% CP and Testing with LA-ICP-MS.

4.7.2.1 Materials and Methods

A number of amalgam discs (n=12) were prepared as before. The discs (10 mm diameter x 2 mm height) were randomly divided into for equal groups. One group was treated with 10% CP gel, the second group with lutrol gel, the third with water and the fourth with Sprite-Light®. Each disc was in turn tested by applying a focussed laser beam onto its surface causing ions to be sputtered from the surface. Excited ions were then passed to the mass spectrometer filtering out the photons and leaving electrons. An electron multiplier counts the number of electrons

allowed through for each mass sequentially for Cu, Ag, Sn and Hg.

4.7.2.2 Statistical Analysis

The data were analysed using One-Way ANOVA followed by Bonferroni Post-Hoc analyses.

4.7.3 Preparation of Amalgam Samples for Bleaching with Hydrogen Peroxide.

The aim of this *in vitro* study was to investigate the effect of varying HP concentrations on metal ion release from dental amalgam.

4.7.3.1 Materials and Methods

The amalgam tested in this investigation was Tytin® (Kerr UK, Peterborough, UK). Like Sybraloy®, this is also a common restorative material based on a high copper, unicompositional spherical alloy. The composition of this alloy is 59% Ag, 28% Sn, 13% Cu and final mercury concentration is 42.5% (w/w).

Preparation of amalgam discs (n = 25) for testing followed a strict protocol to ensure a high degree of sample uniformity. The discs (10 mm diameter by 2 mm final height) were prepared using the jigs described earlier (Figure 4.1) wearing polythene gloves. The discs were polished on the uppermost surface to to as near a mirror finish as possible by using silicone carbide paper (Grit no. 800 and then 1200) followed by polishing on a felt wheel (matted wool cloth) with a 1μm diamond suspension. The surface roughness of the polished surface of each disc was measured with a Talysurf (4.5).

Titrations were carried out as before to establish the actual active concentration of HP in a 30% (w/v) solution. The concentration of the HP solution thus calculated was 30.23%. This solution was diluted to obtain a 1%, 3%, 10% and 30% HP solutions used in these experiments with 0% as the control.

The 25 amalgam discs were randomly divided into 5 equal groups. The 5 discs in a group were individually immersed in either 0%, 1%, 3%, 10% and 30% HP solution (20 ml) for 24 hours at 37°C creating 5 samples of each solution. Each disc was placed in individually numbered tapered centrifuge tube, with all surfaces exposed to the particular HP concentration in

that tube. All the 25 solutions samples were analysed by ICP-MS.

4.7.3.2 Ion Release

As before, all ion release samples were acidified with 200 µl of nitric acid (for Ag determination) or hydrochloric acid (for all other ions). For each analysis, the ICP-MS instrument performs 5 measurements and calculates the mean and relative standard deviation (%) for each element. Thus, with 5 discs tested in each group, the total number of measurements recorded per element was 25.

4.7.3.3 Surface Roughness

The roughness of the polished surface of each disc was measured again by moving the stylus across its diameter. This procedure was repeated (8 replicates) for each disc, altering the orientation each time, and the results averaged.

4.7.3.4 Statistical Analysis

A Two-Way analysis of variance (element by concentration) revealed a significant interaction between concentration and

elements ($p<0.001$) indicating that differences between solutions were different across elements. The Two-Way analysis was followed by a One-Way ANOVA and Dunnett Post-Hoc test for multiple comparisons between solutions for each element. The roughness measurements were analysed using a paired t-test.

4.8 Preparation of Dental Casting Alloy Samples

4.8.1 Materials and Methods

Two typical dental alloys were tested in the current work, a Ni-Cr alloy (Wiron 99, Bego, Bremen, Germany) and a Pd-Cu-Ga alloy (Cerapall II, Metalor,Technologies SA, CH-2009 Neuchatel). The Ni-Cr is a base metal alloy and the Pd-Cu-Ga is a noble alloy as classified by the American Dental Association (125). The composition (% w/w) of the two alloys is presented in Table 4.3 below.

The lost wax technique was used to prepare the discs (n=28 for each alloy). Accordingly, the discs were made in wax from silicone moulds (10 mm x 2 mm for the Ni-Cr discs and 5 mm x 1 mm for the Pd-Cu-Ga discs). The total surface area of a Ni-Cr disc was calculated to be 2.2 cm^2 and that of a Pd-Cu-Ga disc to

be 0.55 cm^2. The discs were identically sprued and invested in phosphate bonded investment. Melting and casting of the alloys was carried out using an induction casting machine (Modular 3S, ASEG Galloni, Italy). The castings were allowed to bench cool, devested and sprues with excess alloy ground away. The cast discs were heat treated for VITA (Vita Zahnfabrik H. Rauter GmbH & Co. KG Postfach 1338 D-7880 Bad Sackingen Germany) porcelain firing cycles (Table 4.4). Each disc was polished on both sides using fine stones, rubber wheels and bristle brushes loaded with universal polish and finally lambs wool mop.

Once again volumetric analysis based on iodine thiosulphate titration was performed to establish the exact active concentration of HP in test solutions. Five titrations were carried out using nominally a 30% HP (w/v) solution. The HP solution was diluted to obtain 3% (w/v), 10% (w/v) as well as 30% (w/v) solutions with high purity distilled water as the control. Testing at 1% (w/v) HP concentration (used in amalgam testing in addition to the above concentrations) was not considered necessary. The pH of the solutions was also measured using a pH meter (Checker 1, Hanna Instruments, UK). The meter was calibrated using two standard buffers of pH 4 and 7.

Ni-Cr Alloy		Pd-Cu-Ga Alloy	
Element	% (w/w)	Element	% (w/w)
Ni	65.0	Au	2.0
Cr	22.5	Pd	78.5
Mo	9.5	Cu	6.9
Si	1.0	Ga	5.5
Nb	1.0	In	4.5
Fe	0.5	Sn	2.0
Ce	0.5	Zn and Ru	1.0 Max
C	0.02 Max		

Table 4.3 Alloy compositions
(from manufacturer's data sheet)

Program	Top Temperature (°C)	Preheat at 600°C Time (m)	Time to Top Temperature (m)	Time Held at Top Temperature (m)	Time Fired Under Vacuum (m)
Oxidation	980	-	-	5	-
Opaque	930	-	3	1	3
Main Vacuum	930	6	6	1	6
1st Correction	920	6	6	1	6
2nd Correction	910	6	6	1	6
Glaze	930	-	3	1	-

Table 4.4 Porcelain firing cycles

4.8.2 Ion Release

For each alloy, the 28 discs were randomly divided into four equal groups. All discs in a group (n=7 per group) were individually immersed in either 0%, 3%, 10% and 30% HP (w/v) solutions (20 ml for Ni-Cr alloy and 10 ml for Pd-Cu-Ga alloy) for 24 h at 37°C creating seven samples of each solution. Each disc was placed in a tapered centrifuge tube, with all surfaces exposed to the particular HP concentration in that tube. The solution samples were analysed by ICP-MS, Agilent 4500.

All ion release samples were acidified with 200 µl of nitric acid (for Ag determination) or hydrochloric acid (for all other ions). For each analysis, the ICP-MS instrument performs five measurements and calculates the mean and relative standard deviation (%) for each element. Thus with the 7 discs tested in each group, the total number of measurements recorded per element was 35.

4.8.3 Surface Roughness

The surface roughness of each disc was measured on both polished surfaces before and after bleaching using a Talysurf instrument. The procedure was repeated several times for each disc altering the orientation after each measurement.

4.8.4 Statistical Analysis

A Two-Way ANOVA (element by concentration) revealed a significant interaction between concentration and elements ($p < 0.001$) for both alloys indicating that differences between solutions were different across elements. The Two-Way ANOVA was followed by a One-Way ANOVA and Dunnett Post Hoc test for multiple comparisons between solutions for each element. The roughness measurements were analysed using a paired t-test.

4.8.5 Biocompatibility Testing

For the purpose of this thesis biocompatibility will be used to represent cytotoxicity/ cytocompatibility.

Biocompatibility of the two alloys before and after bleaching was assessed. Two types of tests were carried out; Alamar blue Assay and SEM. Cellular response was evaluated using Alamar blue (a measure of the respiratory rate of the cells) and SEM for the observation of the cultured cells.

Alamar blue assay provides a quantitative measure of cytocompatibility. It incorporates a fluorometric/volumetric growth indicator based on detection of metabolic activity. This non toxic reagent is readily reduced by living cells and the resulting product can be detected spectrophotometrically by fluorescence or by the measurement of optical density (OD) at 570 nm and 600 nm. In this work the reduced dye was detected by measurement of OD. Under these conditions Alamar blue has a sensitivity of 1000-80,000 cells per well.

SEM can be used to provide a qualitative measure of cytocompatibility.

4.8.5.1 Materials and Methods

A number of similar size discs of the two alloys (Ni-Cr and Pd-Cu-Ga) were also prepared for biocompatibility testing. Sixteen discs of each alloy were prepared making a total of 32 discs.

The 16 discs of each alloy were randomly divided into two equal groups; one group treated with 0% HP and the other with 30% HP. Thus, four groups were created and are defined as follows.

Group 1 – Eight Pd-Cu-Ga discs treated with 0% HP for 24 h at 37°C

Group 2 – Eight Ni-Cr discs treated with 0% HP for 24 h at 37°C

Group 3 – Eight Pd-Cu-Ga discs treated with 30% HP (w/v) for 24 h at 37°C

Group 4 – Eight Ni-Cr discs treated with 30% HP (w/v) for 24 h at 37°C

4.8.5.2 Biocompatibility Assays

Mouse fibroblast cells (L929) were removed from storage in liquid nitrogen and defrosted by warming in a 37 °C water bath. The storage medium was removed and the cells were washed using sterile phosphate buffered saline (PBS). The cells were cultured in a Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal calf serum and antibiotics (Pencillin and Streptomycin). Cells were left to populate the surface of culture plates and, when fully confluent, were passaged using Trypsin (0.25 % w/v) - EDTA (0.2% w/v). The cells were seeded at a density of 1.25×10^4 cells/well into a 48

well plate (8 x 6) containing the test samples. The sample groups were placed into the wells (one group per row). In this way four rows were used for the four groups. In the fifth row, a non-material control was included for comparison. The materials and cells were incubated at 37°C in a 95% air/5% CO_2 atmosphere for 72 h.

Three discs were removed from each group for SEM work. These discs were washed in 0.1 M sodium cacodylate buffer (adjusted to PH 7.4) and fixed using 3% gluteraldehyde fixative. The fixative was removed and the samples washed with buffer three times. Secondary fixation using osmium tetraoxide was then carried out. The osmium solution was then replaced with cacodylate buffer. The samples were dehydrated using a series of graded ethanol, mounted on carbon discs and gold coated for SEM studies. The remaining samples and controls were used for cell viability determined using Alamar blue according to the manufacturers instructions (Biosource) on the remaining samples and controls. The Alamar blue Assay was performed on the 5 samples of each of the four groups as well as the fifth non-material control group after 3 days. The media was removed from each well and replaced with 0.9 ml of the culture medium together with 0.1 ml Alamar blue solution. The samples were

returned to the incubator and incubated for 4 hours. 200 µl samples were then removed from each test well and transferred to a 96 well plate and the OD of the dye determined at a wavelength of 570 nm and also 600 nm.

The cell viability was expressed as a percentage Alamar blue present in the reduced form.

4.8.5.3 Statistical Analysis

A One-Way ANOVA followed by Dunnet's Post Hoc for multiple comparisons between groups were carried out.

5. Results

5.1 Effect of Hydrogen Peroxide Concentration on Bovine Enamel and Dentine.

5.1.1 Ion Release

Ion release data in units of µg/l, as measured by ICP-MS, are plotted in Figures 5.1 a and b to show the relationship between the increase in ion release with increasing HP concentration. The mean and standard deviation values of ion release data (Ca and P) were converted to units of ug/mm^2 at 0%, 3%, 10% and 30% (w/v) HP concentration and presented in Table 5.1 for the enamel and dentine. This was obtained by dividing the total amount of ion release in µg over a 24 h period, for each element, by the total surface area of the corresponding enamel or dentine sample. There was an increase in ion release values of Ca and P with increasing HP concentrations for both enamel and dentine samples. The release of Ca was consistently more than P ion release at all HP concentrations for both enamel and dentine. More ions were released from dentine than enamel at all HP concentrations tested here.

The distribution of the recorded Ca and P ion release data did not follow a normal distribution for both enamel and dentine and therefore a non-parametric statistical test was used. There was a significant change in ion release ($p<0.05$), for both enamel and dentine, between control and all other HP concentrations. Additionally, there was a significant increase ($p<0.05$) in ion release each time the HP concentration increased (Table 5.1).

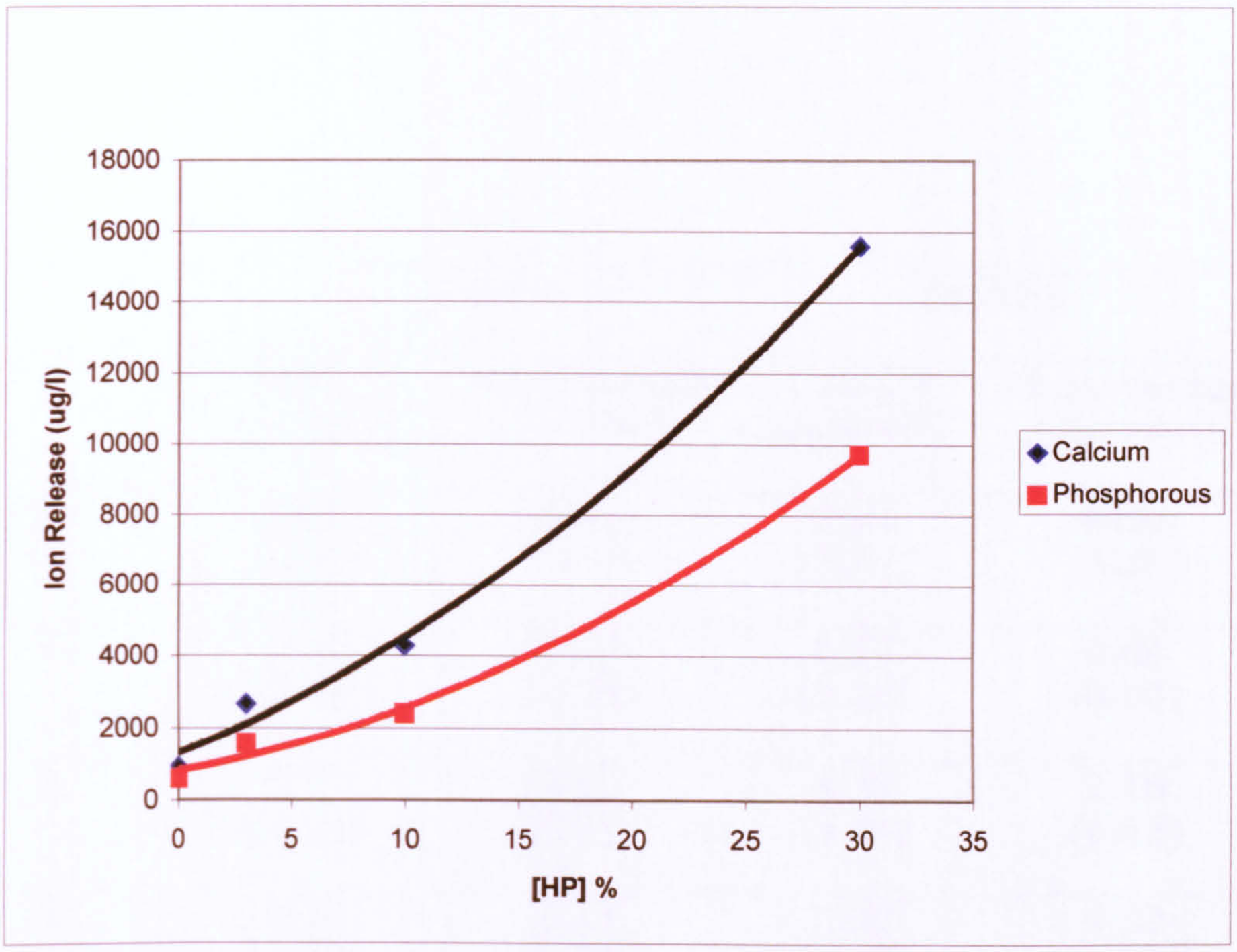


Figure 5.1a Variation of ion release with [HP] - enamel

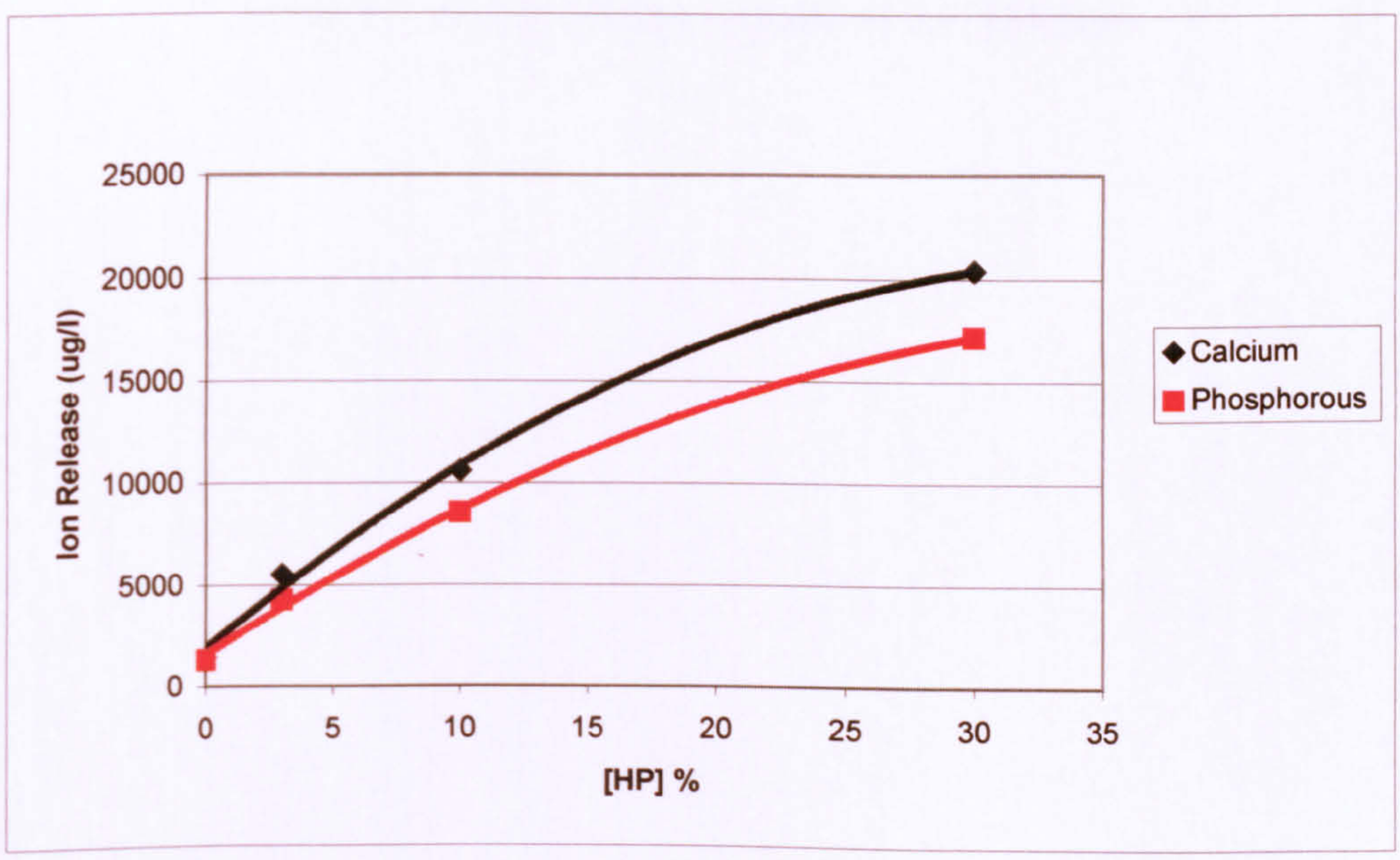


Figure 5.1b Variation of ion release with [HP] - dentine

	Enamel		Dentine	
	Calcium ($\mu g/mm^2$)	Phosphorous ($\mu g/mm^2$)	Calcium ($\mu g/mm^2$)	Phosphorous ($\mu g/mm^2$)
[HP] %	Mean (S.D.)	Mean (S.D)	Mean (S.D.)	Mean (S.D.)
0	0.48 (0.08)	0.31 (0.06)	0.72 (0.25)	0.68 (0.10)
3	1.34 (0.25)	0.80 (0.15)	2.77 (0.51)	2.16 (0.62)
10	2.15 (0.56)	1.21 (0.29)	5.30 (0.45)	4.30 (0.81)
30	7.80 (0.67)	4.87 (0.75)	10.20 (0.57)	8.60 (1.19)

Table 5.1 Mean (S.D.) values of ion release

	Enamel						Dentine					
	Ca			P			Ca			P		
[HP]%	3	10	30	3	10	30	3	10	30	3	10	30
0	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
3	-	0.008	0.008	-	0.032	0.008	-	0.008	0.008	-	0.008	0.008
10	0.008	-	0.008	0.032	-	0.008	0.008	-	0.008	0.008	-	0.008

Table 5.2 Multiple comparisons – p-values

5.1.2 Microhardness Measurements

The average microhardness and standard deviation values for enamel before and after treatment with HP solutions are shown in Figure 5.2a. Paired t-tests showed significant difference ($p<0.05$) in mean microhardness values of enamel before and after bleaching in all the groups. The average recorded microhardness values before bleaching were 264, 250 and 271 at 3%, 10% and 30% HP concentration. The corresponding values after bleaching were 222, 216 and 198. These represent a reduction of 16%, 14% and 27% at 3%, 10% and 30% HP concentration, respectively. The mean recorded microhardness values before and after bleaching for dentine (Figure 5.2 b) were 129±13 and 120±30, 155±27 and 158±15, 126±20 and 125±24 at 3%, 10% and 30% HP (w/v) respectively. Paired t-test showed no significant difference ($p>0.05$) in mean microhardness values of dentine before and after bleaching in all the groups.

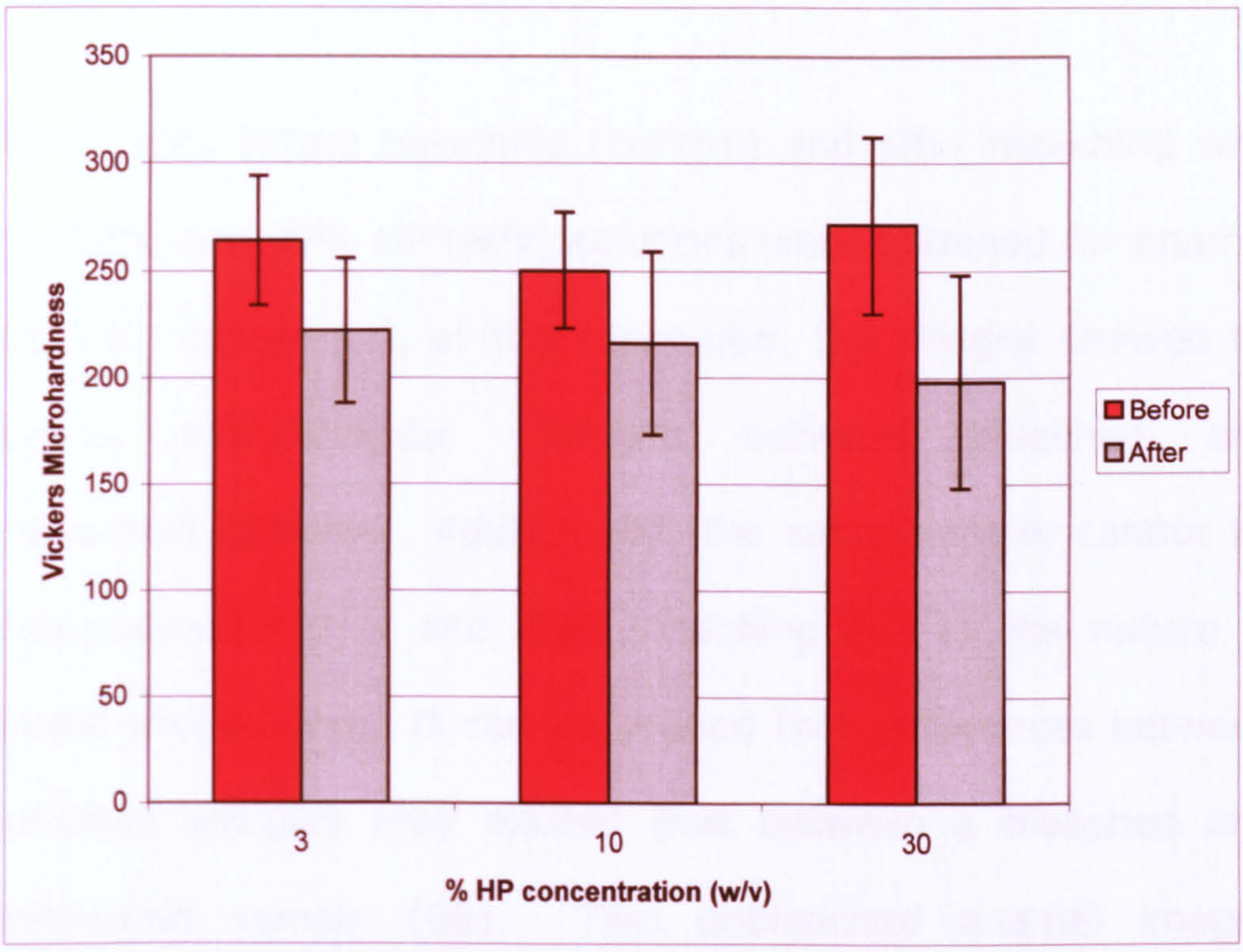


Figure 5.2a Enamel microhardness values before and after bleaching at different HP concentrations. Paired t-tests showed statistically significant differences ($p<0.05$) in microhardness before and after bleaching

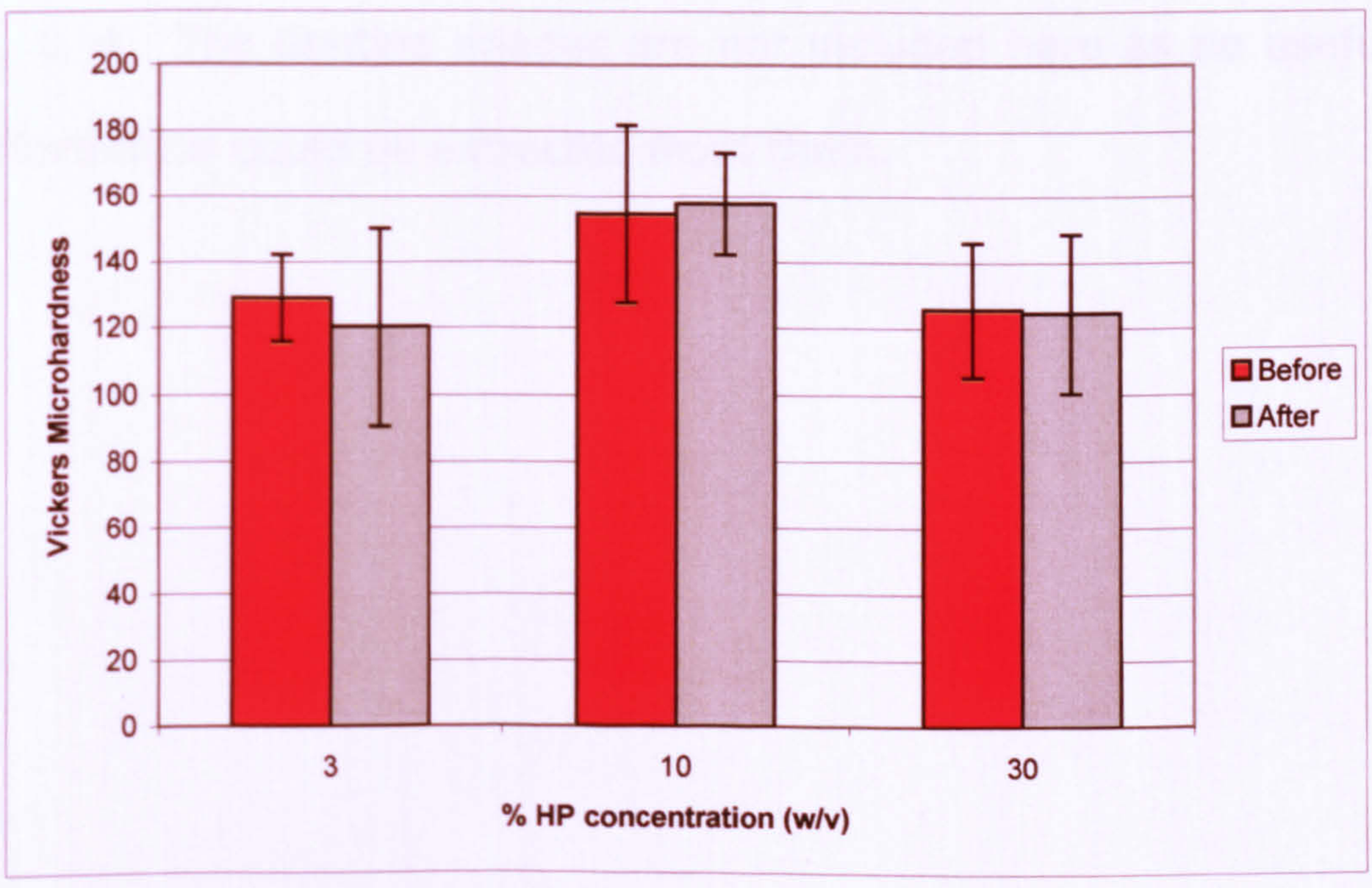
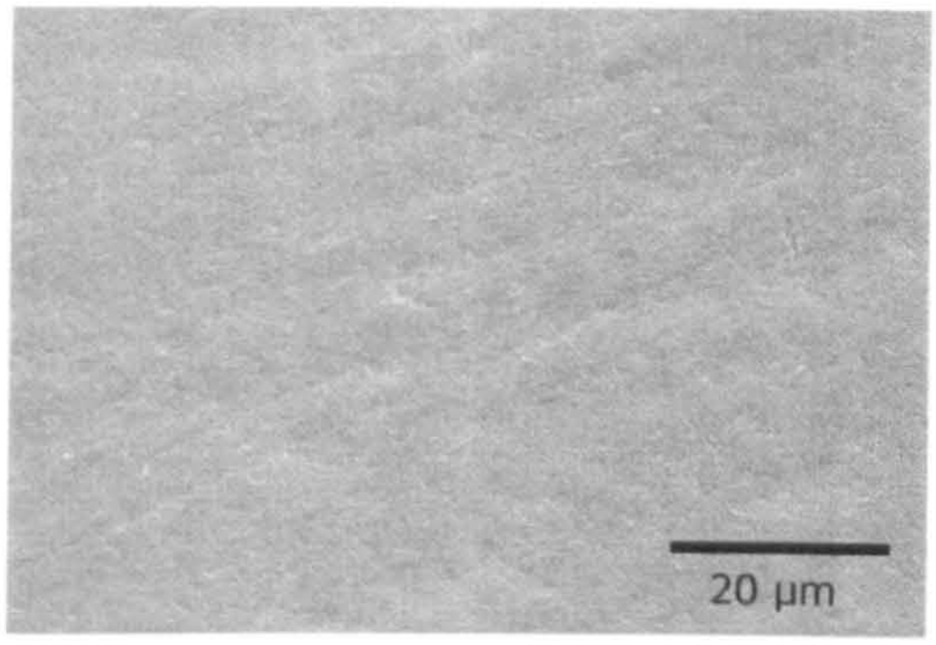


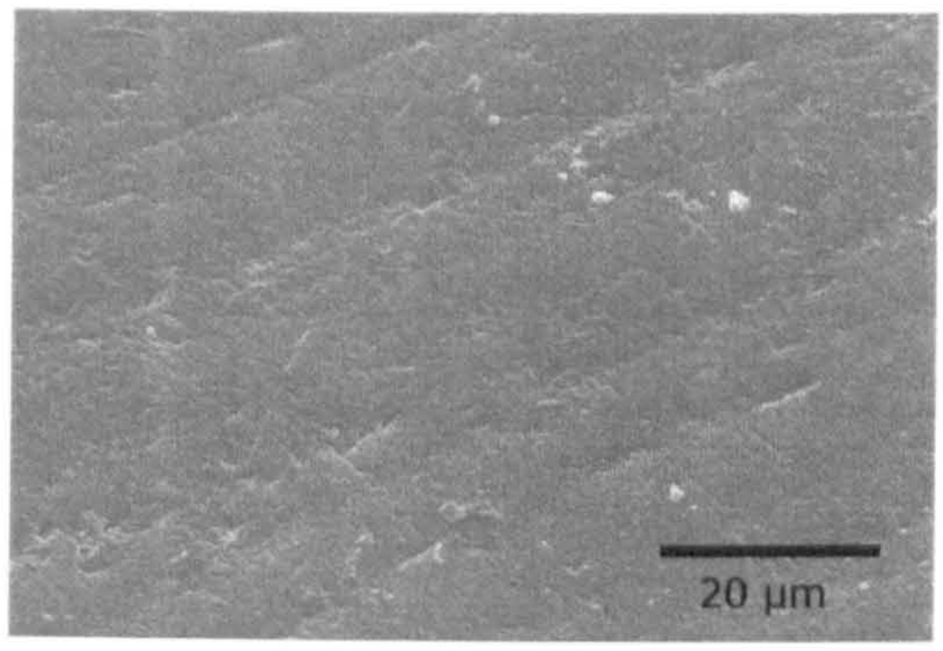
Figure 5.2b Dentine microhardness values before and after bleaching at different HP concentrations. Paired t-tests showed no statistically significant differences ($p>0.05$) in microhardness before and after bleaching

5.1.3 Scanning Electron Microscope Images

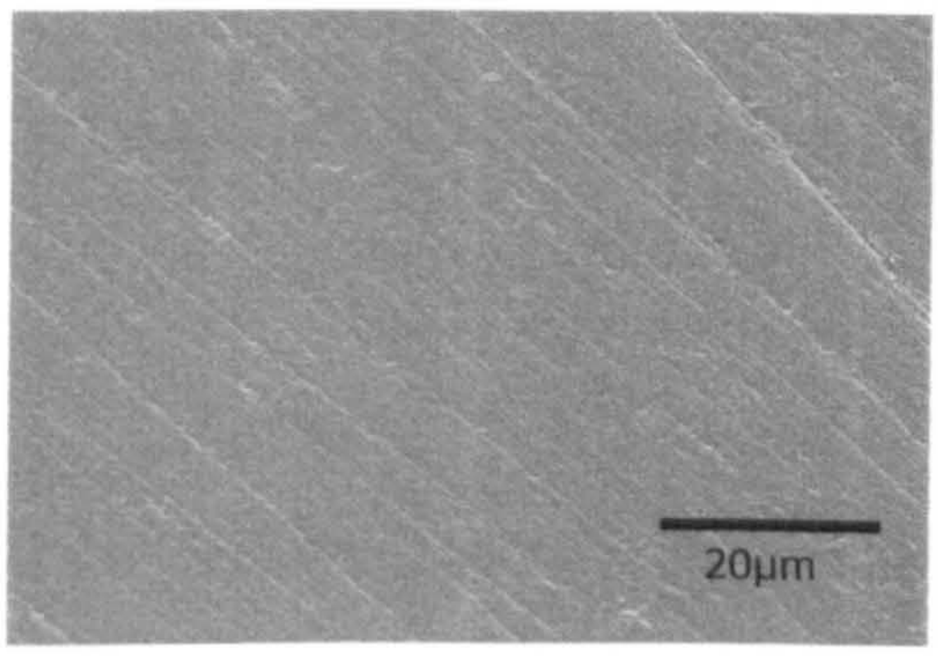
SEM images before bleaching (control) and after bleaching with 3%, 10% and 30% HP (w/v) solutions were obtained for enamel samples. In general, at this resolution, the images showed no obvious morphological changes between bleached and unbleached samples. Additionally, the same sample cannot be photographed before and after bleaching due to the nature of sample preparation. It can be argued that differences between individual samples may exceed that between a bleached and unbleached sample (58). Two unbleached enamel images (control) and two bleached enamel images at 30% HP were selected for comparison purposes and are shown in Figure 5.3 a, b, c, d. The dentine images are not included here as no useful information could be extracted from them.



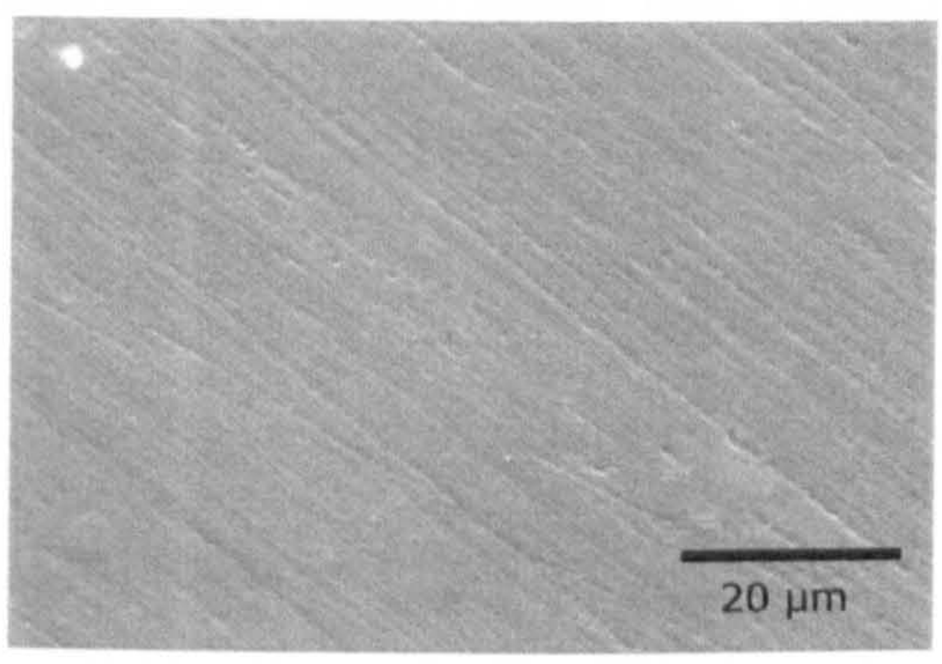
a



b



c



d

Figure 5.3 SEM enamel images - (a and b) control, (c and d) bleached at 30% HP

5.1.4 Translucency Measurements and Colour Change

5.1.4.1. Transmittance Measurements

Variation of transmittance (calculated as a % of total transmittance with no specimen in the transmittance port) with wavelength is plotted in Figure 5.4 for bleached and unbleached enamel sample. As can be seen, there is a large increase in the recorded values of both total transmittance and diffuse transmittance when the sample was bleached. The increase was larger at 380 nm (violet) than at 700 nm (red) end of the wavelength. It is also of interest to note that the direct transmittance is higher for the bleached samples at wavelength values less than 616 nm; the cross over point of the bleached and unbleached traces. The direct transmittance of the unbleached specimen, thereafter, becoming higher.

The dentine sample was tested in a similar manner and the data are plotted in Figure 5.5. The trends exhibited by T(tot) and T(dif) curves are fairly similar to that of enamel. However, the values of T(tot) and T(dif) start at 35% and 30% at 380 nm for the bleached dentine rising to 61% and 47% at 700 nm, respectively. The corresponding figures for the bleached enamel

are 36% and 27% rising to 73% and 54%, respectively. Clearly, therefore, although the values of T(tot) and T(dif) for enamel and dentine are fairly similar at 380 nm the corresponding values at 700 nm are almost 15% higher for the enamel as compared to dentine. The values of T(dir) for dentine before and after bleaching are almost identical at all wavelengths (380-700 nm).

The ratio of T(dif)/T(tot) for enamel as a function of wavelength is plotted in Figure 5.6 before and after bleaching. T(dif)/T(tot) for the bleached is consistently higher than that for unbleached sample. One explanation for this is that bleaching etches the enamel surface leading in turn to an increase in T(dif). Ratios of T(dir)/T(tot) are also plotted on the same graph with values of T(dif)/T(tot) + T(dir)/T(tot) = 1 at every point on the graphs. The corresponding data for dentine is plotted in Figure 5.7 exhibiting a similar trend to that of the enamel.

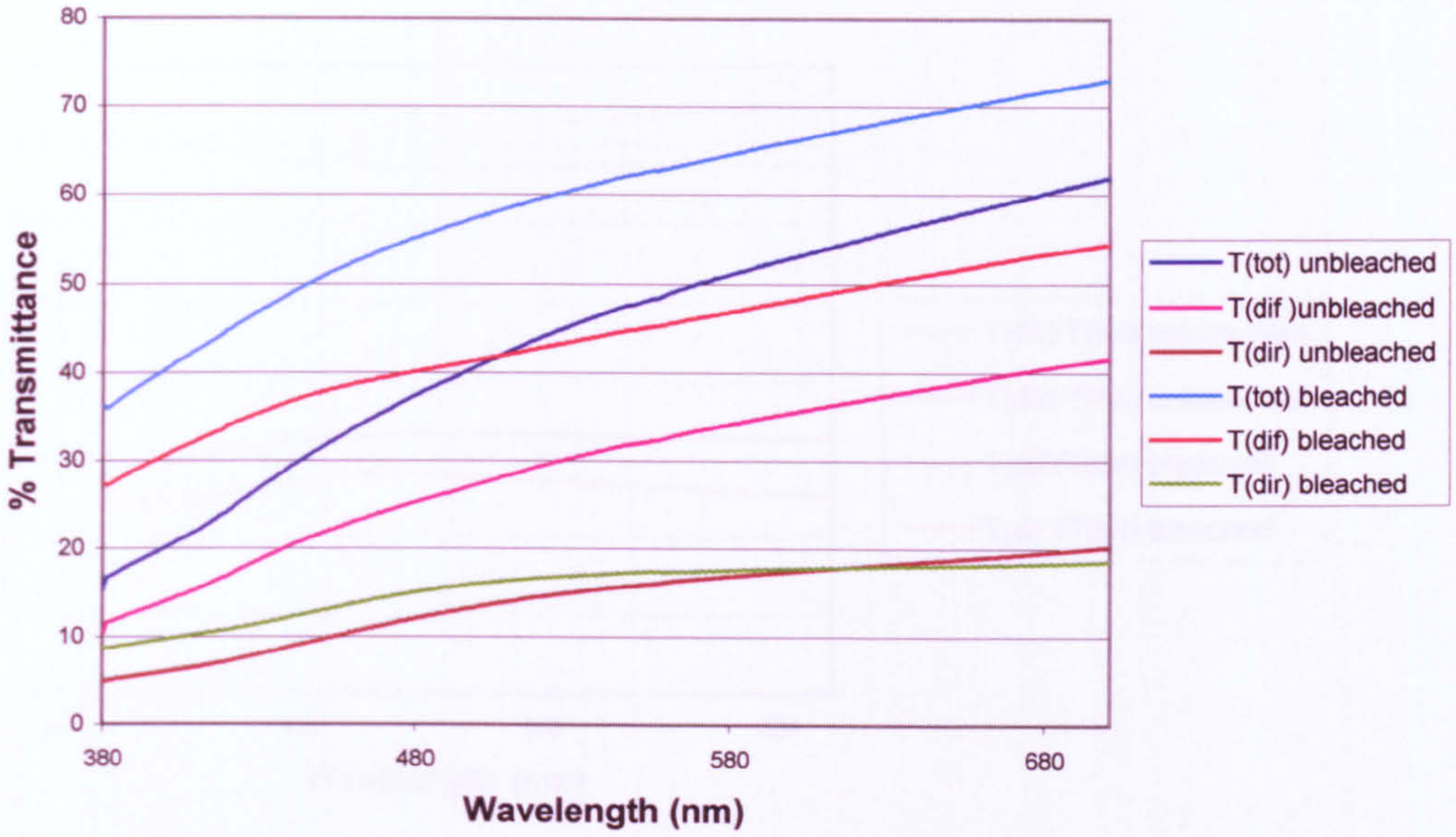


Figure 5.4 Variation of transmittance with wavelength-enamel

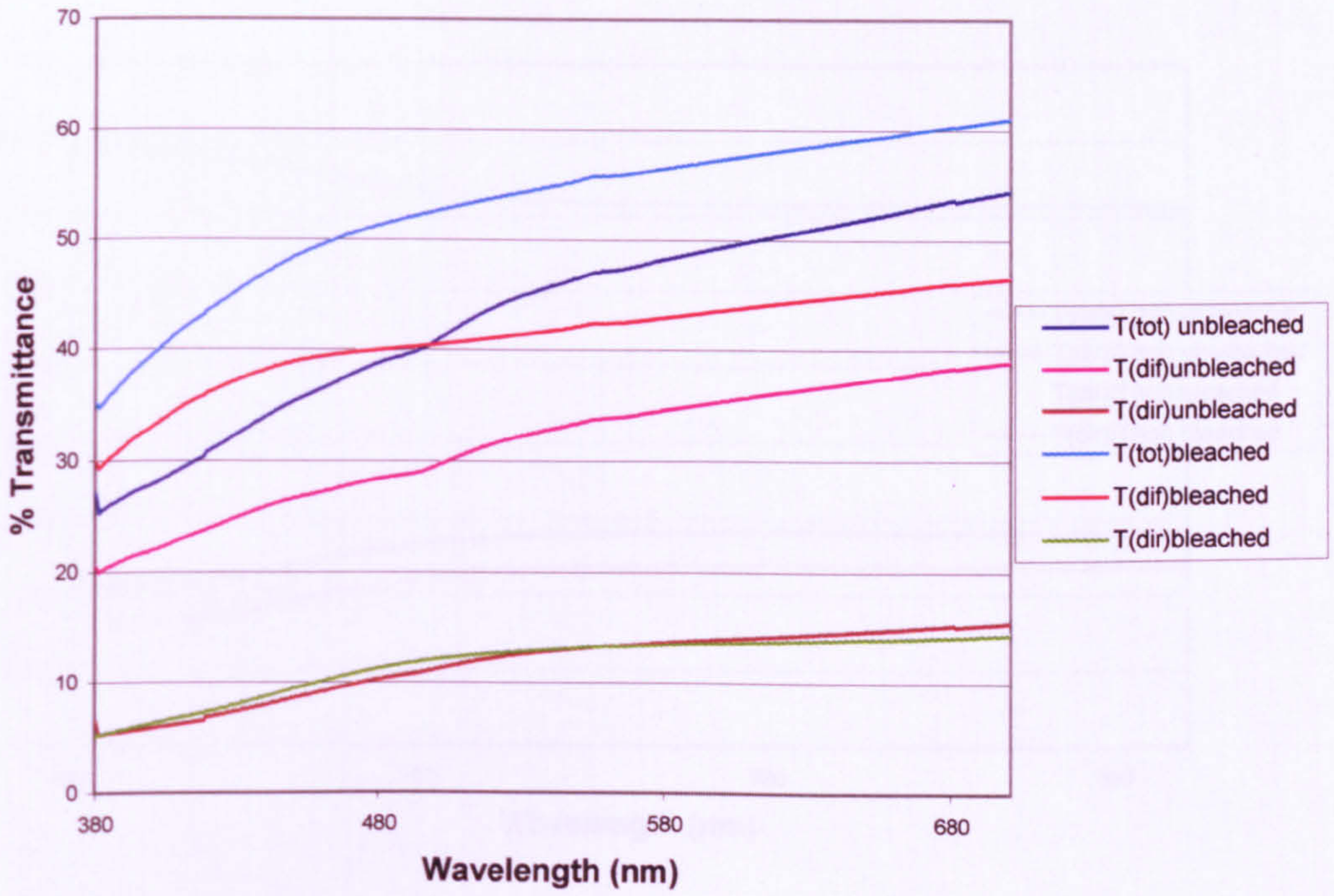


Figure 5.5 Variation of transmittance with wavelength- dentine

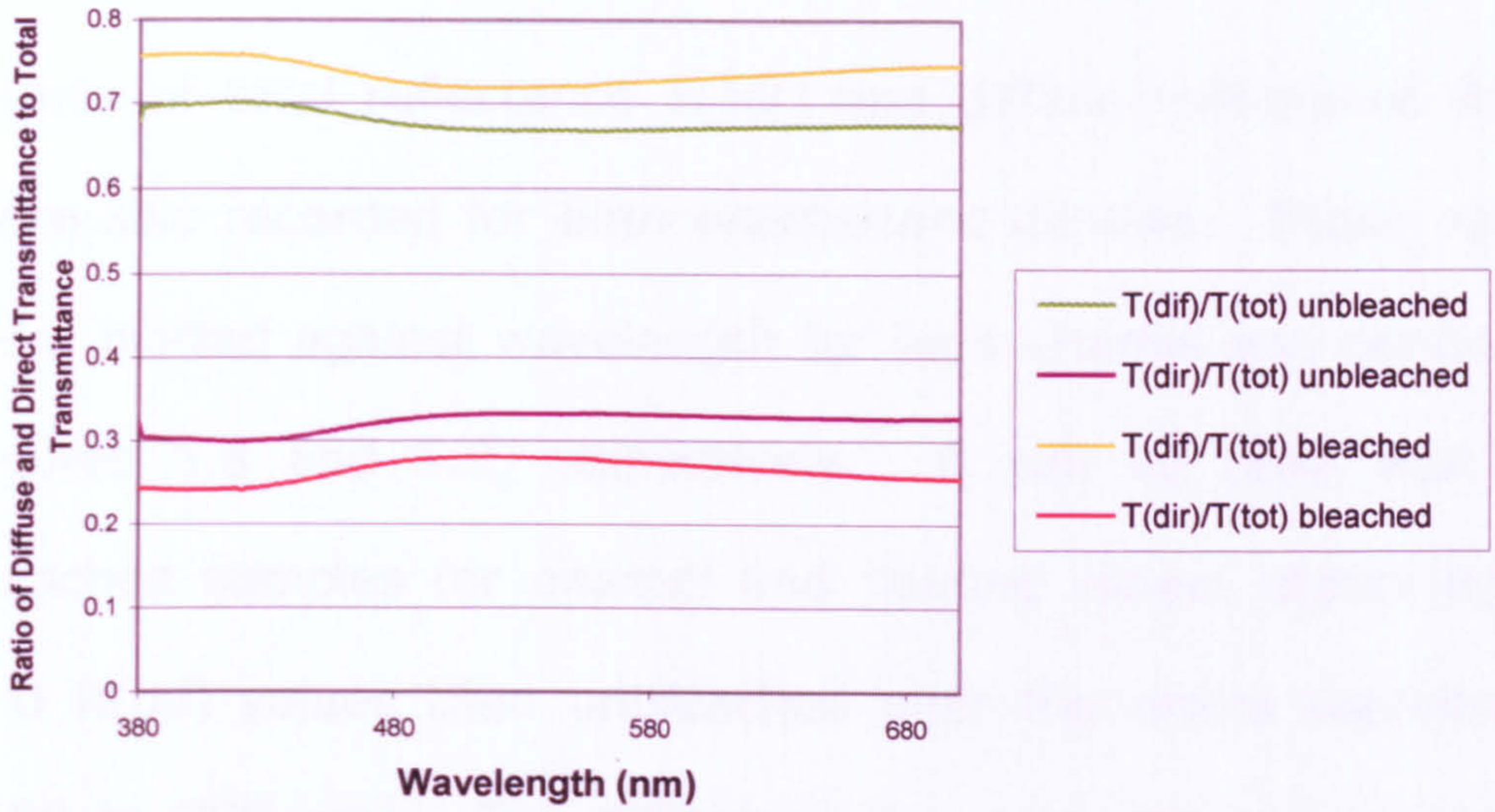


Figure 5.6 Variation of transmittance ratios with wavelength - enamel

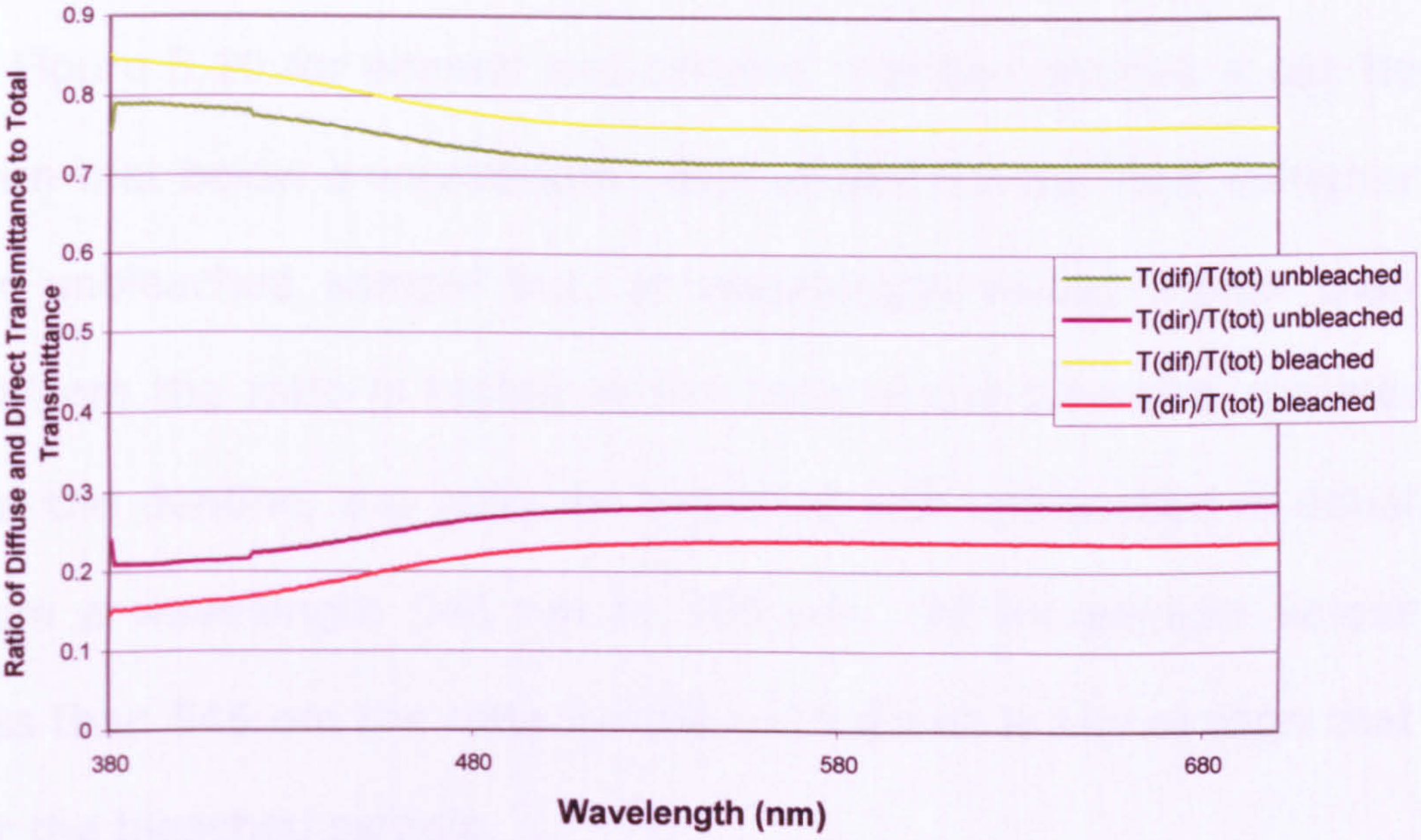


Figure 5.7 Variation of transmittance ratios with wavelength - dentine

5.1.4.2 Reflectance Measurements

Values of total reflectance R(tot) and diffuse reflectance R(dif) were also recorded for both enamel and dentine. These values were plotted against wavelength for both enamel and dentine in Figures 5.8 and 5.9, respectively. It can be seen that the bleached samples for enamel and dentine reflect higher R(tot) and R(dif) values than unbleached over the entire wavelength (380 to 700 nm). The difference in values between bleached and unbleached being higher at 380 nm wavelength (violet end) as was the case with transmittance.

The ratio of R(dif)/R(tot) as a function of wavelength is plotted in Figure 5.10 for enamel and dentine. For the enamel, it can be seen that below a wavelength value of 507 nm the ratio is higher for unbleached sample but, at wavelength values higher than 507 nm the ratio is higher in the case of the bleached sample. For the dentine, the ratio for bleached and unbleached is equal from a wavelength 546 nm to 700 nm. At wavelength values less than 546 nm the ratio for the unbleached is higher than that for the bleached sample.

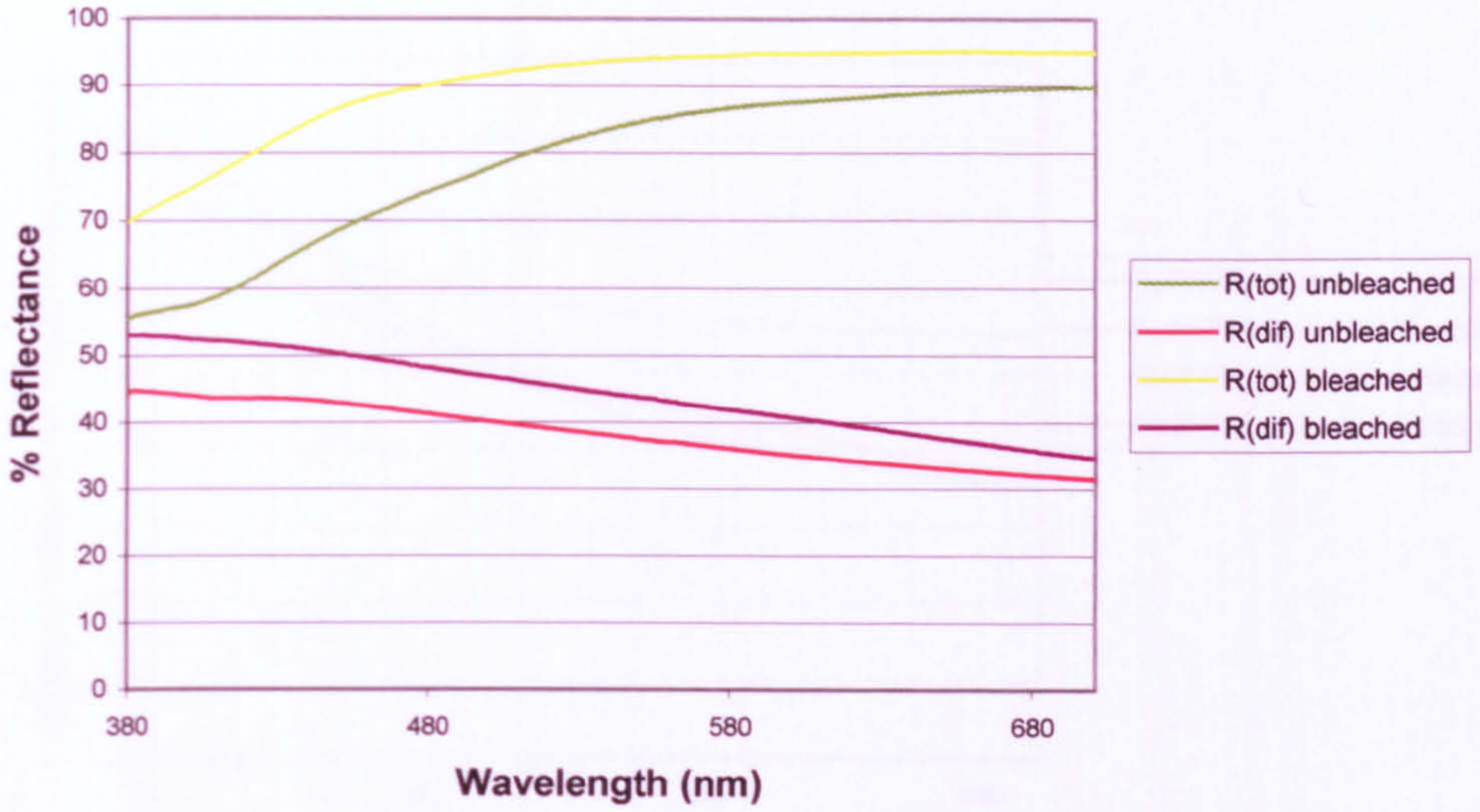


Figure 5.8 Variation of reflectance with wavelength - enamel

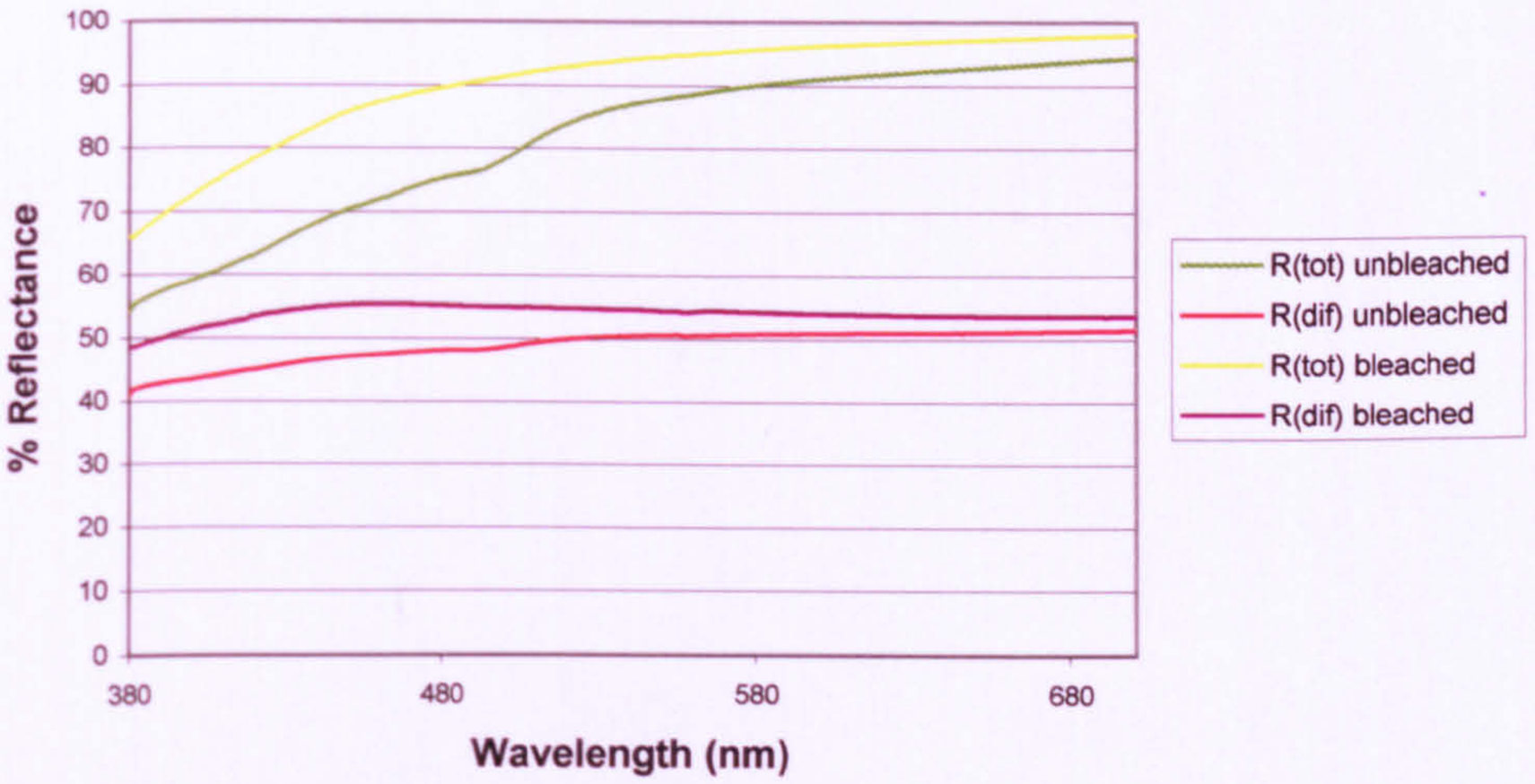


Figure 5.9 Variation of reflectance with wavelength - dentine

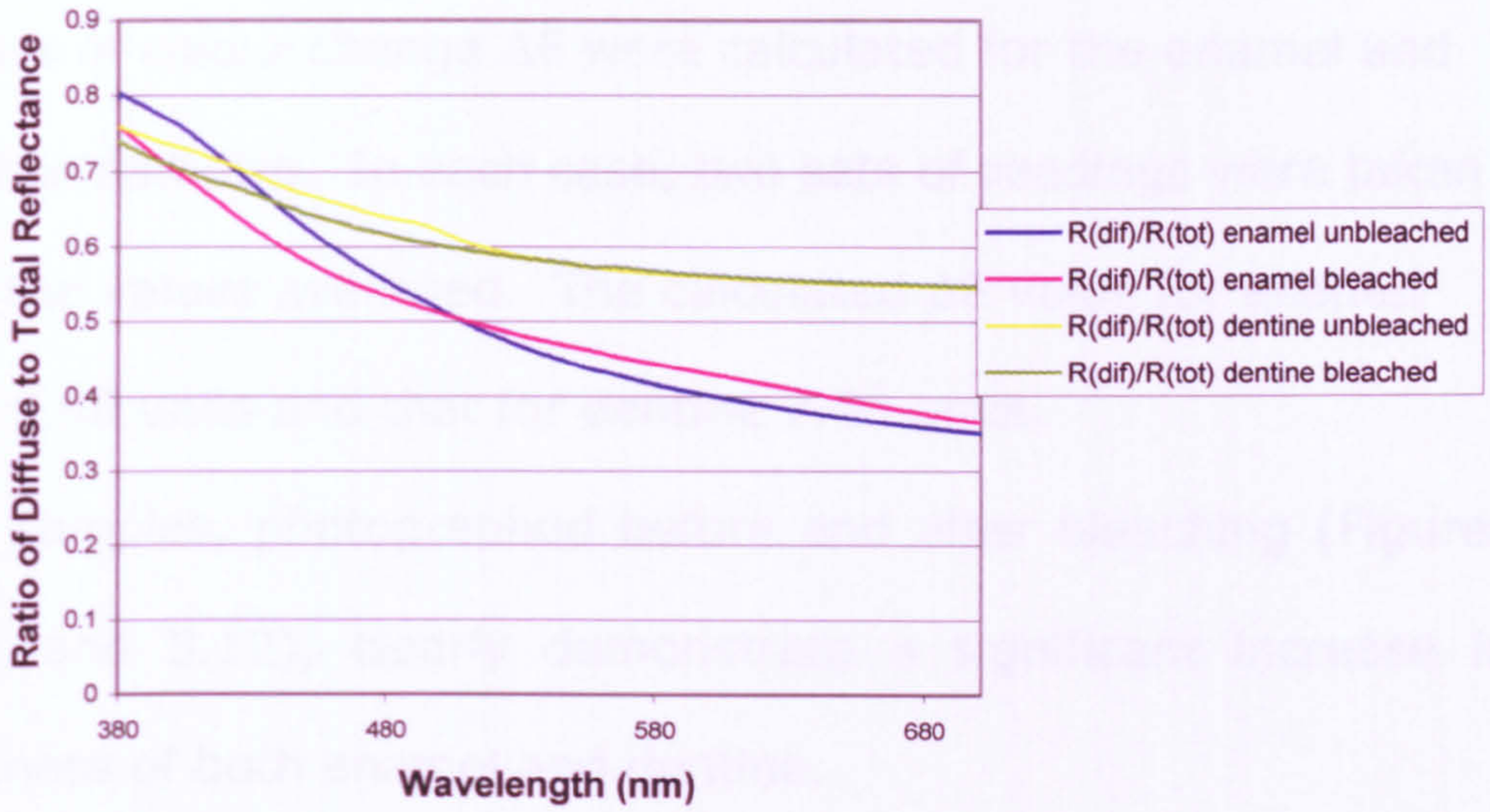
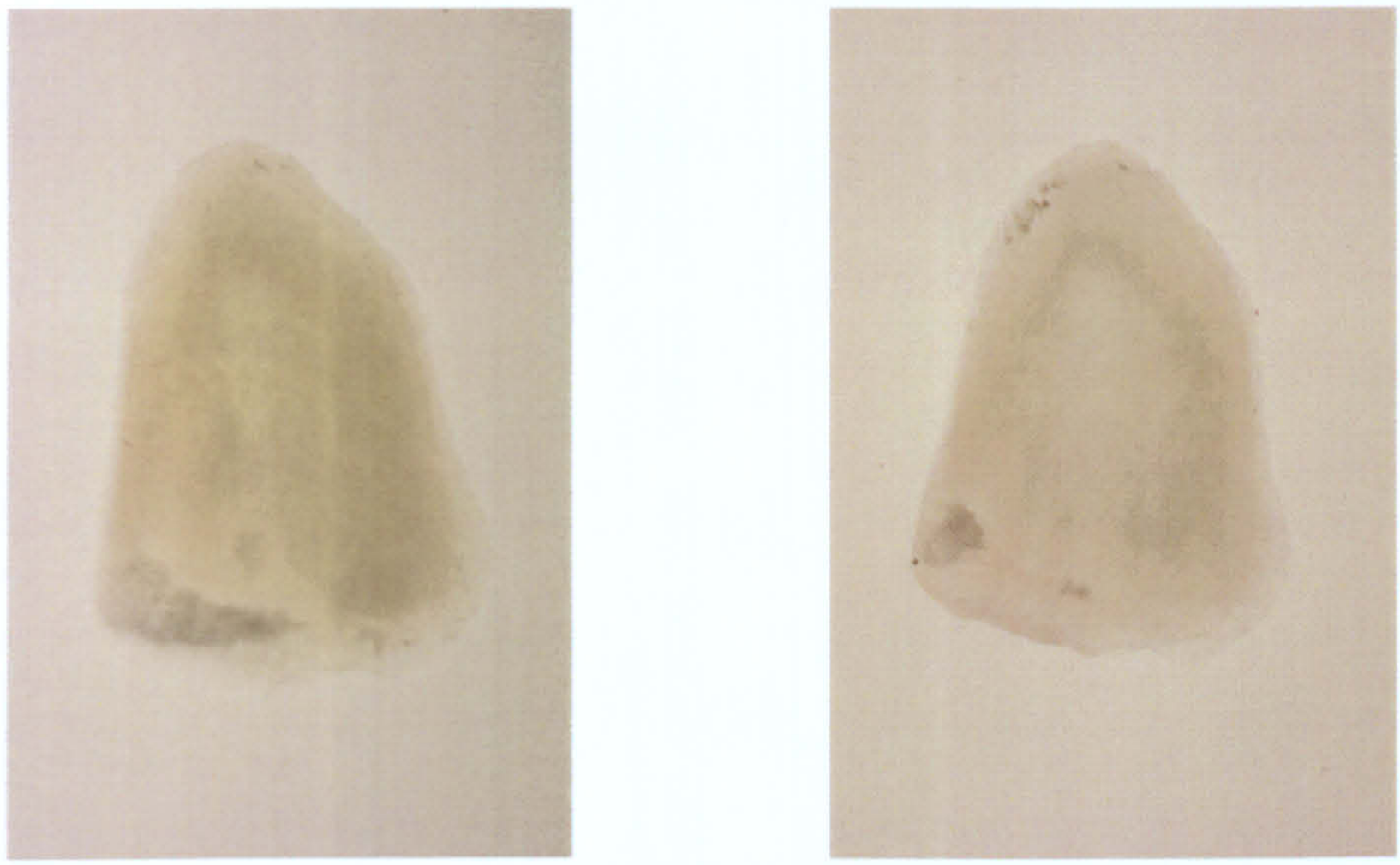


Figure 5.10 Variation of reflectance ratio with wavelength for enamel and dentine

5.1.4.3 Colour Change

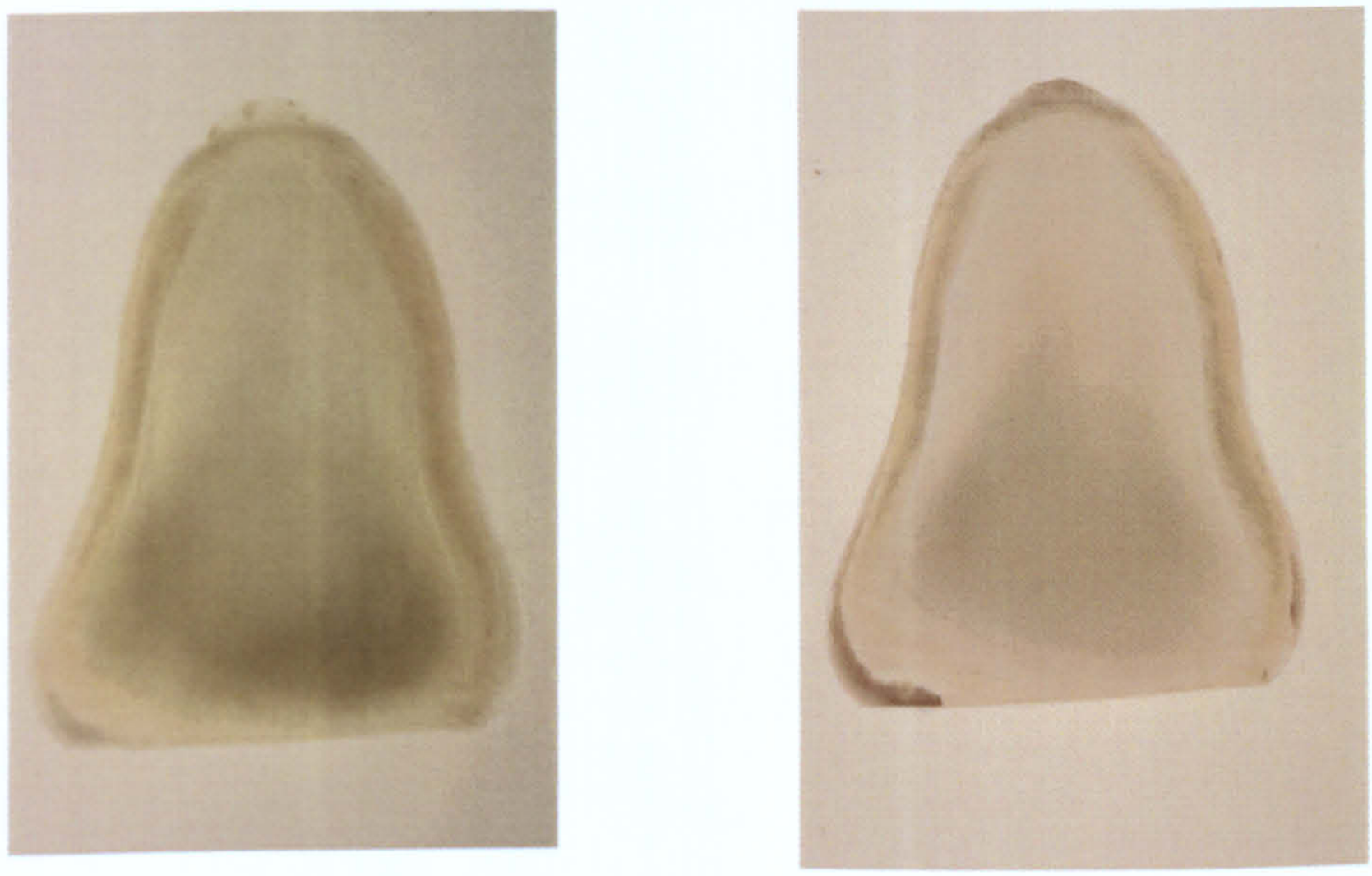
Values of colour change ΔE were calculated for the enamel and dentine samples. In each case, two sets of readings were taken and the values averaged. The calculated ΔE value for enamel was 9.96 units and that for dentine 7.06 units.

The samples, photographed before and after bleaching (Figures 5.11 and 5.12), clearly demonstrate a significant increase in lightness of both enamel and dentine.



a b

Figure 5.11 Enamel (a) pre and (b) post bleaching



a b

Figure 5.12 Dentine (a) pre and (b) post bleaching

5.2 Bleaching Amalgam Discs

5.2.1 Bleaching Amalgam Discs with 10% Carbamide Peroxide

5.2.1.1 Ion Release

Metal ion release data for mercury, silver, tin and copper are shown in Figures 5.13 – 5.16, respectively. It can be seen that treatment with Sprite-Light® did not result in significant release of mercury or silver, but gave the highest release of copper. There were no significant differences in metal ion release between 10% CP and 0% CP gel ($p>0.05$). There were, however, significant differences between 10% CP gel and Sprite-Light® ($p<0.05$) for mercury and copper release.

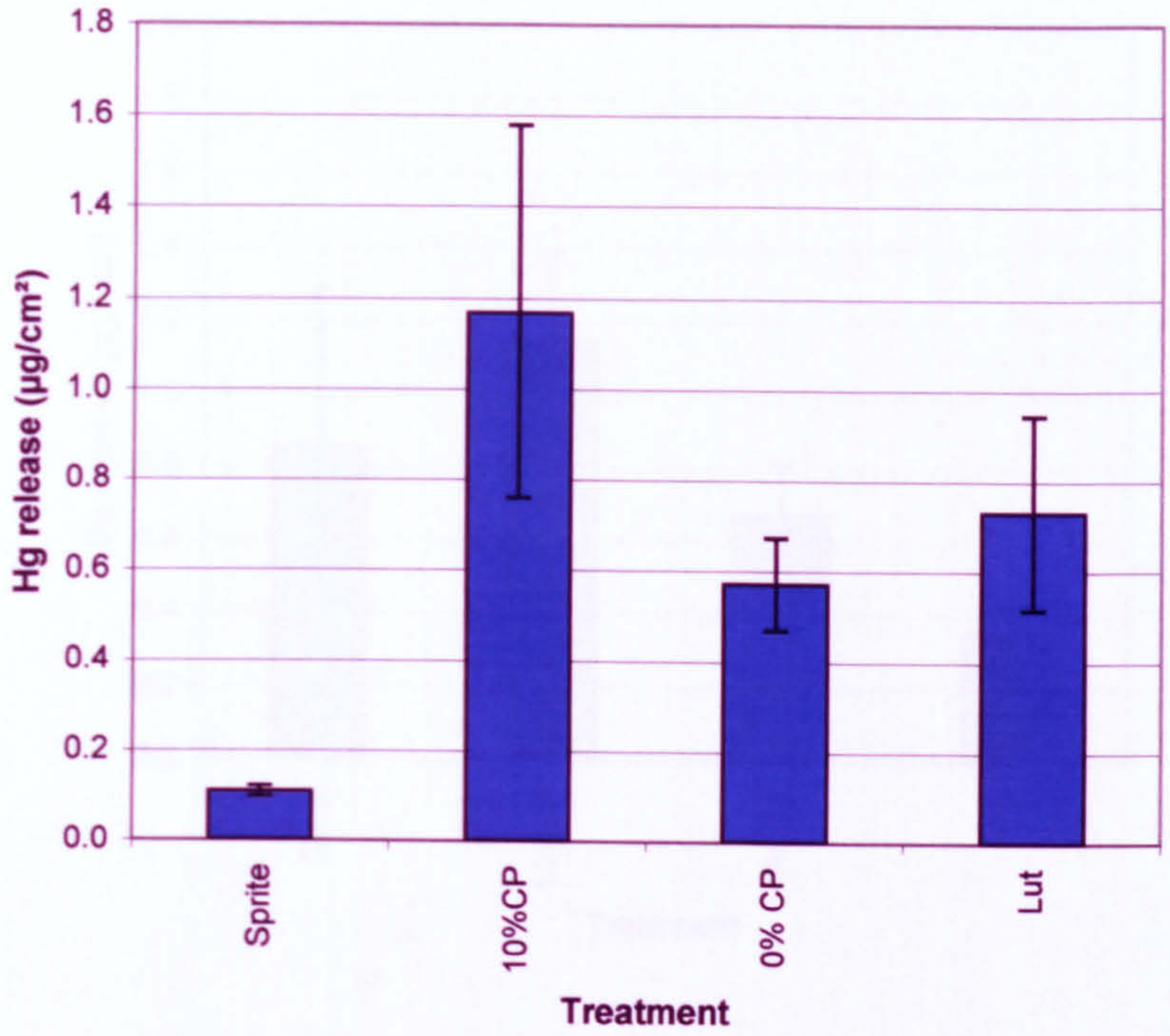


Figure 5.13 Mercury ion release from discs following treatment with 10% CP and control materials. Results are the mean of five samples (error bars show standard error of mean)

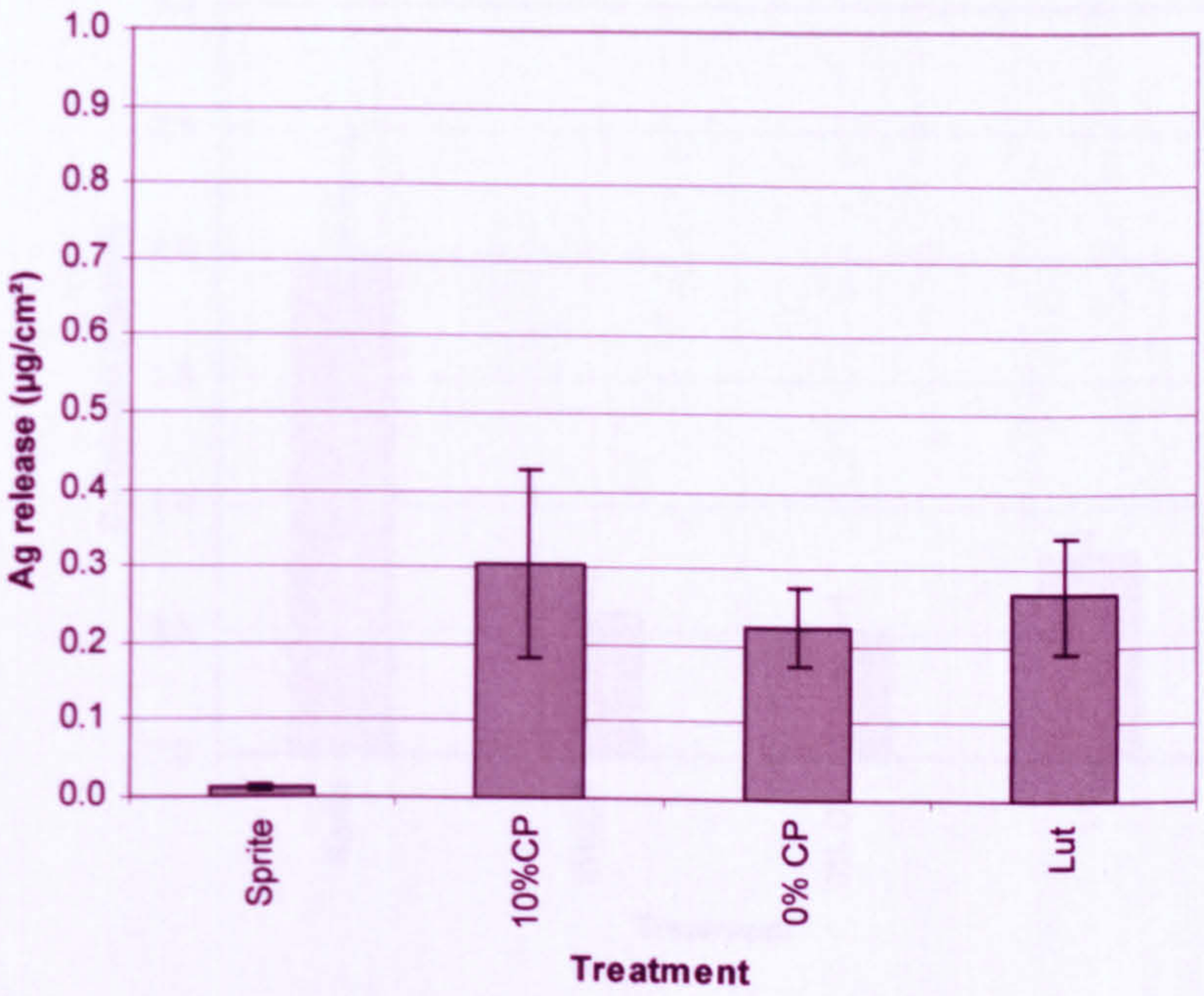


Figure 5.14 Silver ion release from discs following treatment with 10% CP and control materials. Results are the mean of five samples (error bars show standard error of mean)

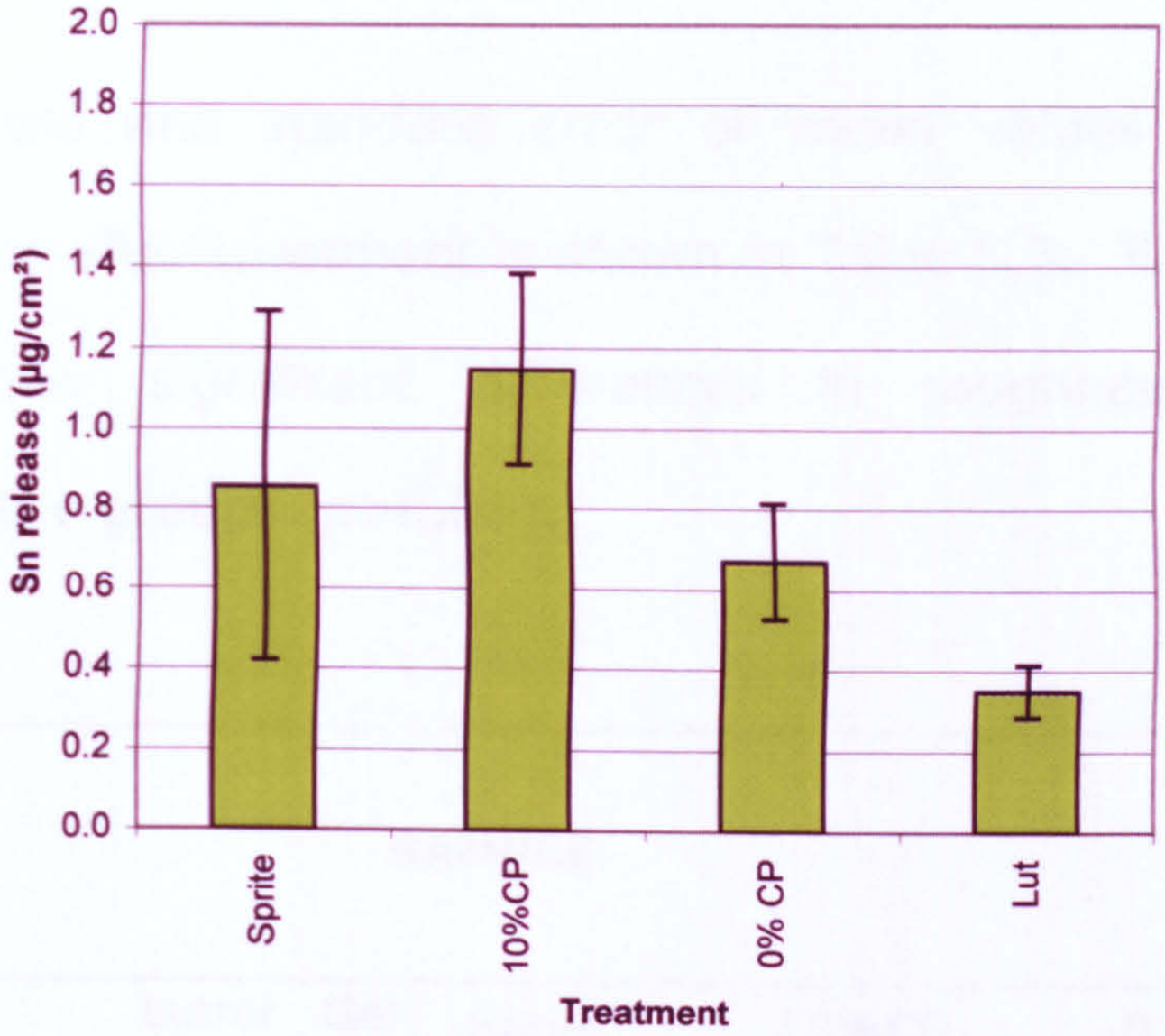


Figure 5.15 Tin ion release from discs following treatment with 10% CP and control materials. Results are the mean of five samples (error bars show standard error of mean)

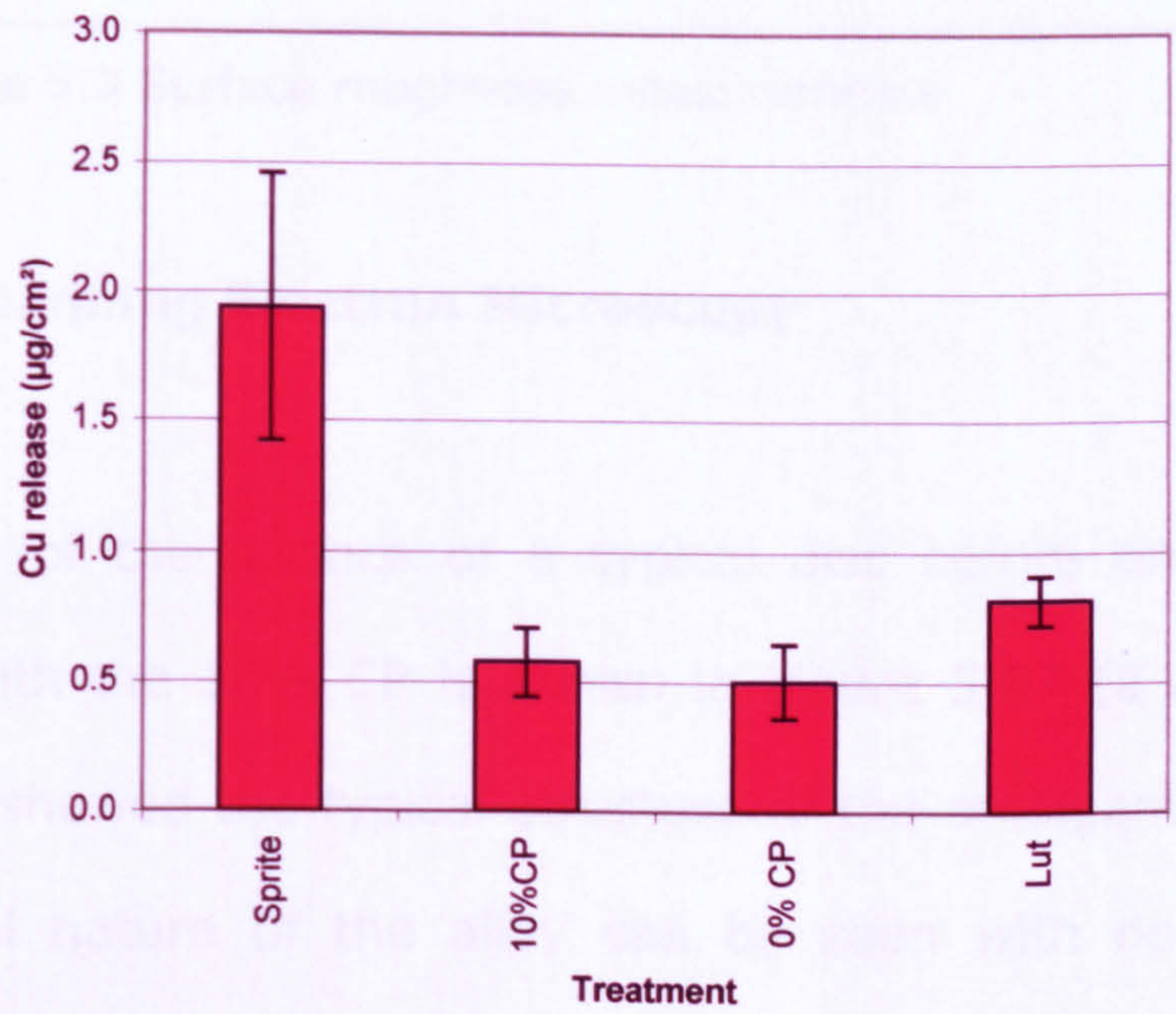


Figure 5.16 Copper ion release from discs following treatment with 10% CP and control materials. Results are the mean of five samples (error bars show standard error of mean)

5.2.1.2 Surface Roughness

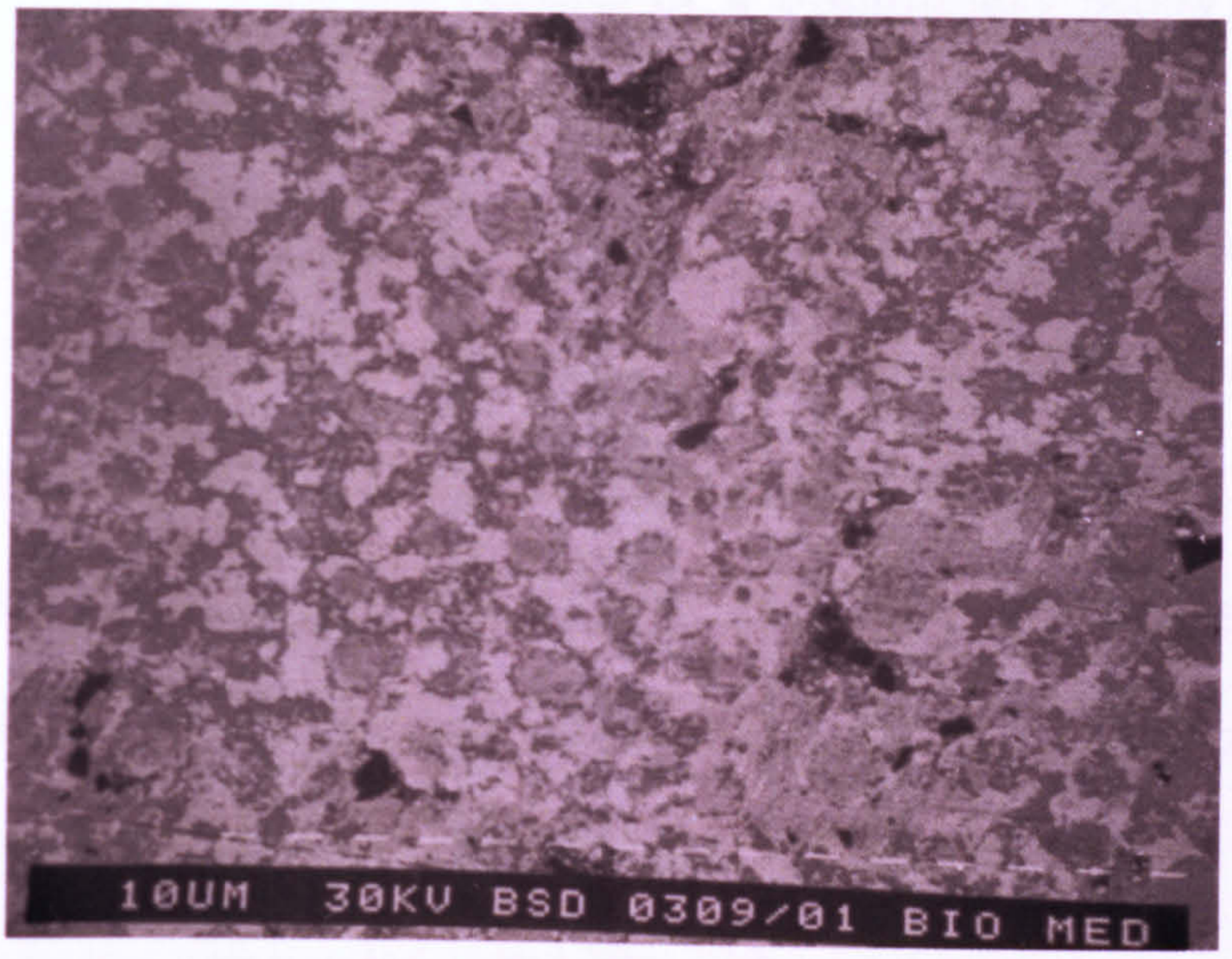
The roughness and standard error of mean values for each group of discs after treatment is shown in Table 5.3. There were no statistically significant differences in roughness values between all the groups ($p>0.05$).

	SAMPLE			
	Lutrol Gel	Sprite	10%CP	0 % CP
Roughness(μm)	1.23	1.91	2.23	1.74
(S.E. of mean)	(0.12)	(1.23)	(0.47)	(0.16)

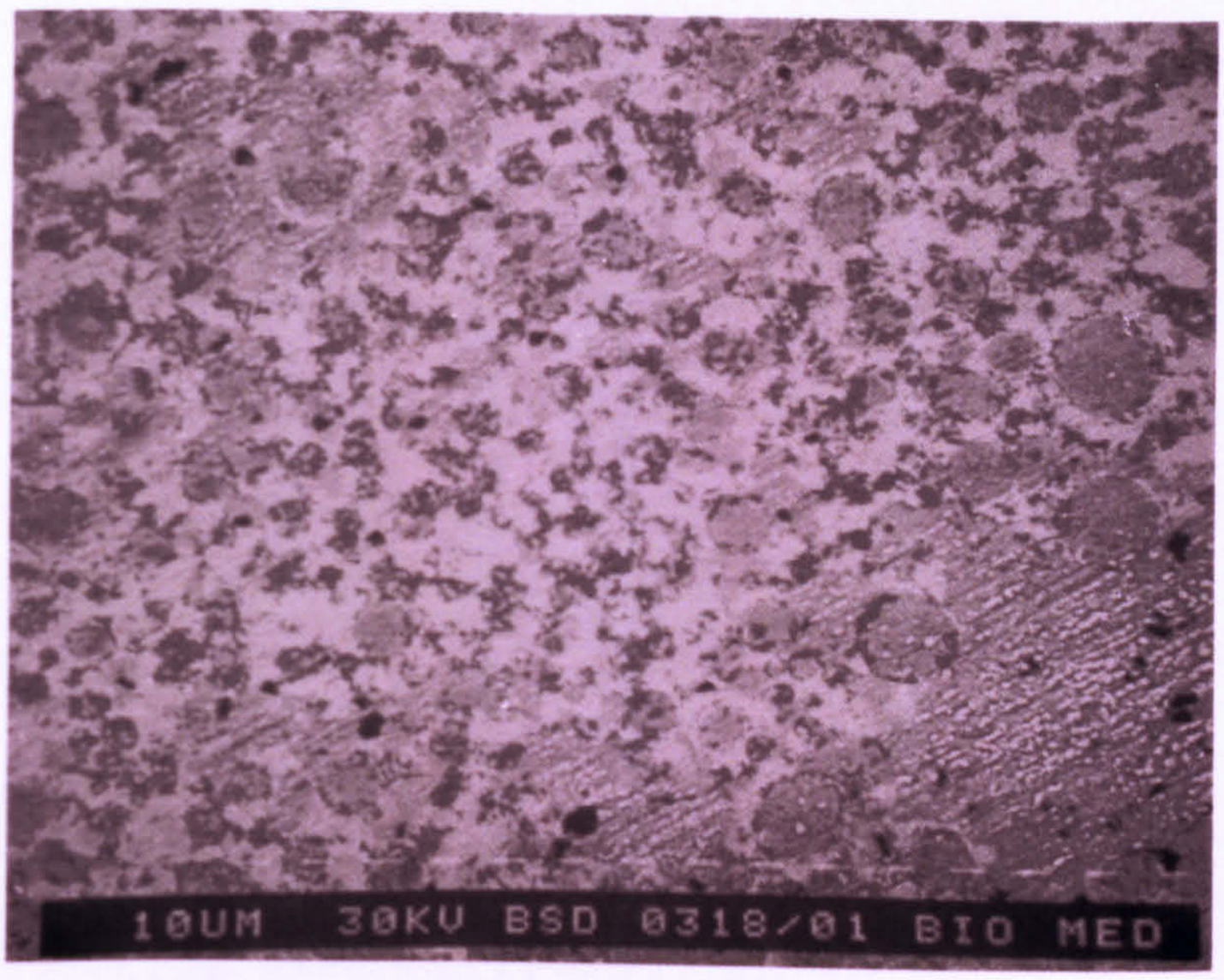
Table 5.3 Surface roughness measurements

5.2.1.3 Scanning Electron Microscopy

SEM images of the surface of a typical disc before and after treatment with the 10% CP is shown in Figure 5.17 (a and b). The images showed the typical structure of the amalgam where the spherical nature of the alloy can be seen with no major differences before and after treatment.



a



b

Fig. 5.17 Scanning electron micrograph showing typical amalgam surface (a) before and (b) after treatment with a CP gel

The above were the results of a preliminary study, thus no data were available for power calculations. Based on these data and using Altman's (161) method of power calculations, a sample of 30 discs would be required to yield an 80% power to find a difference of the magnitude observed for mercury ion release in these data at an alpha of 0.05. The sample size could be reduced by increasing the effect of the bleaching agent (for example by using a higher concentration) and by reducing the variability between samples (for instance by standardising surface preparation).

5.2.2 The Use of LA-ICP-MS for Testing Amalgam Samples

The intensity of the various elements released from the surface of each disc after application of LA-ICP-MS was continuously measured in counts per second (CPS) as the laser beam traversed the surface of the disc. For each element, 333 analyses were taken in the central portion (plateau of each curve) shown in Figure 5.18. The data were statistically analysed and values of mean and standard deviation for each element are shown in Table 5.4 for all the groups. The highest ion releases were those for mercury, followed by silver and tin and finally copper. The LA-ICP-MS technique proved suitable for determining the relative levels of ion emissions of the various metals. The main limitation of this technique, at present, was that no quantitative data was obtained. Clearly further work is needed to overcome this problem.

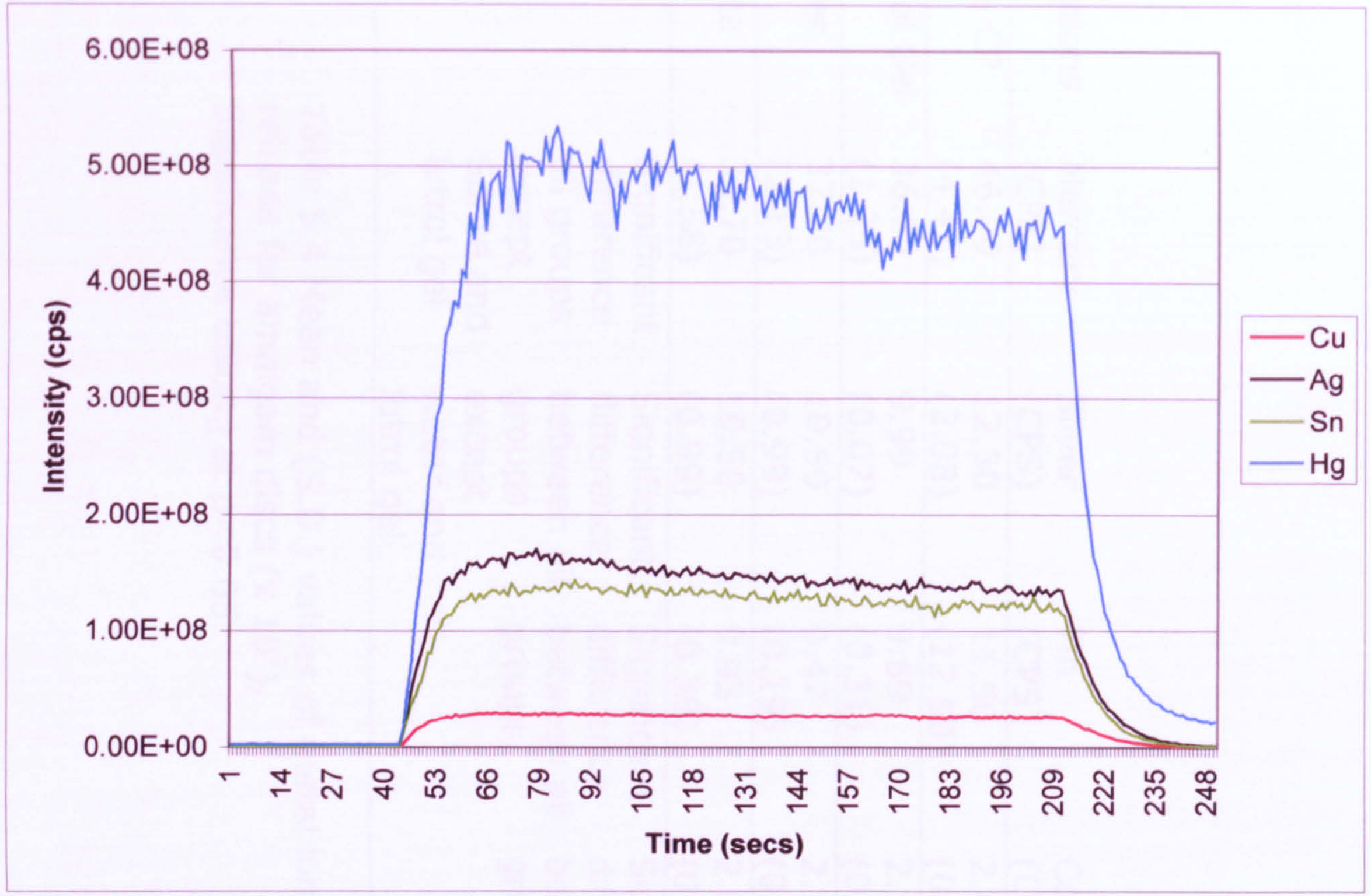


Figure 5.18 Line spectra for analysis of an amalgam disc treated with 10% CP

Solutions	Mercury (CPS)	Silver (CPS)	Tin (CPS)	Copper (CPS)
10% CP	46.00 (4.41)	12.30 (2.08)	11.50 (12.50)	2.48 (0.19)
Lutrol Gel	36.60 (1.25)	9.99 (0.07)	9.69 (0.31)	2.10 (0.07)
Water	37.70 (2.13)	10.50 (0.99)	9.47 (0.19)	2.10 (0.13)
Sprite	36.70 (1.58)	10.50 (0.99)	9.93 (0.38)	2.04 (0.06)
	Significant difference all groups except Sprite and lutrol gel	Significant difference between all groups except water and lutrol gel	Significant difference between all groups	Significant difference between all groups

Table 5.4 Mean and (S.D.) values of metal ion release for amalgam discs (x 10^7).
Significance testing at $p<0.05$

Following from the preliminary work on bleaching amalgam discs with 10% CP, a more comprehensive study was designed to test the effect of varying HP concentrations (0%-30% w/v) on metal ion release from dental amalgam. Samples were taken from the actual HP solutions, rather than the eluents (washings) for ICP-MS analysis. Additionally, a protocol was set in place for sample preparation.

5.2.3 Bleaching Amalgam Discs with Varying Concentrations of Hydrogen Peroxide (0-30% w/v)

5.2.3.1 Ion Release

The recorded ion release data in µg/l at different HP concentrations are presented in Figure 5.19. Values of the mean and standard deviation of elemental ion release data were also converted to units of µg/cm^2 and are shown in Table 5.5, at 1%, 3%, 10% and 30% HP concentrations. This was obtained by dividing the total amount of ion release in µg over a 24 h period for each element by the total surface area of the corresponding disc.

For all elements, metal ion release increased with increasing hydrogen peroxide concentration (Figure 5.19). The highest ion releases were those for mercury followed closely by silver then tin and finally copper. The distribution of the recorded ion release data did not follow a normal distribution and was positively skewed for all elements. Accordingly, it was decided to analyse the data using a natural logarithmic transformation. The normality was explored graphically using a histogram, normal probability plot and a detrended normal plot (Figures 5.20 to 5.22 for mercury data). These graphs suggest that the variable, mercury ion release, is significantly positively skewed. Following the transformation, Figure 5.23 shows the variables falling more closely onto the straight line and Figure 5.24 shows a closer clustering of points around a horizontal line through zero. Figure 5.25 shows the transformed histogram following a more normal distribution. Clearly, a natural logarithmic transformation proved to be an appropriate choice. The same process was used to assess normality of recorded copper, silver and tin metal ion releases. Figures 5.26 (a and b) to 5.28 (a and b) show the distributions of these elements before and after a natural logarithmic transformation. All elements exhibited a more normal distribution following the transformations thus justifying the decision to use such a transformation. The p-

values for the One-Way ANOVA and Dunnets Post Hoc test are shown in Table 5.6. The difference in metal ion release between 0% HP (control) and all other concentrations (1%, 3%, 10% and 30%) was statistically significant ($p<0.05$) for all elements. Additionally, for tin, there was a significant change in ion release data every time the HP concentration changed. For mercury, silver and copper there was a significant difference in ion release between 30% and each of 1% and 3% but, no significant difference between 30% and 10% HP concentration.

5.2.3.2 Surface Roughness

Values of the average surface roughness and standard deviation for each group before and after treatment is shown in Figure 5.29. A paired t-test showed no significant difference ($p>0.05$) in mean surface roughness values before and after bleaching at all concentrations.

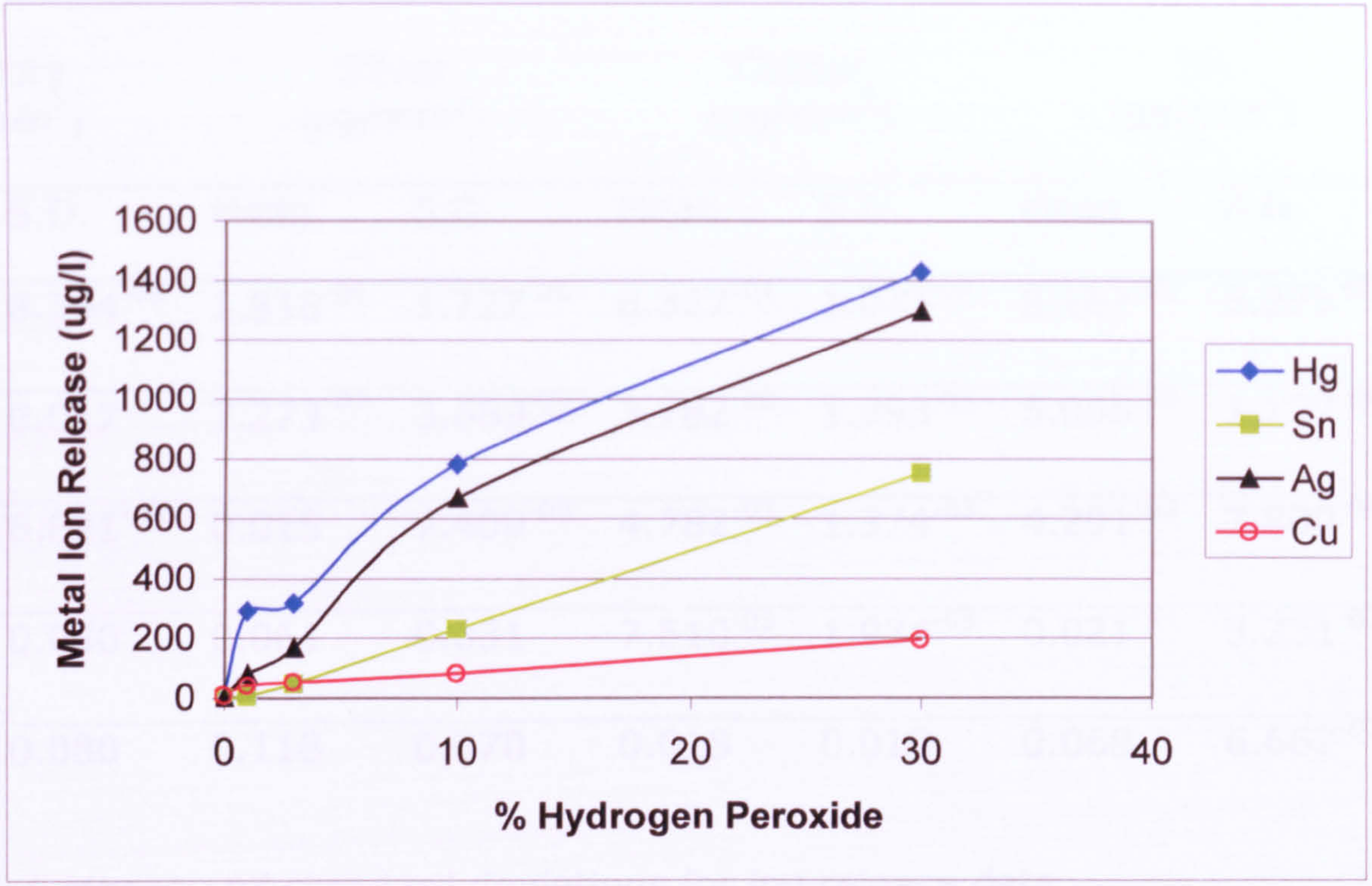


Figure 5.19 The effect of HP concentration on metal ion release

	Mercury ($\mu g/mm^2$)		Silver ($\mu g/mm^2$)		Copper ($\mu g/mm^2$)		Tin ($\mu g/mm^2$)	
[HP]	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0%	2.455^{-04}	8.364^{-05}	1.818^{-06}	1.727^{-05}	8.327^{-04}	1.073^{-04}	8.182^{-05}	3.273^{-05}
1%	0.033	0.017	7.273^{-03}	3.689^{-03}	3.782^{-03}	1.393^{-03}	5.055^{-04}	1.127^{-04}
3%	0.029	6.821^{-03}	0.015	7.409^{-03}	4.782^{-03}	1.374^{-03}	4.291^{-03}	7.800^{-04}
10%	0.071	0.040	0.061	0.031	7.510^{-03}	1.934^{-03}	0.021	3.231^{-03}
30%	0.130	0.080	0.118	0.070	0.018	0.010	0.068	6.662^{-03}

Table 5.5 Means and standard deviations for ion release data

Transformed p values (unequal variance)																
	Mercury				Silver				Copper				Tin			
[HP]	1%	3%	10%	30%	1%	3%	10%	30%	1%	3%	10%	30%	1%	3%	10%	30%
0%	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.001	0.001	<0.001	<0.001	<0.001
1%	-	1.000	0.623	0.047	-	0.279	0.005	<0.001	-	0.868	0.036	0.009	-	<0.001	<0.001	<0.001
3%	1.000	-	0.437	0.028	0.279	-	0.049	0.003	0.868	-	0.212	0.023	<0.001	-	<0.001	<0.001
10%	0.623	0.437	-	0.749	0.005	0.049	-	0.617	0.036	0.212	-	0.161	<0.001	<0.001	-	<0.001

Table 5.6 Multiple comparisons – p - values

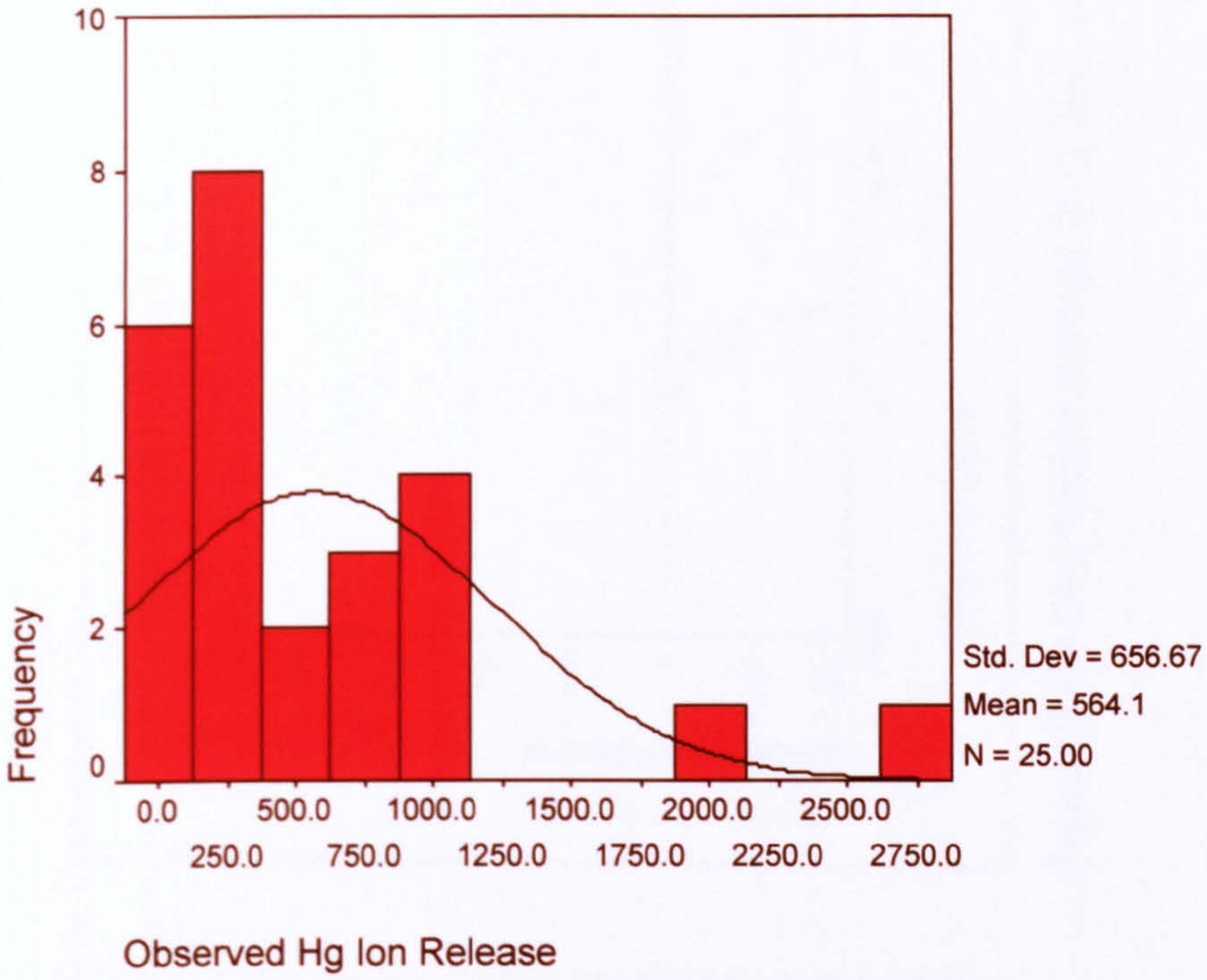


Figure 5.20 Mercury ion release – untransformed data

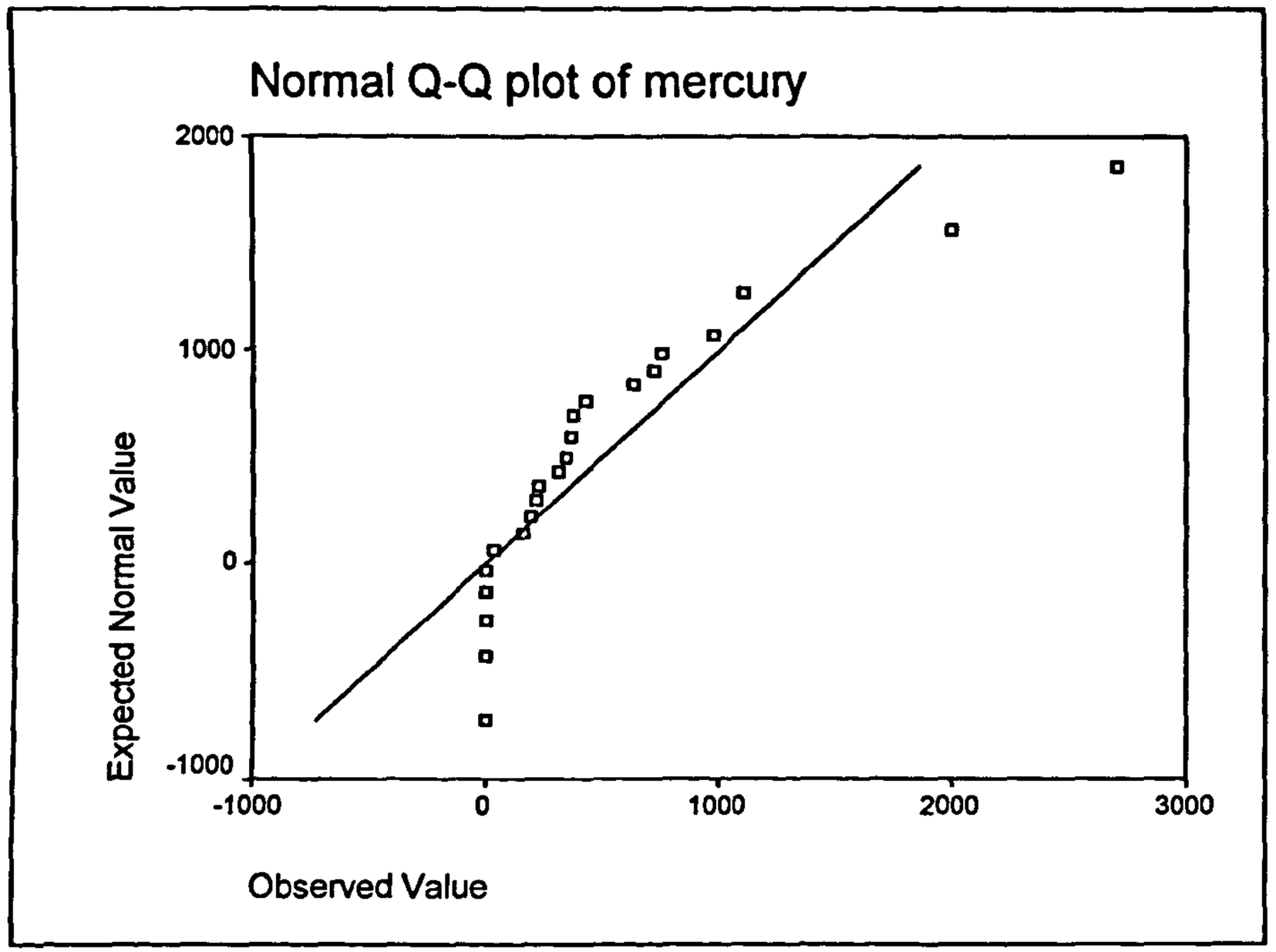


Figure 5.21 Normal Q-Q plot, observed value

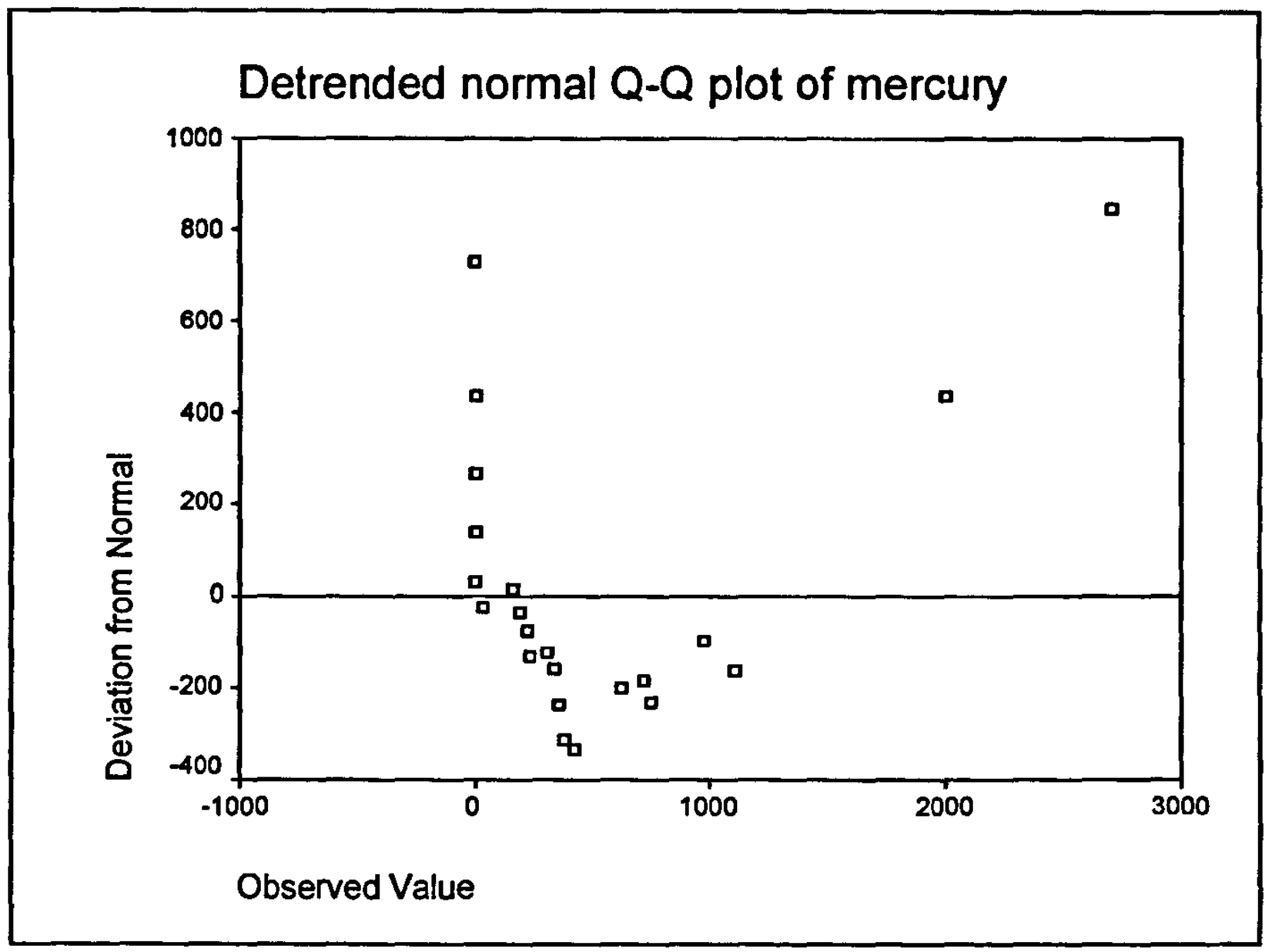


Figure 5.22 Detrended normal Q-Q plot, observed value

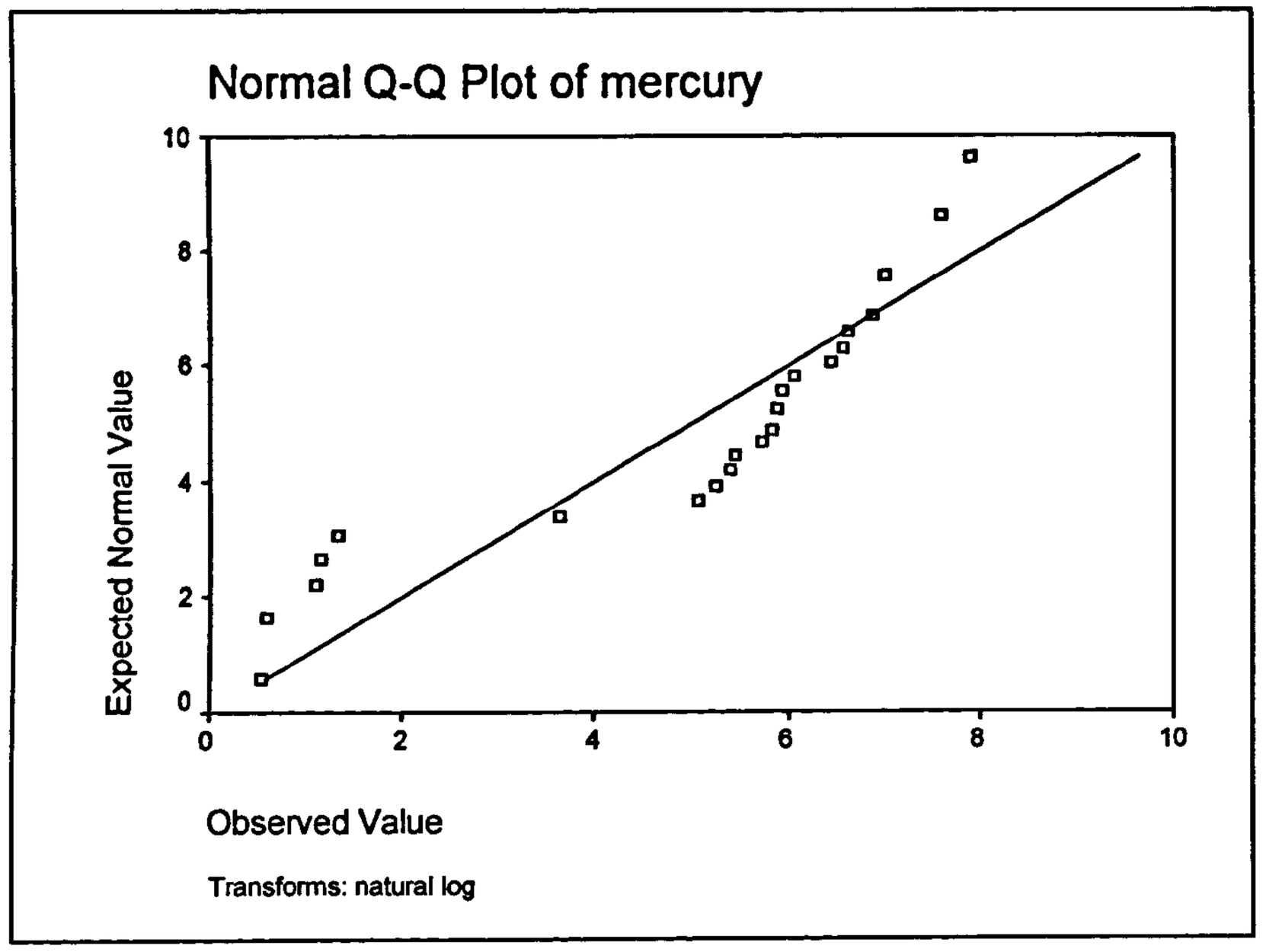


Figure 5.23 Normal Q-Q plot, transformed natural log

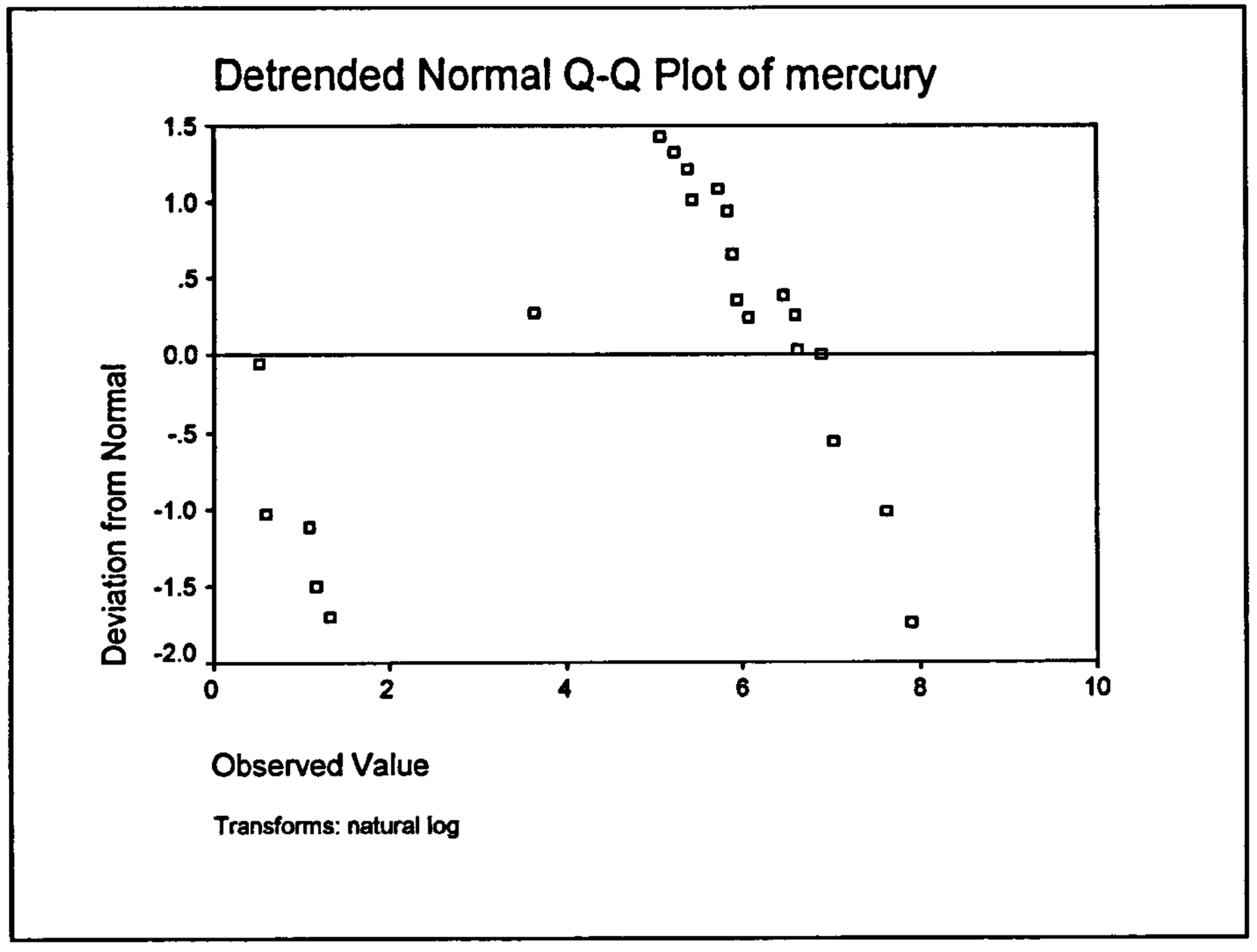


Figure 5.24 Detrended normal Q-Q plot, transformed natural log

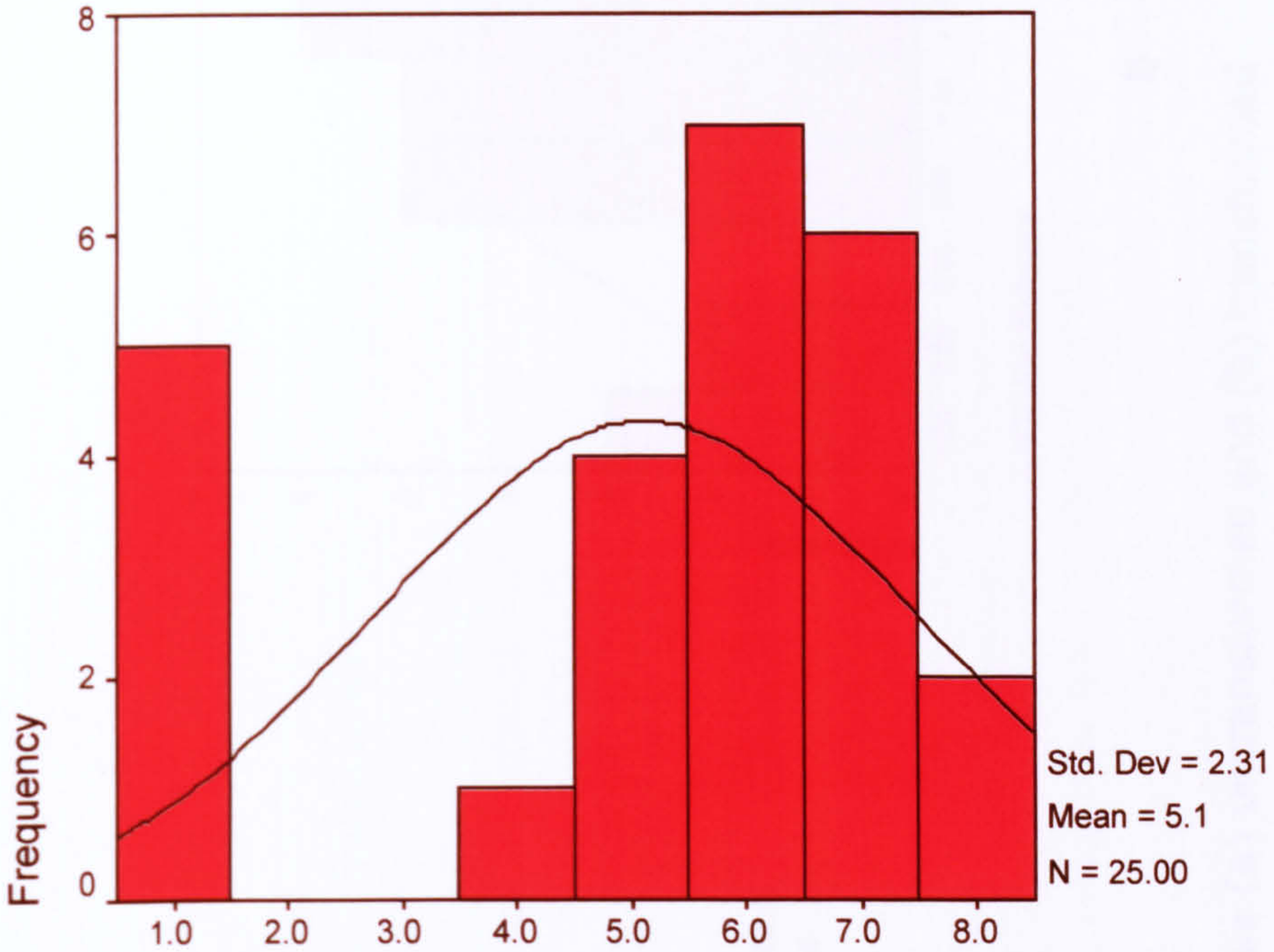
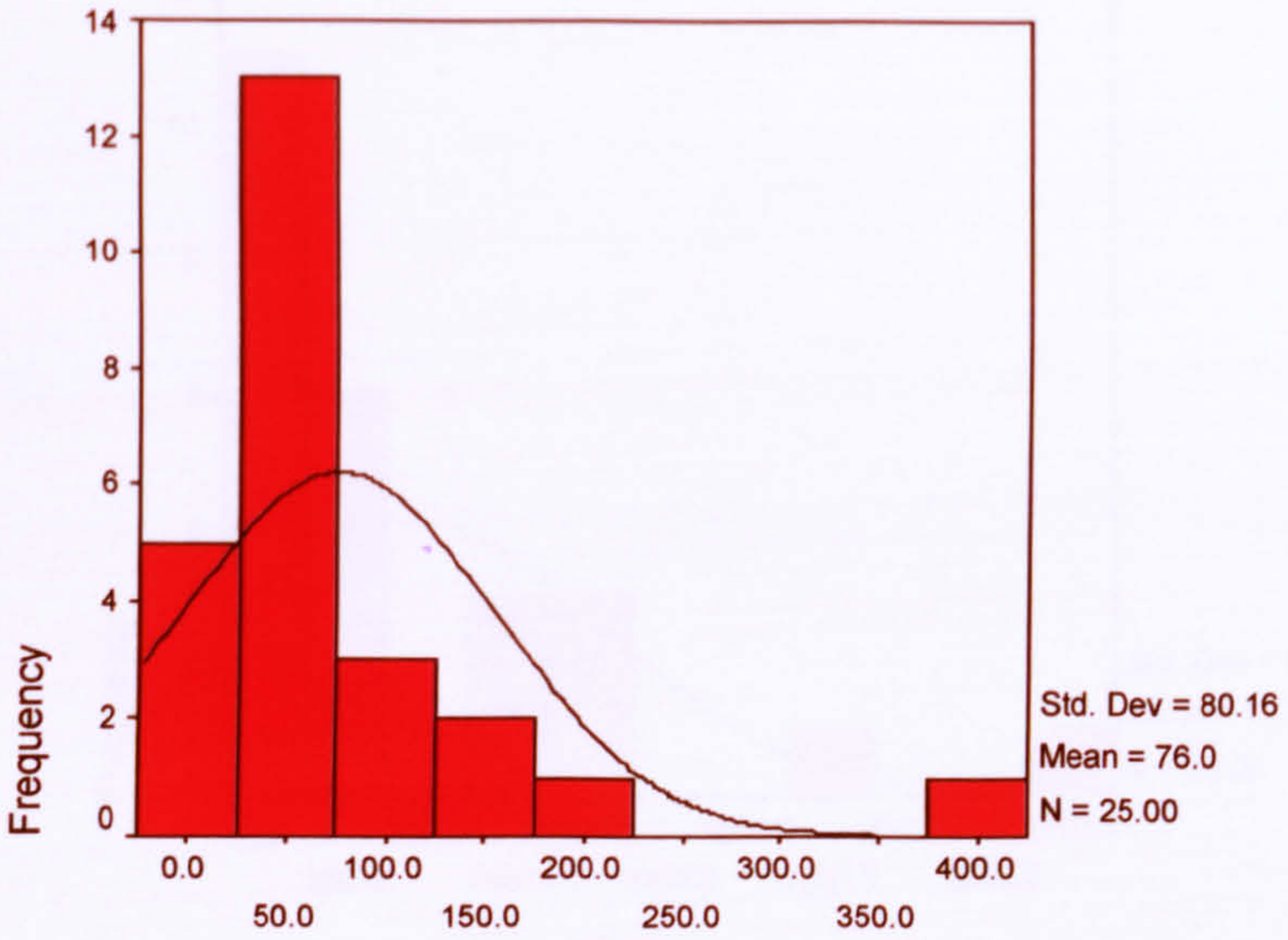
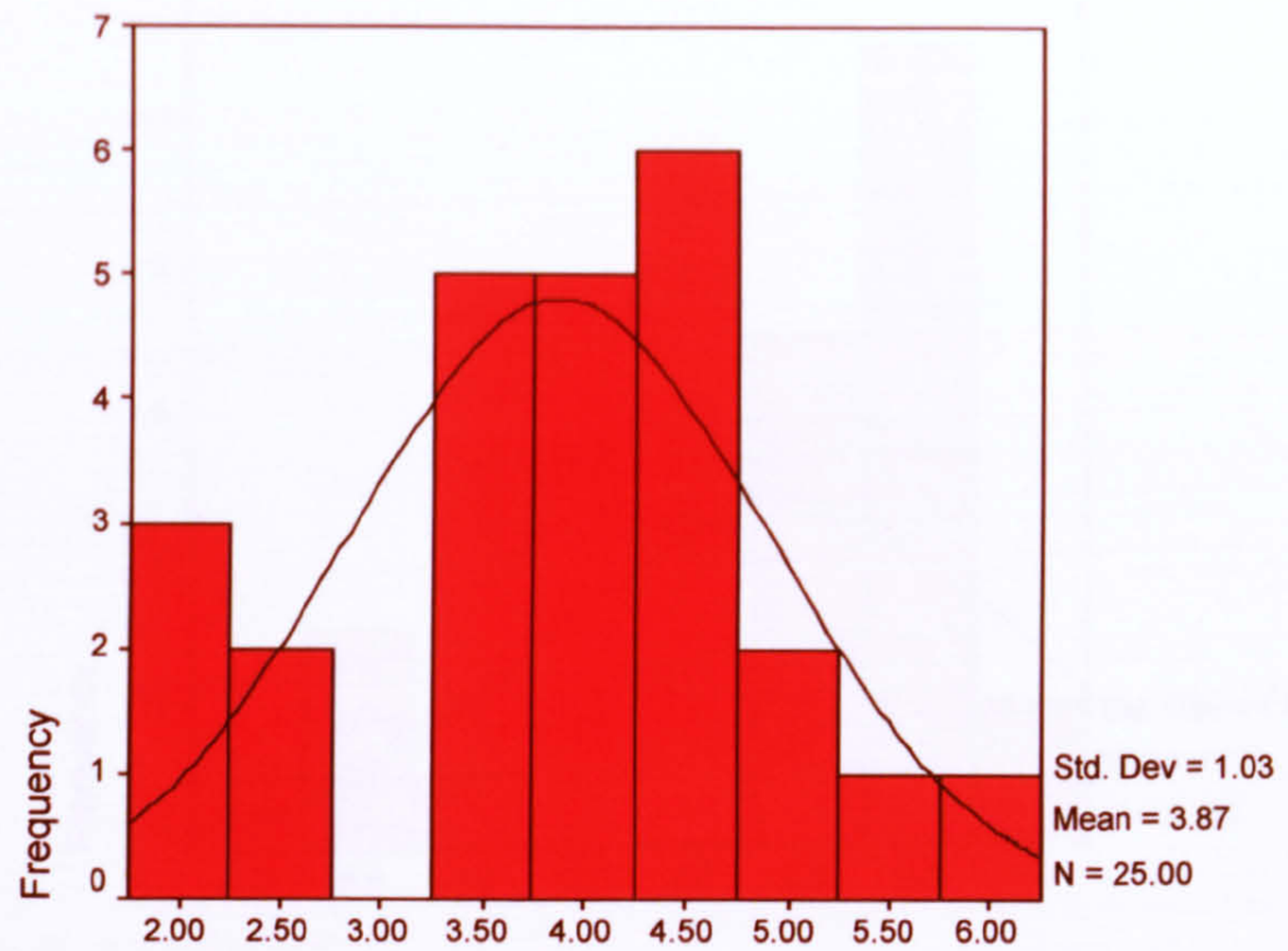


Figure 5.25 Mercury ion release, transformed natural log

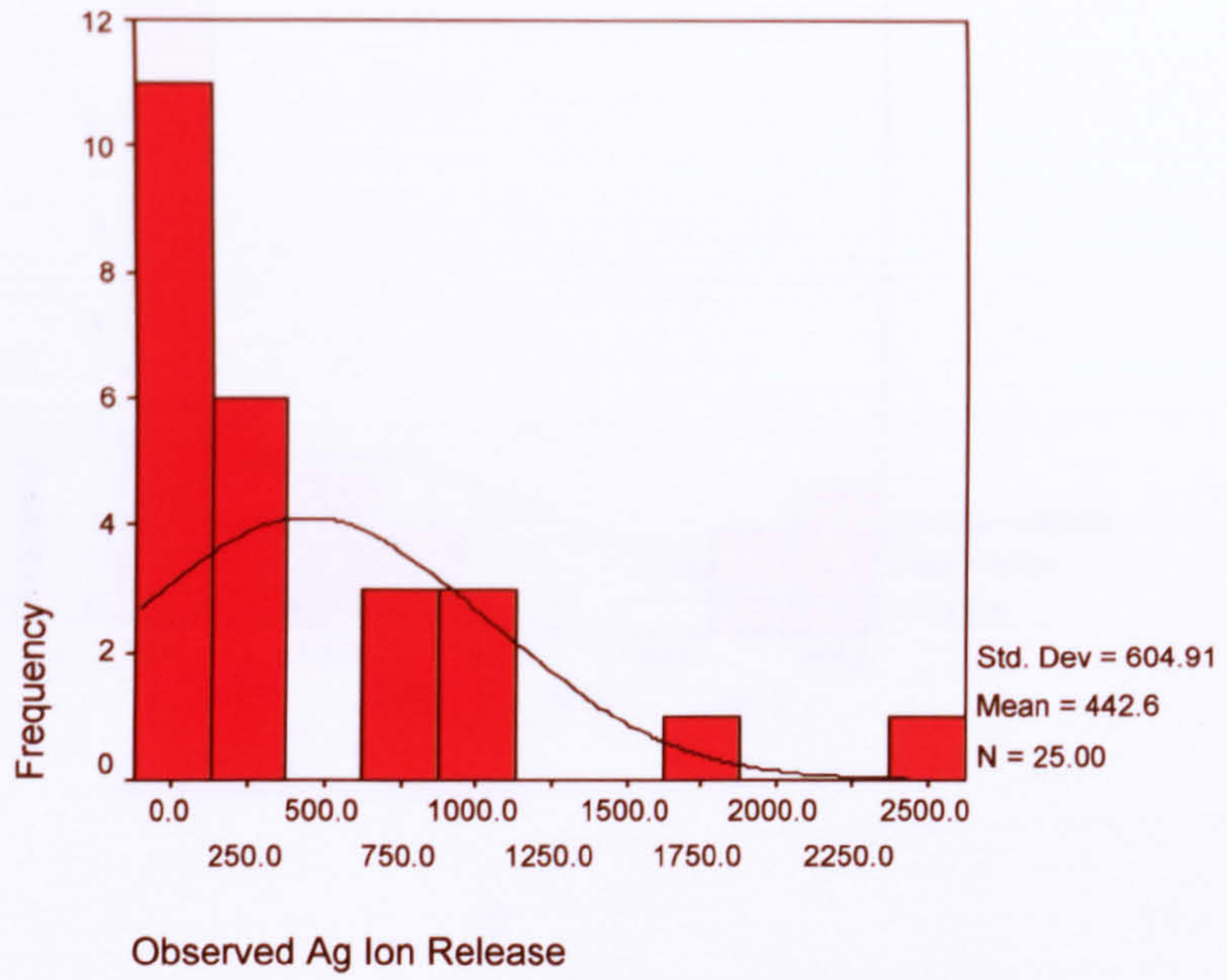


a

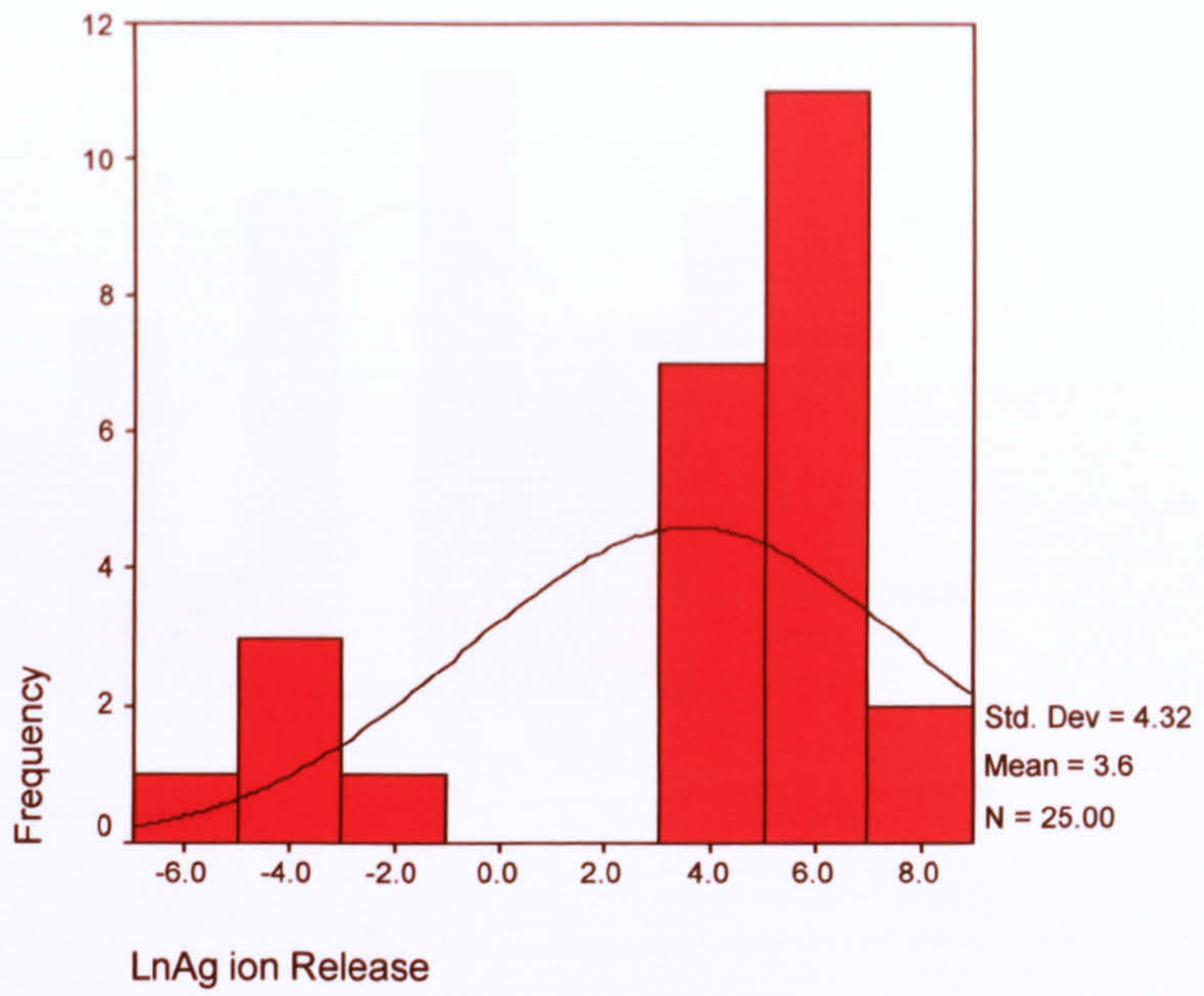


b

Figure 5.26 Copper ion release (a) untransformed and (b) transformed

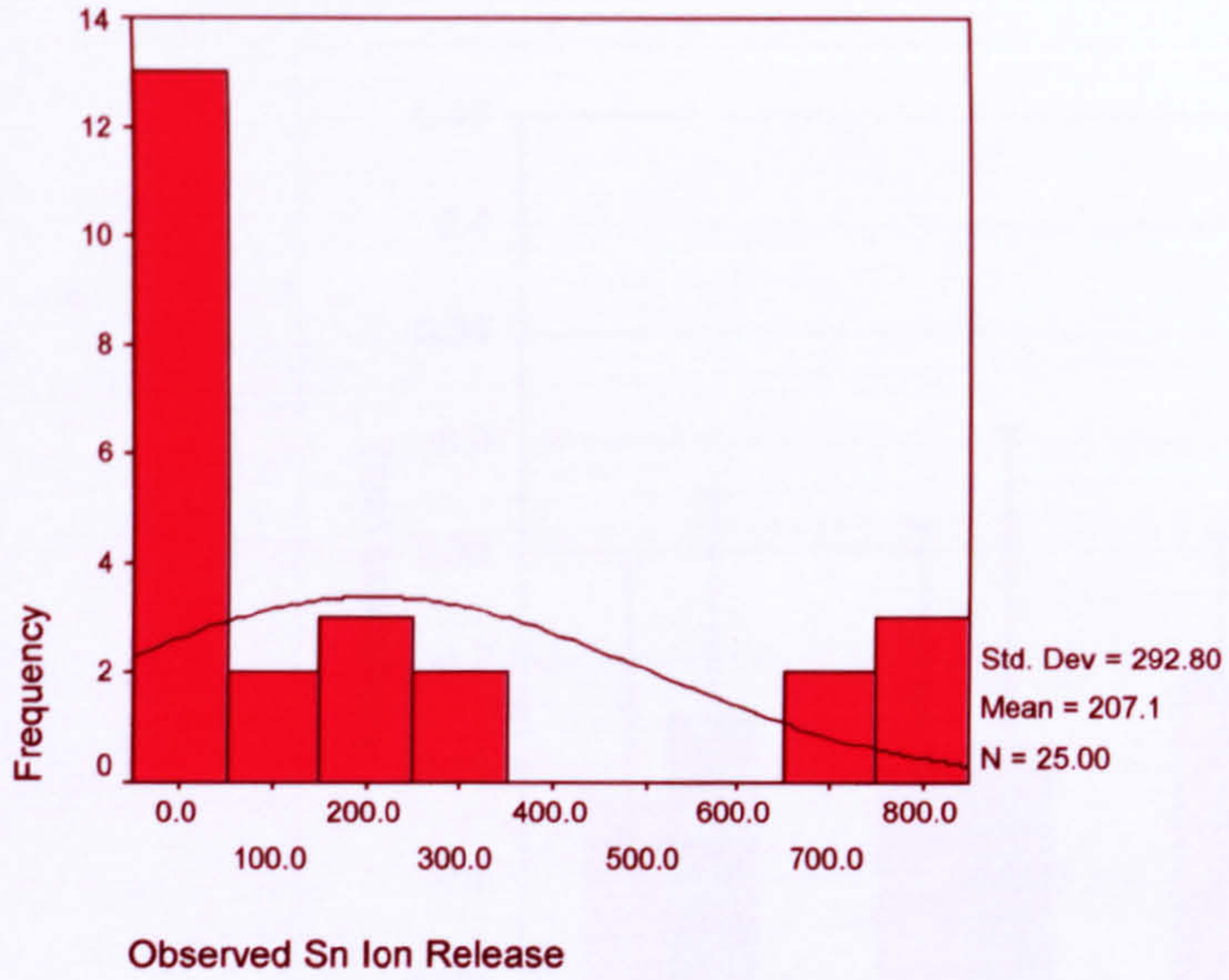


a

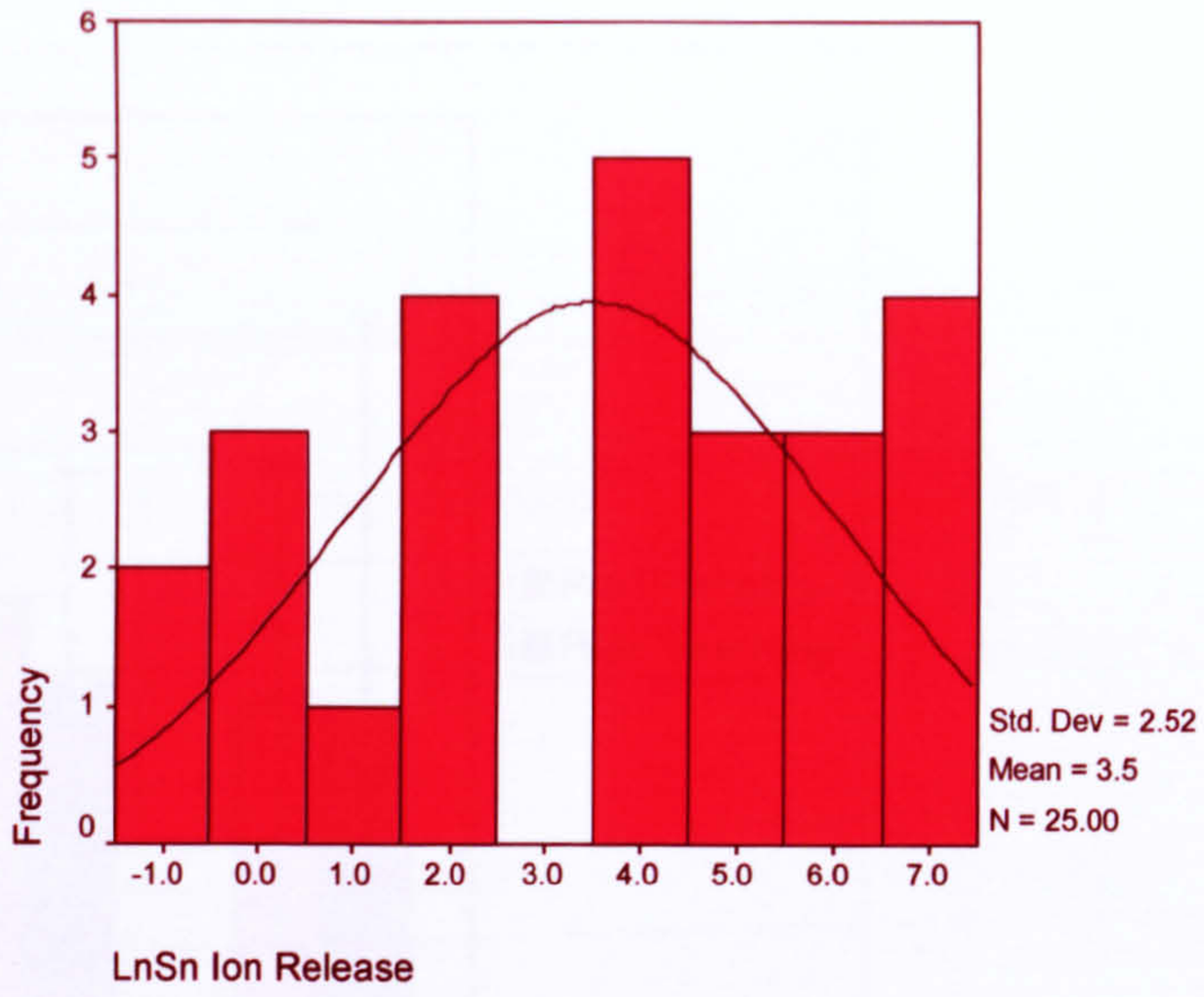


b

Figure 5.27 Silver ion release (a) untransformed and (b) transformed



a



b

Figure 5.28 Tin ion release (a) untransformed and (b) transformed

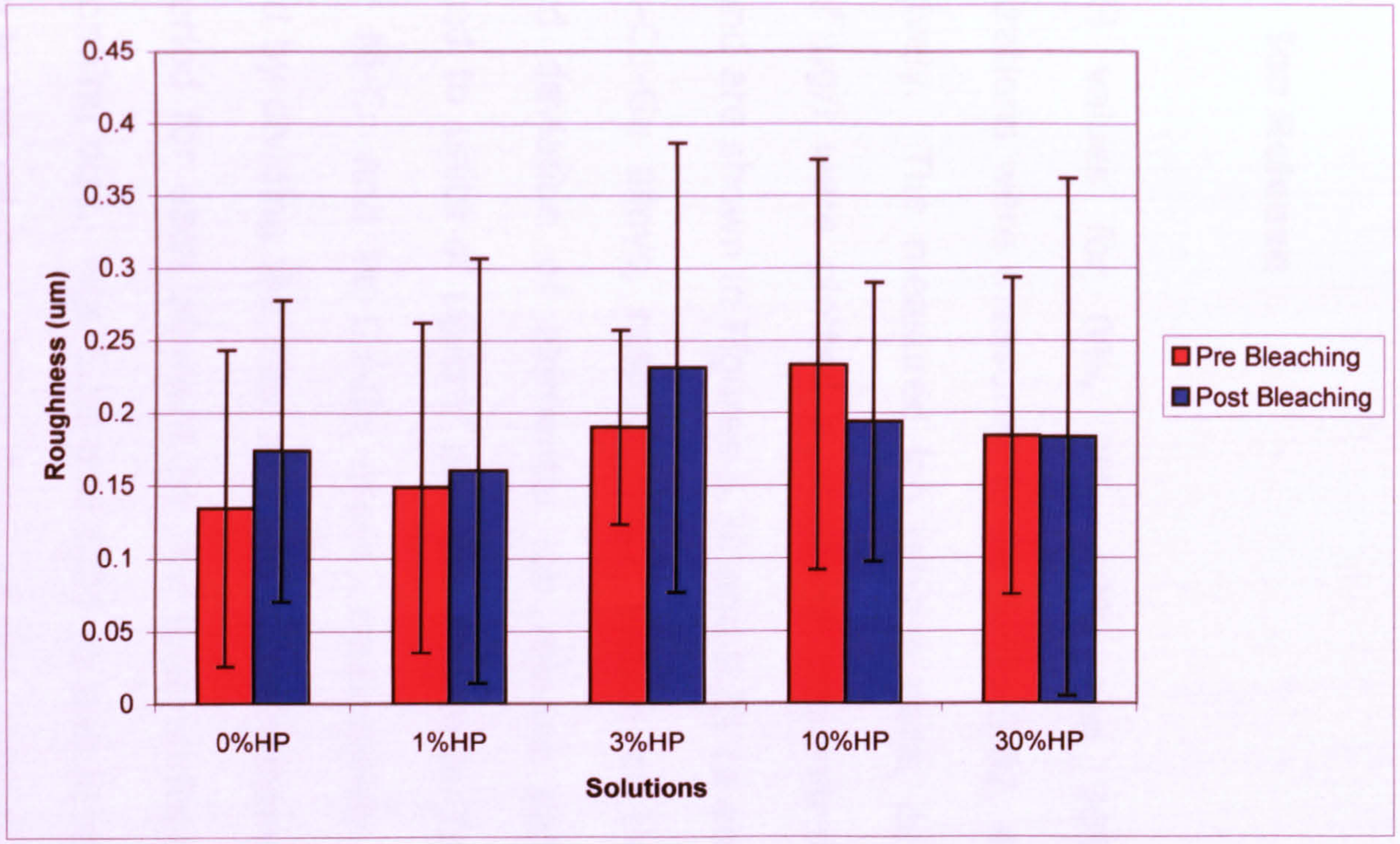


Figure 5.29 Roughness measurements before and after bleaching

5.3 Bleaching Dental Casting Alloys with Varying Concentrations of Hydrogen Peroxide (0-30% w/v)

5.3.1 Ion Release

The pH values for 0%, 3%, 10% and 30% HP (w/v) concentrations were measured to be 6.41, 5.62, 4.75 and 3.83 respectively. The measured ion release data, as recorded, in units of µg/l were plotted against HP concentration for both alloys and are shown in Figures 5.30 and 5.31 (a and b) for Ni-Cr and Pd-Cu-Ga alloys, respectively. Values of the mean and standard deviation of elemental ion release data were also converted to units of $\mu g/cm^2$ and are shown in Tables 5.7 and 5.8 for Ni-Cr and Pd-Cu-Ga alloys, respectively. This were obtained by dividing the total amount of ion release in µg over a 24 h period for each element by the total surface area of the corresponding disc. Figs 5.30 and 5.31 (a and b) show a steady increase in ion release values from all the constituent elements of the two alloys, except gold, with increasing HP concentration. The constituents with percentage weight of 1% or less in the two alloys were not included in the above graphs and Tables. For each sample tested, the ICP-MS recorded ion release values of

elements in the periodic table present in the particular sample. It was noted that the mean ion release value of Pd from the Ni-Cr alloy was 0.00015 μg/cm^2 and that of Ni from the Pd-Cu-Ga alloy was 0.002 μg/cm^2 at 30% HP concentration. These values are negligibly small which in turn adds weight to the accuracy of the recorded data.

The distribution of the recorded ion release data was normal for all elements. The p-values for the One-Way ANOVA and Dunnets Post Hoc test are shown in Tables 5.9 and 5.10 for Ni-Cr and Pd-Cu-Ga alloys, respectively. The difference in metal ion release between 0% HP (control) and all other concentrations (3%, 10% and 30%) was statistically significant ($p<0.05$) for all elements except gold (the p-values for gold are not included in Table 5.10). Additionally, there was a significant change in ion release data at each increase in HP concentration except for Mo (Table 5.10) where there was no significant difference ($p>0.05$) when HP concentration was increased from 10% to 30% and indium (Table 5.10) when HP concentration changed from 3% to 10%.

5.3.2 Surface Roughness

The average roughness values for Ni-Cr and Pd-Cu-Ga alloys before and after treatment is shown in Figures 5.32 and 5.33. Paired t-tests showed no significant difference ($p>0.05$) in mean surface roughness values before and after bleaching in all the groups.

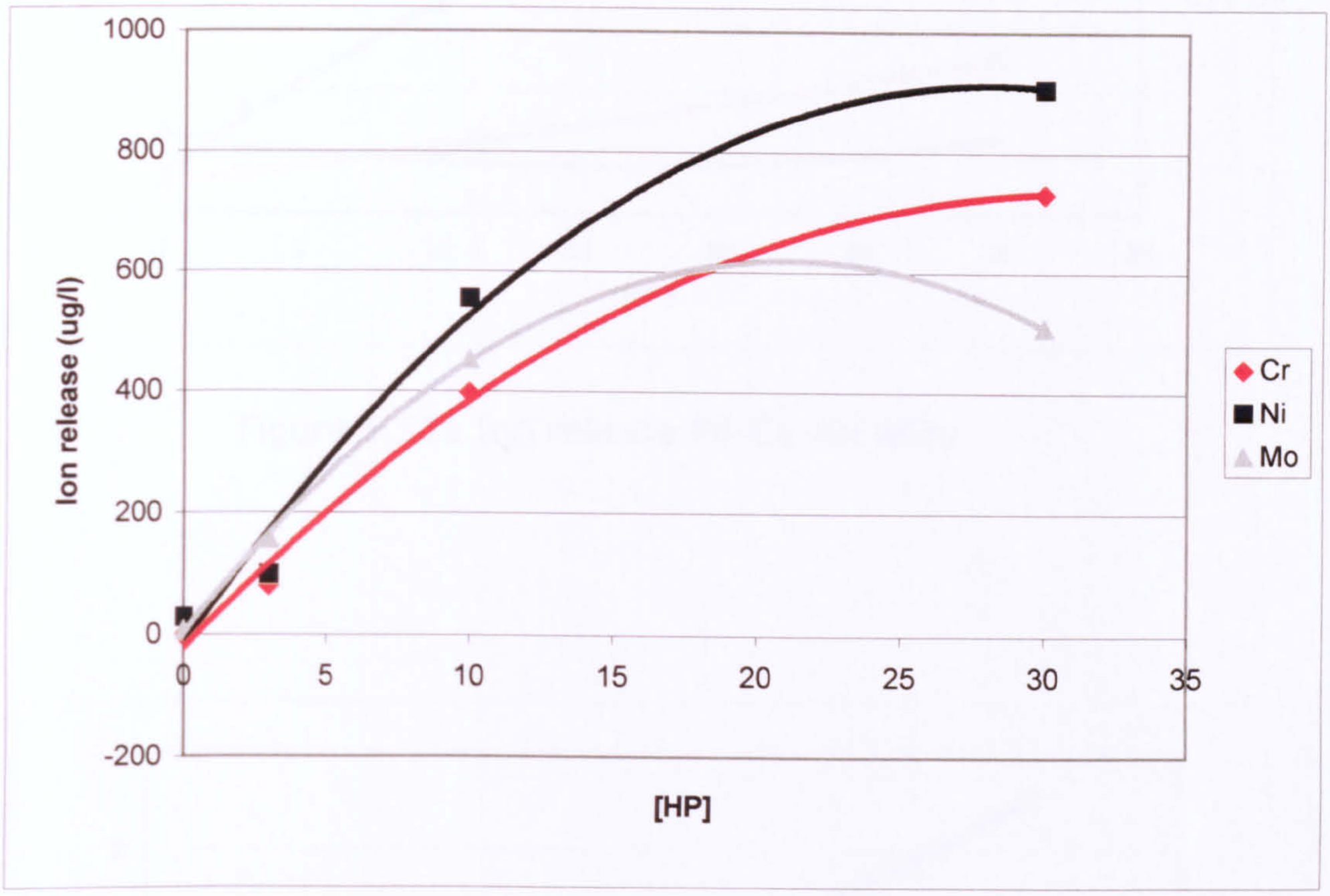


Figure 5.30 Ion release Ni-Cr alloy

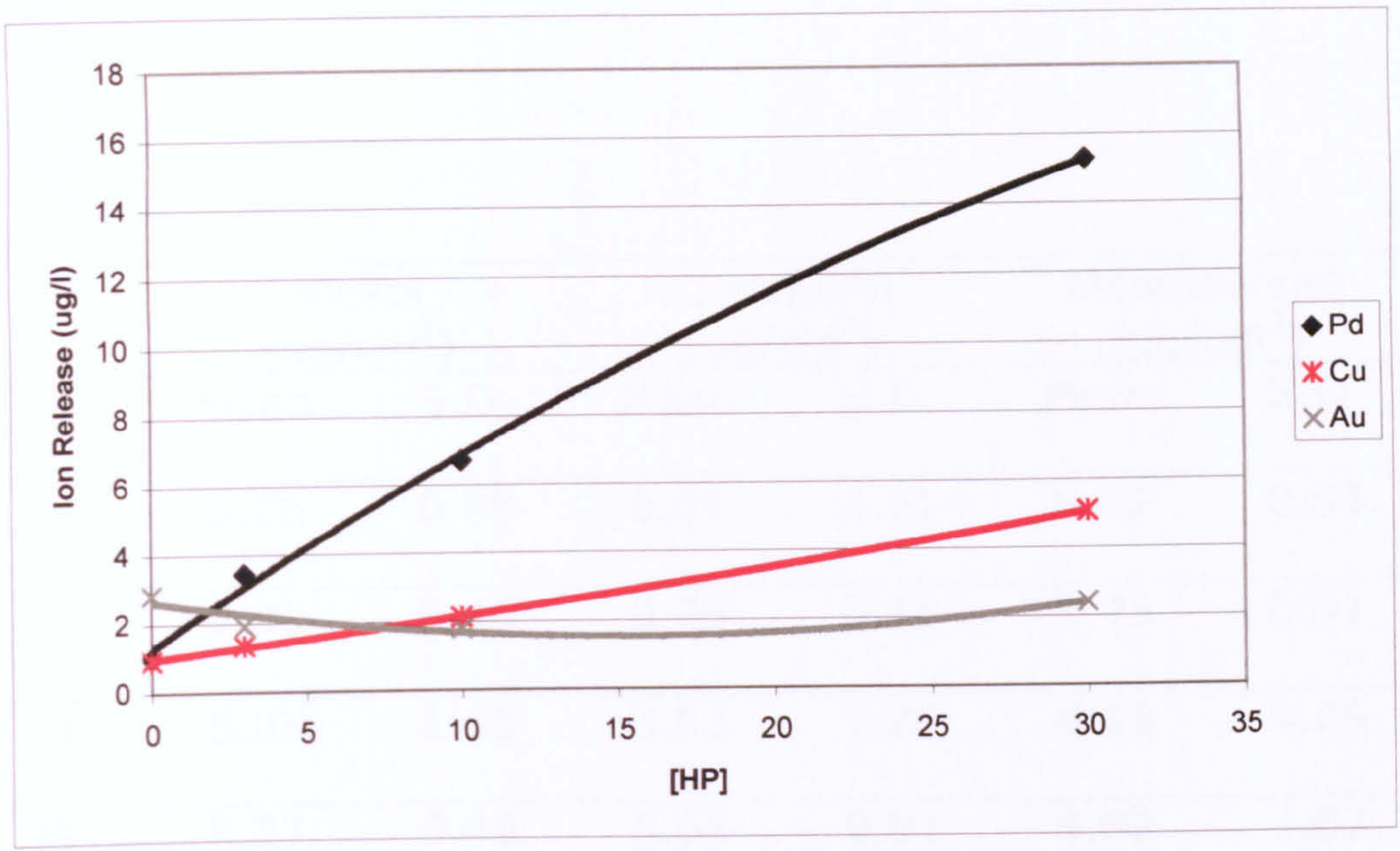


Figure 5.31a Ion release Pd-Cu-Ga alloy

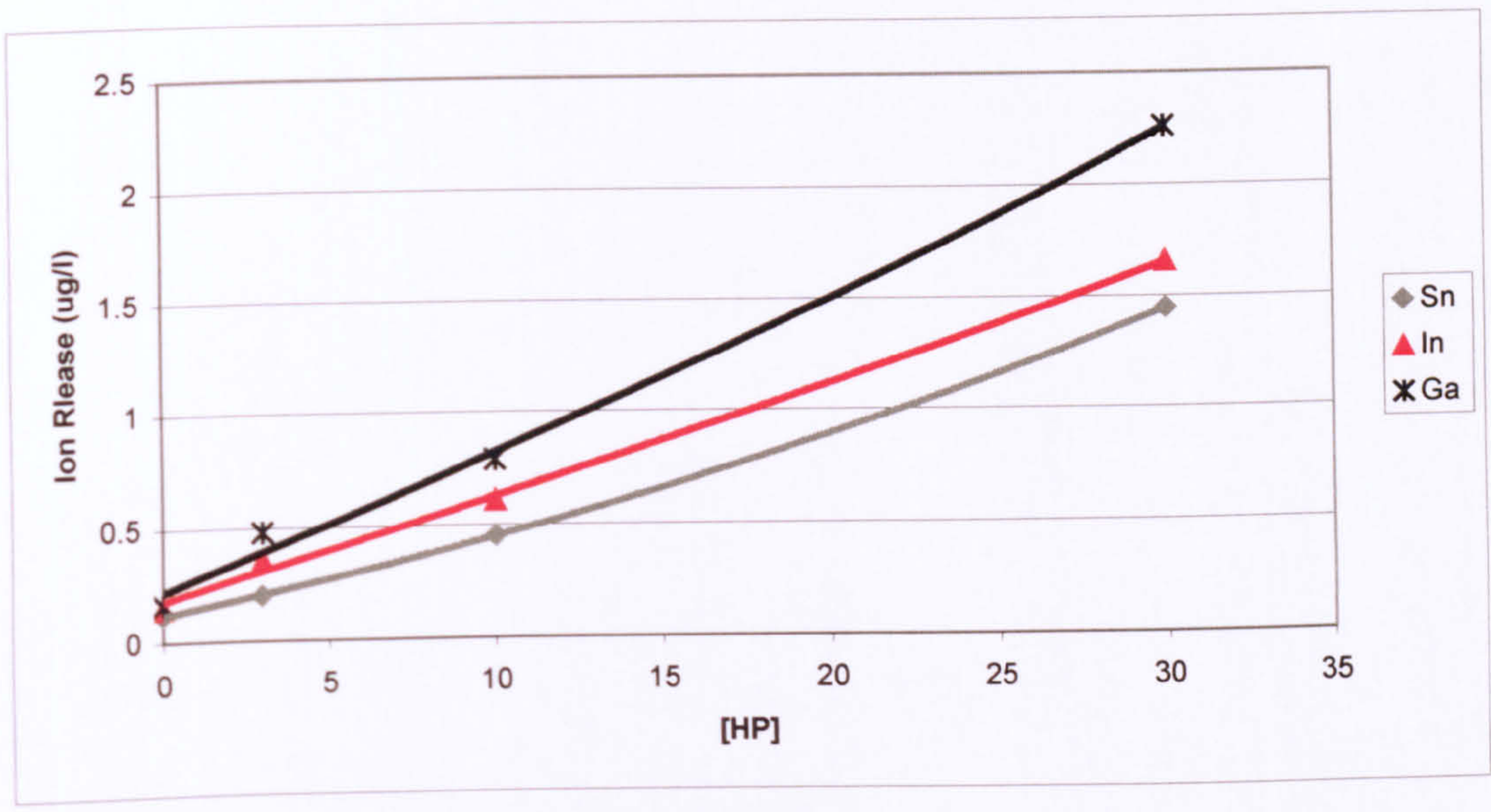


Figure 5.31b Ion release Pd-Cu-Ga alloy

	Nickel ($\mu g/cm^2$)		Chromium ($\mu g/cm^2$)		Molybdenum ($\mu g/cm^2$)	
[HP]	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	0.26	0.08	0.01	0.01	0.07	0.03
3	0.90	0.16	0.75	0.11	1.43	0.31
10	5.05	1.50	3.63	1.21	4.11	0.76
30	8.23	0.49	6.65	0.91	4.62	1.07

Table 5.7 Mean and (S.D.) Ni-Cr alloy

	Palladium (µg/cm^2)		Copper (µg/cm^2)		Gallium (µg/cm^2)		Indium (µg/cm^2)		Tin (µg/cm^2)		Gold (µg/cm^2)	
[HP]	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	9.90^{-03}	2.70^{-03}	1.70^{-02}	2.42^{-03}	3.04^{-03}	7.27^{-04}	2.91^{-03}	8.91^{-04}	2.24^{-03}	3.27^{-04}	0.05	0.02
3	0.06	7.82^{-03}	2.48^{-02}	4.91^{-03}	8.73^{-03}	1.64^{-03}	6.55^{-03}	1.55^{-03}	3.69^{-03}	4.55^{-04}	0.04	5.42^{-03}
10	0.12	0.01	0.04	6.73^{-03}	0.01	3.05^{-03}	0.01	3.55^{-03}	8.24^{-03}	1.30^{-03}	0.03	5.29^{-03}
30	0.28	0.02	0.09	0.01	0.04	6.55^{-03}	0.03	0.01	0.26	2.05^{-03}	0.04	7.38^{-03}

Table 5.8 Mean and standard deviation Pd-Cu-Ga alloy

P values (unequal variance)									
	Nickel			Chromium			Molybdenum		
[HP]	3	10	30	3	10	30	3	10	30
0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3	-	<0.001	<0.001	-	<0.001	<0.001	-	<0.001	0.001
10	<0.001	-	<0.001	<0.001	-	<0.001	<0.001	-	0.840

Table 5.9 Multiple comparisons – p-values for Ni-Cr alloy

P values (unequal variance)															
	Palladium			Copper			Tin			Indium			Gallium		
[HP]	3	10	30	3	10	30	3	10	30	3	10	30	3	10	30
0	<0.001	<0.001	<0.001	0.026	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.003	0.003	<0.001	<0.001	<0.001
3	-	<0.001	<0.001	-	0.005	<0.001	-	<0.001	<0.001	-	0.069	0.005	-	0.011	<0.001
10	<0.001	-	<0.001	0.005	-	<0.001	<0.001	-	<0.001	0.069	-	0.015	0.011	-	<0.001

Table 5.10 Multiple comparisons- p-values for Pd-Cu-Ga alloy

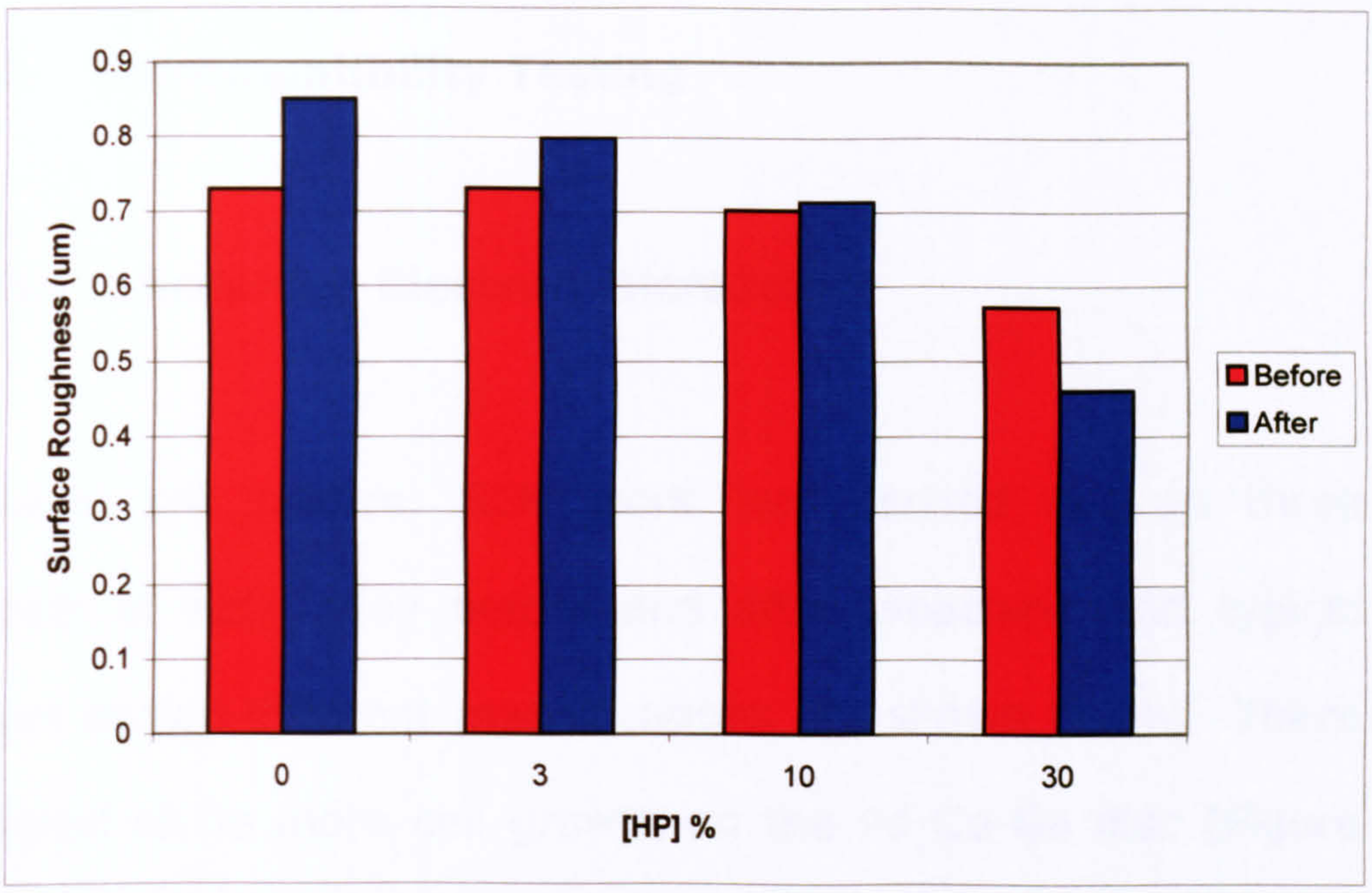


Figure 5.32 Mean surface roughness values for Ni-Cr alloy. Paired t-tests showed no significant differences ($p>0.05$) in surface roughness values before and after bleaching.

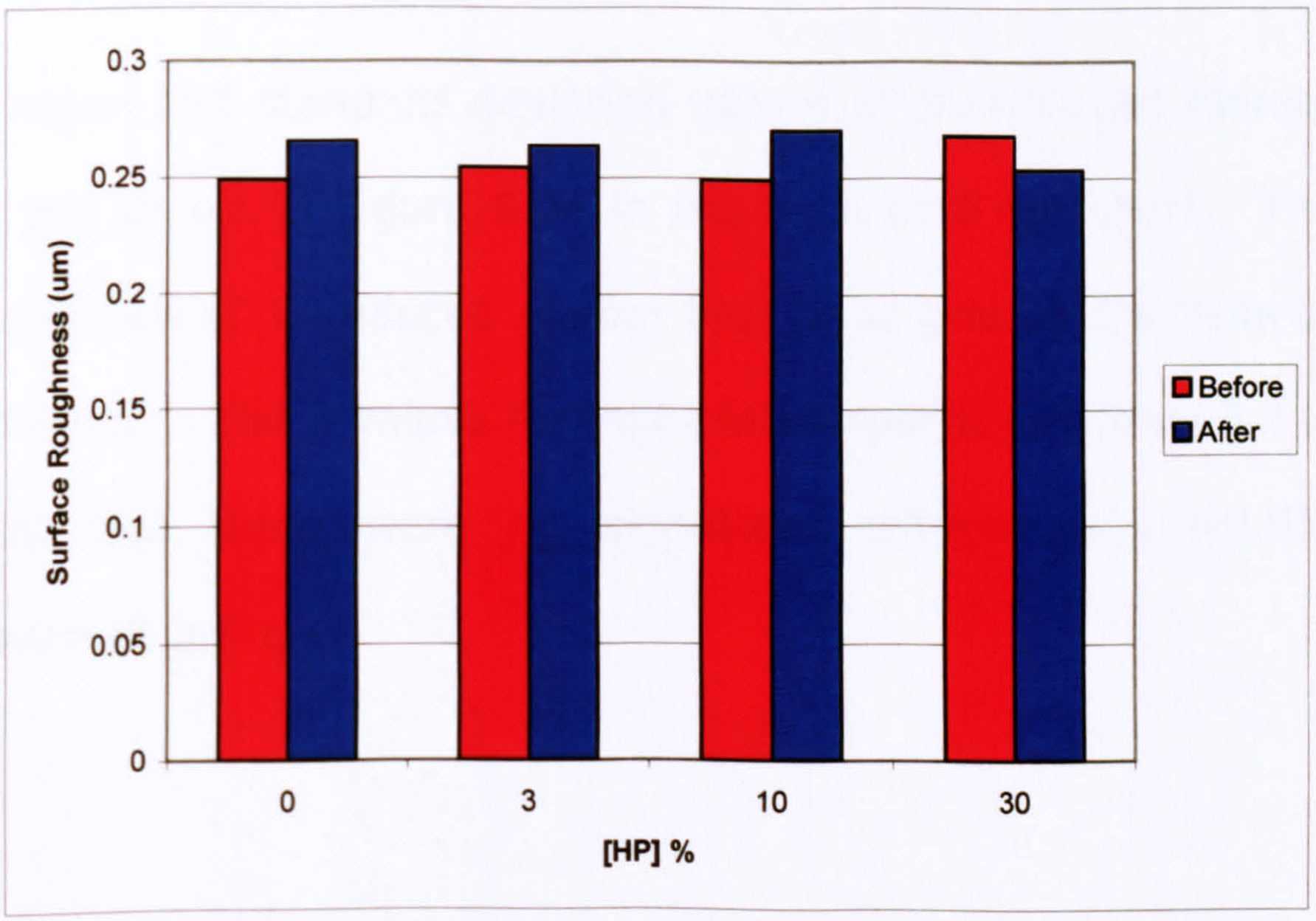


Figure 5.33 Mean surface roughness values for Pd-Cu-Ga alloy. Paired t-tests showed no significant differences ($p>0.05$) in surface roughness values before and after bleaching.

5.3.3 Biocompatibility Testing

5.3.3.1 Scanning Electron Microscopy

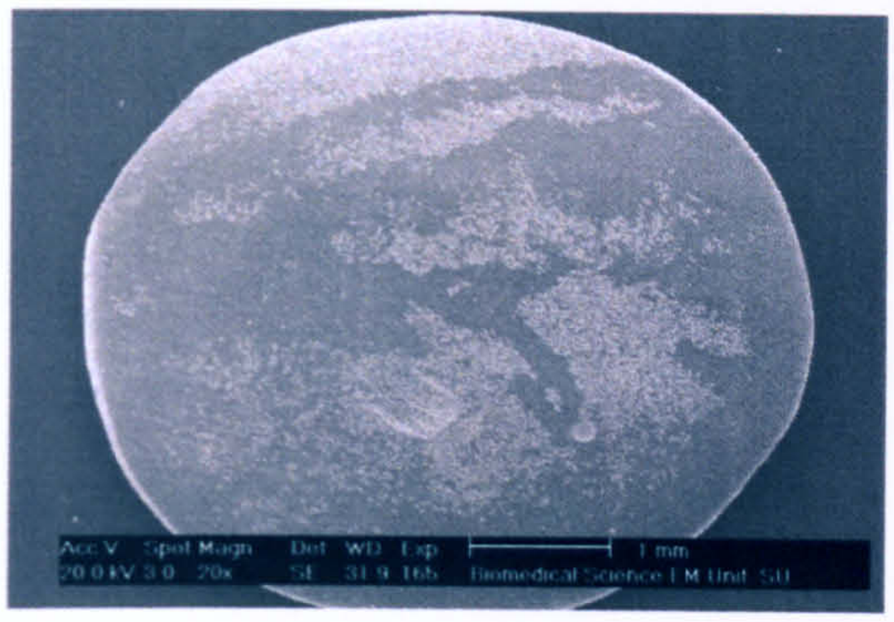
Following cell culture, SEM work was carried out on three samples of each alloy before and after bleaching and typical images at two different magnifications are shown below. There appeared to be more cell growth on the Pd-Cu-Ga disc (Figure 5.35) than on the Ni-Cr disc (Figure 5.36).

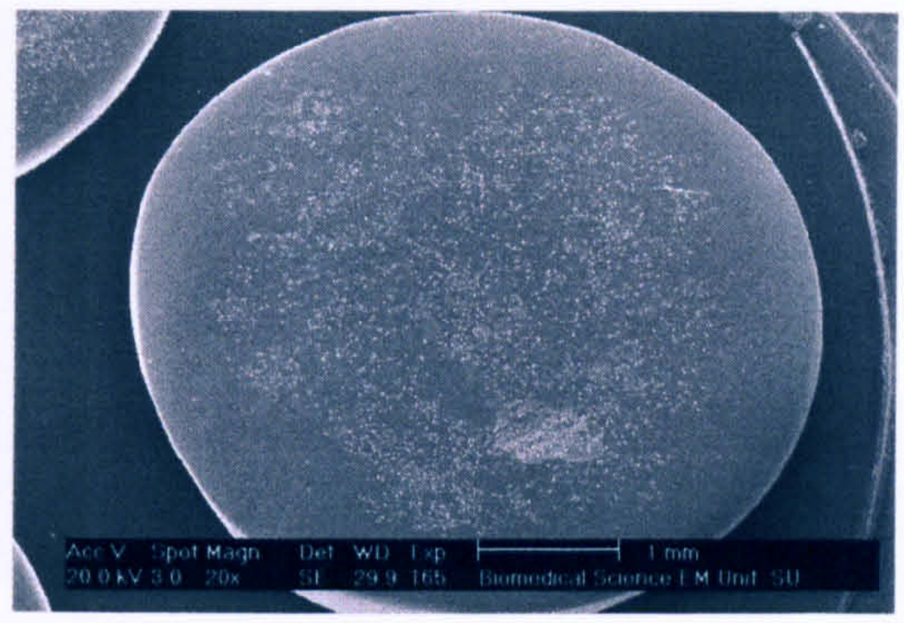
5.3.3.2 Alamar Blue

The mean and standard deviation values of % reduced Alamar blue are shown in Figure 5.34 in the form of a bar chart. The mean values of % reduced Alamar blue of all groups are close to each other. The p values for multiple comparisons (Table 5.11) shows that there were no significant differences ($p>0.05$) between all groups.

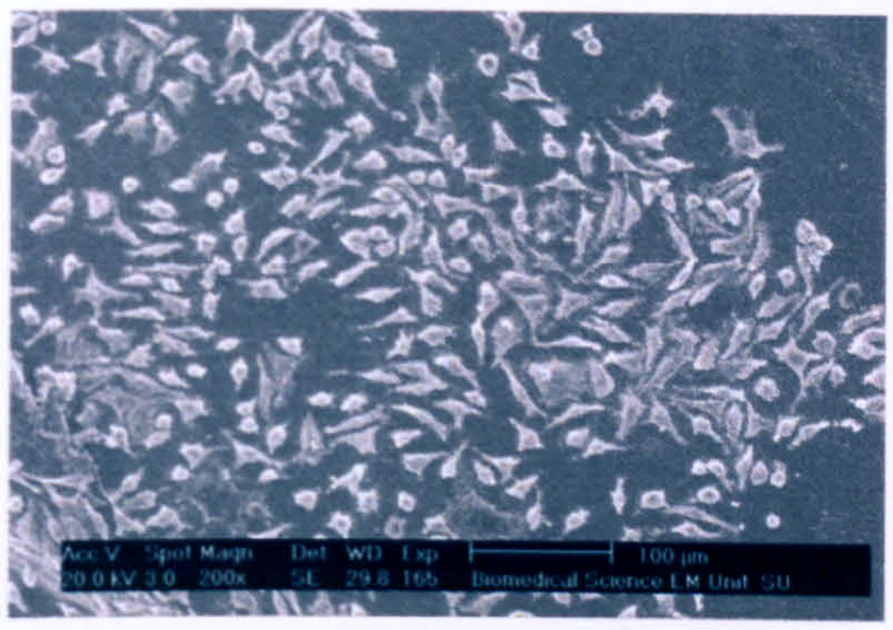
0% HP

30% HP

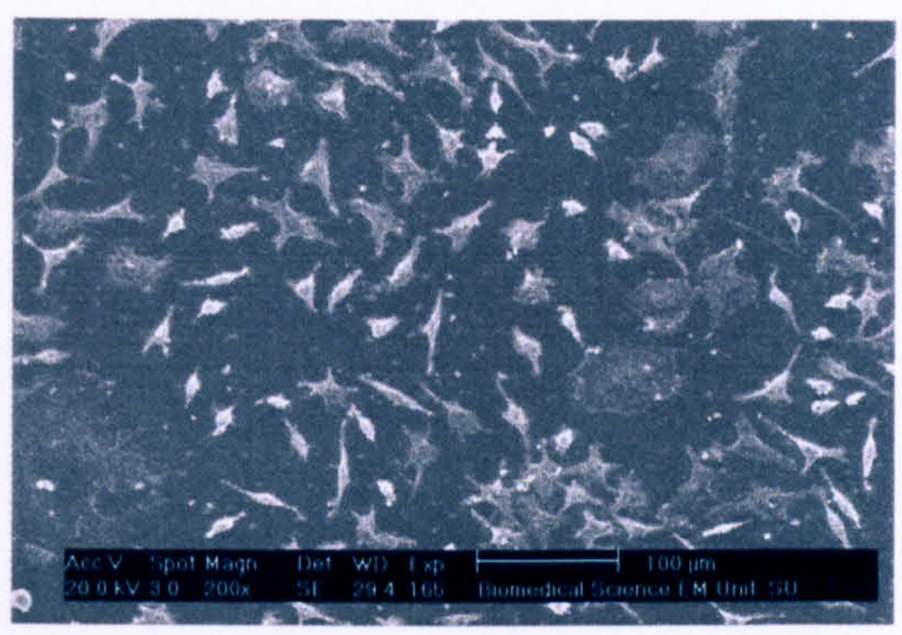




a



Fibroblastic cell morphology characteristic of the L929 lines



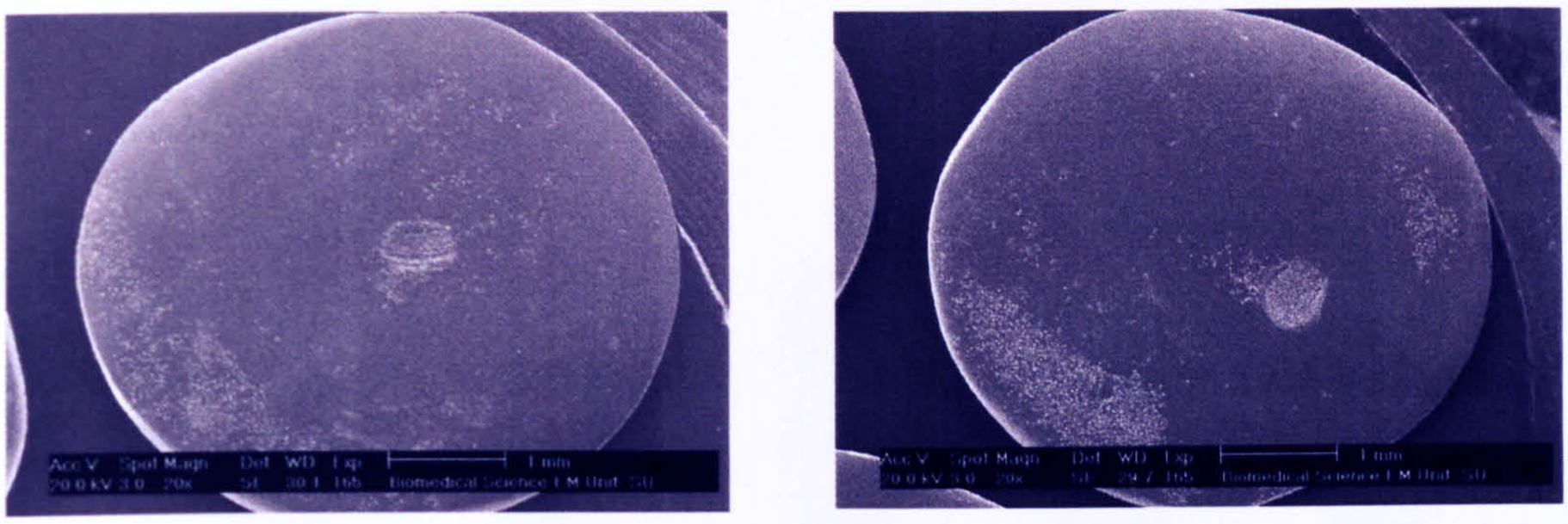
While morphology was generally unaffected, there are fewer cells in field and some cells appear less flat

b

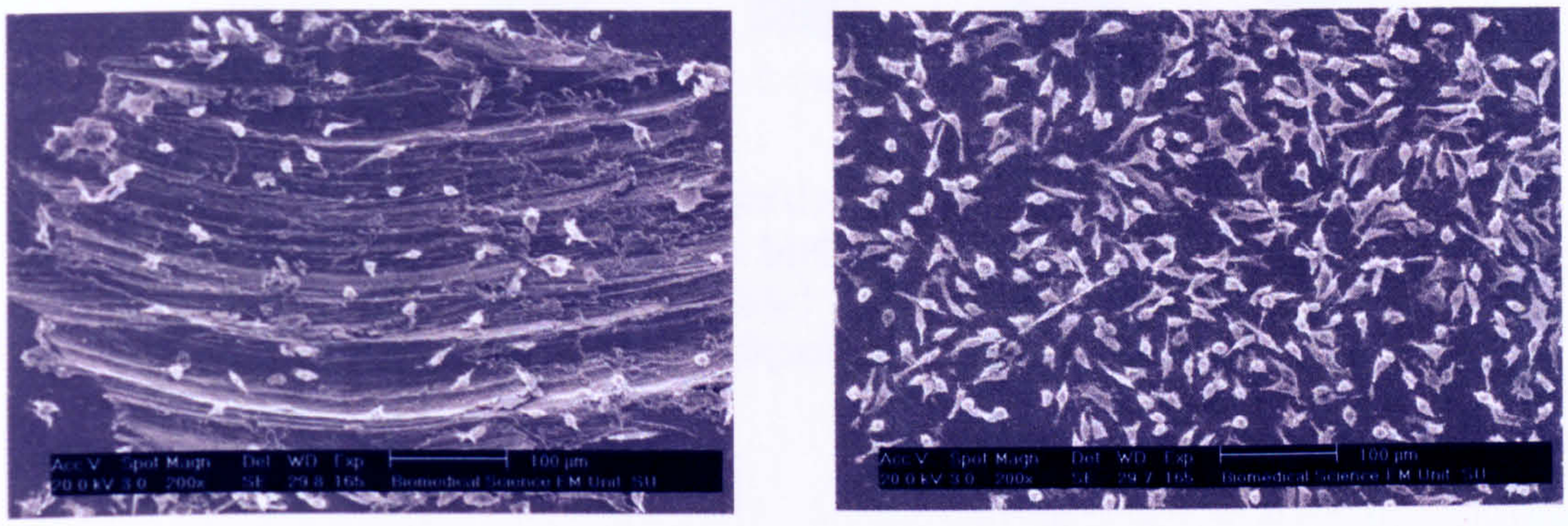
Figure 5.34 SEM images of Pd-Cu-Ga alloy before and after bleaching

0% HP

30% HP



a



Fibroblastic cell morphology characteristic of the L929 lines. Fewer cells in field

Fibroblastic cell morphology characteristic of the L929 lines

b

Figure 5.35 SEM images of Ni-Cr alloy before and after bleaching

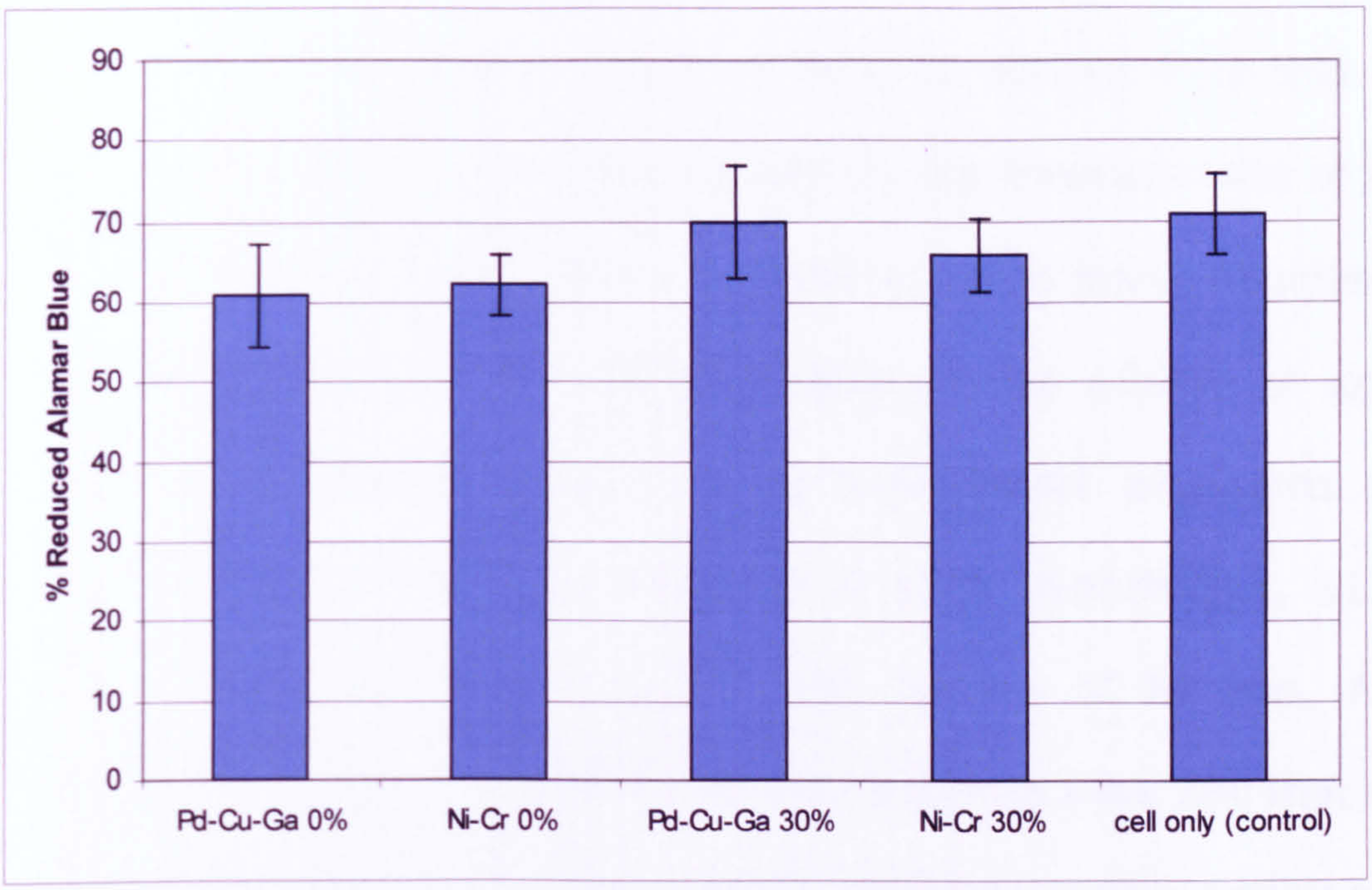


Figure 5.36 Mean and standard deviation of reduced Alamar blue for Pd-Cu-Ga and Ni-Cr before and after bleaching together with control (cell only). The One-Way ANOVA showed no statistically significant differences ($p>0.05$) between groups.

Item	Control	Pd-Cu-Ga 0%	Ni-Cr 0%	Pd-Cu-Ga 30%	Ni-Cr 30%
Pd-Cu-Ga 0%	0.15	-	1.00	0.39	0.39
Ni-Cr 0%	0.05	1.00	-	0.39	0.83
Pd-Cu-Ga 30%	1.00	0.39	1.00	-	0.92
Ni-Cr 30%	0.53	0.81	0.83	0.93	-

Table 5.11 Multiple comparisons- p-values

6. Discussion

The work on ion release presented here, as distinct from that of other investigators, was characterised by the measurement of all periodic table elements, present in the samples being analysed. This was important as it provided data on the release of ions such as Ag, Sn, Cu as well as Hg from dental amalgams as opposed to only Hg in the majority of other reports (14, 113, 114). In testing casting alloys, the release of Ni ions, for example, was also measured from the Pd-Cu-Ga alloy and that of Pd release from Ni-Cr alloy. These provided useful additional controls, again not reported by others (143, 149). The data obtained in the current work were statistically analysed and presented in the form of graphs and/or Tables. The results, presented in Chapter 5, are discussed below.

6.1 Bleaching Tooth Tissue

Calcium release into the HP solutions from bovine enamel and dentine was greater than that of phosphorous at all concentrations, effectively reducing Ca/P ratios in the bleached samples. Decreased Ca/P ratios in bleached samples was reported by other workers (68, 72). Lee *et al.* (72) also reported that Mg/Ca ratio to be 0.06 when testing mineral loss from

bovine enamel tested with 30% HP. The loss of Mg is a sign of the demineralisation process (72, 162). For comparison purposes, the ratio of Mg/Ca in the 30% HP solution was found to be 0.056 which is almost exactly the same value reported by Lee *et al.* (72). The release of Ca and P ions from dentine was more than that from enamel at all HP concentrations up to 30% tested here. The gradients of the curves in Figures 5.1a and 5.1b, however, suggest that at higher HP concentrations (>35%), the release of Ca and P from enamel may exceed that from dentine. In clinical situations the enamel surfaces will be fully exposed to the bleaching agent whereas dentine exposure is via peroxide diffusing through the enamel to reach enamel dentine junction before reaching dentine regions (55). In cases of defective restorations and non-vital bleaching procedures, the dentine may also be directly exposed to the bleaching agent.

McCracken *et al.* (11) reported calcium loss of 0.27 $\mu g/mm^2$ and 1.06 $\mu g/mm^2$ from human enamel exposed for 6 h to distilled water and 10% CP respectively. These figures are close to the results obtained in the current study of 0.48 $\mu g/mm^2$ and 1.34 $\mu g/mm^2$ for Ca loss from bovine enamel exposed for 24 h to distilled water and 3.0% HP (10% CP is equivalent to about 3.6% HP) respectively. The higher values of Ca loss reported in

the present work is due in part to the longer exposure time (24 h as opposed to 6 h) and can also be due to morphological differences between human and bovine enamel. McCracken *et al.* (11) also reported a slightly higher value (1.25 μg/mm^2) for Ca loss from human teeth when exposed to a cola beverage for 2.5 minutes, the time equivalent to drinking a 16 oz beverage. Similarly, Grobler *et al.* (70) reported mean Ca loss value of 1.9 μg/mm^2 from enamel after exposure to a Pepsi Cola drink for 2 minutes. In a further paper, Grobler *et al.* (71) reported fairly similar values of Ca loss from enamel exposed to orange juice. Diet Pepsi Cola showed smaller losses.

The amount of Ca loss from bovine enamel exposed to 30% HP over 24 h period reported here was 7.8 μg/mm^2 (Table 5.1). This equates to drinking the equivalent of about 6 cola beverage drinks, based on McCraken *et al.* (11) results, or about 4 Pepsi Cola or orange drinks, based on Grobler *et al.* (70) results, in a 24 h period. The corresponding number of drinks equating to bleaching with 10% HP are 1.72 and 1.13. These figures are intended to be used as a rough guide as they are based on the assumption that Ca loss from bovine and human enamel are equal following exposure to similar environments. The data presented here clearly established the effect of bleaching on

tooth demineralisation. The amount of Ca loss, however, during bleaching with up to 10% HP should not give cause for concern when compared with Ca loss from teeth exposure to daily intake of soft drinks. The demineralisation with 30% HP is clearly much larger and caution should be exercised when bleaching teeth with 30% HP.

The initial mean Vickers microhardness values for the three groups ranged from 250 to 271 for the enamel (Figure 5.2a). These values fall well within the range 200 to 350 reported by Wang *et al.* (163) for bovine teeth. The mean Vickers microhardness values for enamel before and after bleaching (Figure 5.2a) for the 3 bleached groups showed a statistically significant ($p<0.05$) decrease in microhardness value in each group after bleaching. The largest decrease in the enamel microhardness was at 30% HP. Lewinstein *et al.* (56) also reported that 30% HP causes a significant reduction in enamel microhardness. The decrease in microhardness values reported here were 16%, 14% and 27% at 3%, 10% and 30% HP concentrations respectively. Although, a decrease in microhardness is associated with bleaching, no definite relationship has been established here between the percentage decrease in microhardness with the percentage increase in HP

concentration of the bleaching agent. The microhardness values of the dentine were much lower than enamel. The bleached and unbleached data reported here did not exhibit any particular trend with no statistical differences ($p>0.05$) between the bleached and unbleached samples in all the three groups.

Bleaching one sample of each enamel and dentine at 30% HP (w/v) over 24 h period resulted in a colour change of 9.96 units for enamel (Figure 5.11) and 7.06 units for dentine (Figure 5.12). The transmittance data, for the bleached and unbleached samples, also showed larger increase for enamel than dentine upon bleaching. More work is required in this area before any definite conclusions are made.

6.2 Bleaching Amalgam Discs with 10% Carbamide Peroxide

Mercury, silver, tin and copper ions were detected in water samples (eluents) following the treatments including controls (Figures 5.13-5.16). The highest mercury release followed treatment with the 10% CP gel. This was not, however, significantly greater than the 0% CP gel treated group. This finding suggested that the physical removal of gel from the

surface of the amalgam disc itself contributed to subsequent metal ion release. Tin ion release was of the same order as that detected for mercury following treatments with 10% CP and 0% CP. Tin release, however, was higher than mercury following treatment with Sprite-Light®. Treatment with Sprite-Light® (pH 2.84) resulted in relatively low levels of mercury release compared to 10% CP gel, although copper ion release was increased. Sprite-Light® was selected as an additional control solution because it has been commonly employed in dental studies (164) and has been compared to tooth bleaching agents (46, 68). The ion release profiles for acidic Sprite-Light® and the gel treatments were consistent with the operation of different processes. The active gel oxidized the surface of the material whereas, the carbonated beverage acted on the amalgam by acidic attack.

SEM images (Figure 5.17) did not show any differences between samples. The images showed the structure of the amalgam, and the spherical nature of the alloy was apparent. Additionally, there were no statistical differences in surface roughness results ($p > 0.05$) between the surface of amalgam discs treated with 0% and 10% CP gel. Similar results were reported by Potocnik *et al.* (68) on the effects of 10% CP on human enamel as substrate.

The release of mercury from restorations is proportional to the surface area of the restoration and is also time dependent (165). The amount of Hg release was about 1.2 μg/cm^2 in 24 h after treatment with 10% CP. Assuming a surface area of 1 cm^2 is equivalent to about four mercury amalgam surfaces *in vivo*, the mercury release value from the current study would mean that four bleached amalgam surfaces would subsequently release 1.2 μg of mercury into the oral cavity in 24 h. This is well within the World Health Organisation's maximum acceptable daily intake (ADI) for mercury of 40 μg (166). At this rate, a patient would require mercury to be released from about 134 amalgam restorations to exceed the ADI for mercury.

The quantity of product applied in these experiments, can be considered to be in excess of *in vivo* applications since there was no elution of the gel from the amalgam surface. This is in contrast to the *in vivo* situation where it is known that peroxide levels within bleaching products are depleted during use (167). It is therefore unlikely that metal ion release from amalgam following contact with tooth bleaching gels containing up to 10% CP constitutes a health hazard.

The data from the laser ablation tests (Figure 5.18 and Table 5.4) demonstrated the potential for the use of the LA-ICP-MS for amalgam testing. However, this method needs careful calibration before the recorded ion releases can be quantified. The ratio of Hg ion release to Ag measured by the ablation method was almost identical to that reported in Figures 5.13 and 5.14 for 10% CP. Corresponding ratios of Hg/Sn (Figures 5.13 and 5.15) was, however, higher and Hg/Cu (Figures 5.13 and 5.16) was much higher.

In the second series of tests on amalgam, sample preparation and testing procedure were further refined. Samples for metal ion release measurements were taken, after bleaching, from the bleaching agents instead of the eluents. In addition, a strict protocol for sample preparation was put in place to ensure sample uniformity.

6.3 Bleaching Amalgam discs with Varying Hydrogen Peroxide Concentrations

Mercury, silver, tin and copper ions were detected in all analysed samples following experimental treatments. Ion release increased with increasing HP concentrations for all elements.

The release of mercury ions was the highest at all HP concentrations (Figure 5.19). The p-values of the transformed data (Table 5.6) show that there are significant differences between the control and each of the four HP concentrations for all elements ($p < 0.05$). The statistical tests for the surface roughness data, however, showed no statistical significance before and after bleaching at all concentrations ($p > 0.05$). The ion release data for mercury and silver follow a similar trend especially at high HP concentrations. Tin was the most responsive to changes in HP concentration exhibiting a fairly linear relationship between ion release and HP concentration (Figure 5.19). The release of copper ions was the lowest at 10 and 30% HP (w/v) concentrations.

The quantity of mercury released from dental amalgam as reported in scientific journals varies considerably (Table 2.3). For comparison purposes, the data reported by Rotstein *et al.* (14) may be recalculated to show between 0.60 and 4.24 $\mu g/mm^2$ of mercury released directly into CP solutions. In a further paper by Rotstein *et al.* (155) the amount of mercury released from amalgams treated with 10% CP was very similar to that released by 10% HP. Similarly, the data reported for mercury release by Hummert *et al.* (114) and Mackert and Berglund (115) were

recalculated to be between 0.014 and 0.020 μg/mm^2 and 0.016 μg/mm^2, respectively. Recalculation of Robertello *et al.* (113) data, for example, was not possible due to the lack of experimental details given in their paper.

As mentioned earlier, the release of metal ions from restorations had been reported to be proportional to the surface area of the restoration and time dependent (165). The average amount of mercury release, recorded in this study, from a typical sample was 7.2, 6.3, 15.6 and 28.6 μg/day for 1%, 3%, 10% and 30% HP concentrations, respectively. The total surface area of the cylindrical amalgam discs was 220 mm^2. Therefore average mercury release from a typical sample over a 24 h period was 0.033, 0.029, 0.071 and 0.130 μg/mm^2. Assuming the area of a typical restoration in the mouth to be 5 mm x 5 mm approximately, based on these data a single restoration will release on average 0.818, 0.718, 1.775 and 3.245 μg/day at 1%, 3%, 10% and 30% HP concentrations (w/v), respectively. Clearly, to exceed WHO's maximum acceptable intake of 40 μg/day would require mercury release from 49, 56, 23 and 12 restorations when treated with 1%, 3%, 10% and 30% HP concentrations, respectively. Although, exposure to mercury ions is a potential hazard it is, however, unlikely that metal ion

release from amalgam following contact with tooth bleaching agents containing up to 10% HP constitutes a hazard to health. While this is encouraging, it should be noted that some manufacturers advocate the use of even higher concentrations of HP such as the 30% reported here. This consideration, combined with the trend towards direct marketing of tooth bleaching products to the general public, suggests that the situation should be closely monitored by government and the dental profession. The data for mercury release reported here for 10% HP is about 0.07 mg/mm^2; an order of magnitude less than that reported by Rotstein *et al.* (14) but about five times higher than those reported by Hummert *et al.* (114) and Mackert and Berglund (115). Large fluctuations in the values of measured mercury ions are not uncommon. It is partly due to difficulties involved in measuring accurately the released mercury ions, and great care has been taken in this study to overcome these problems. The ICP-MS instrument used for ion release measurements was, therefore, carefully calibrated each time using known standards.

6.4 Bleaching Dental Casting Alloys

Ion release increased with increasing HP concentration for all elements, except gold, for both alloys (Figures 5.30 and 5.31).

The amounts of ion release of all elements from Ni-Cr alloy were higher than those released from the elements in the Pd-Cu-Ga alloy at 3%-30% HP concentrations (Tables 5.7 and 5.8). Generally, Pd-Cu-Ga alloys are considered to be more corrosion resistant than non precious metal based alloys. The Ni-Cr alloy used here was a Ni high Cr alloy, which is the most resistant of the Ni based group having a Cr content of 22.5 % (w/w). The corrosion of these alloys is far better than the Ni-Cr-Be alloys (as Be lowers the corrosion resistance of these alloys) but, not as good as the noble alloy groups (125, 138, 168, 169).

The p values reported in Tables 5.9 and 5.10 showed that there were significant differences ($p<0.05$) between the control and each of the three HP concentrations for the elements in the two alloys given in these Tables. Additionally, there was also a significant change in ion release data every time the HP concentration changed (except for molybdenum between 10% and 30% HP and indium between 3% and 10% HP). This indicated an increase in corrosion with increasing HP concentrations. The statistical tests for the surface roughness data (Figures 5.32 and 5.33) showed no statistically significant differences, for both alloys, before and after bleaching at all concentrations ($p>0.05$).

It is difficult to compare our data with those in the published literature (Table 2.4) due to lack of standardisation in previously reported testing procedures as was the case with the amalgam data. There are differences, for example, in the units of the data presented, exposure times, pH of the treatment solutions and HP concentration. Clearly, therefore, to facilitate a meaningful comparison, it was necessary first to convert the units of our measured ion release data from ug/l, as recorded by the ICP-MS instrument to $\mu g/cm^2$ to correspond to the units quoted, for example, in references (143) and (149) (Table 2.4). Secondly, it was necessary to account for the differences in the exposure times. For this purpose, an attempt was made to calculate the ion release data by Tufekci *et al.* (149) at 24 h exposure time by applying a linear interpolation to their recorded data at 7 h and 70 h. Thus, ion release values of 5.67 $\mu g/cm^2$, 4.50 $\mu g/cm^2$, 4.69 $\mu g/cm^2$, 0.15 $\mu g/cm^2$ and 0.05 $\mu g/cm^2$ were calculated for Pd, Cu, Ga, Sn and In, respectively when treated with a solution of pH 2.24. Our corresponding ion release data, expressed in $\mu g/cm^2$, at 30% HP (w/v) are much lower than the values reported by Tufekci *et al.* (149). The differences can be attributed in part to differences in pH values, as Tufekci *et al.* investigated corrosion under more acidic conditions (pH 2.24 compared with 3.83). Wataha *et al.* (143) reported ion release

data of about 0.02 μg/cm² for Pd and 0.01 μg/cm² for Cu from a Pd-Cu-Ga alloy exposed to a solution of pH value of 4 for a period of 30 minutes (Table 2.4) . Data presented here shows much higher release figures than the corresponding ones reported by Wataha *et al*. The differences may be partly due to differences in exposure times and pH values. Further work is needed in this area, in particular with regard to the standardisation of the testing procedures.

Estimates of daily intake in the diet of some of the elements in dental alloys which are of interest to the present work are 400 μg for Ni and Mo, 240 μg for Cr, and 3110 μg for Cu (124). Assuming the surface area for a typical restoration fabricated from dental alloys to be about 0.33 cm² for the exposed palatal metal collar of a metal ceramic crown and about 1.50 cm² for a full coverage metal crown. For Ni release, based on our measured data, the daily diet figures equate to ion releases from about 147 metal ceramic crowns or 32 full coverage crowns treated with 30% HP concentration over 24h period. The corresponding numbers for the other three elements quoted are 262 and 58 crowns for Mo, 109 and 24 crowns for Cr, 104,714 and 23,037 crowns for Cu. There are a large number of other elements taken daily in the diet which are also released from

dental alloys. A number of these elements are needed for normal body function (e.g. Zn), whereas, others (e.g. Pd) if introduced in excess could become toxic. As elements released from casting alloys are introduced to the oral cavity, these elements are believed to be eliminated from the body in a relatively short space of time (124). Whereas the risk of systemic adverse reactions due to elements released from dental alloys though clearly elevated is unlikely to constitute a health hazard (140). A dental crown can, however, extend below the level of the gingival margin, forming a sulcus between the gingiva and the alloy (124). Elements from the alloy released into the sulcus, may reach high enough concentration to cause a local adverse reaction (15).

Biocompatibility tests *in vitro* are limited in their ability to predict clinical behaviour or toxicity. In the present work, some biocompatibility testing was carried out on the two casting alloys as described in sections 4.8.5 and 5.3.3. There has been very few if any such work, to the author's knowledge, linking exposure to HP, ion release, and effects on biocompatibility. The tests using Alamar blue showed that there were no significant differences ($p>0.05$) in percentage reduced Alamar blue between the control group and all other groups. This indicated that the

unbleached and bleached Ni-Cr and Pd-Cu-Ga samples had similar *in vitro* biocompatibility. The SEM images suggested more cell growth had occurred on Pd-Cu-Ga disc compared to Ni-Cr disc. This was also substantiated by p-values (Table 5.11) of 0.15 between control and Pd-Cu-Ga (0% HP) compared with a value of 0.05 between control and Ni-Cr (0% HP). Corresponding p-values between control and Pd-Cu-Ga (30% HP) and control and Ni-Cr (30% HP) were 1.00 and 0.53. Additionally, the ion release data reported in Tables 5.7 and 5.8 show significantly higher metal ion release from Ni-Cr alloy compared with Pd-Cu-Ga alloy.

The null hypothesis was rejected and the alternative hypothesis was accepted that there were significant differences in ion release from tooth tissue, dental amalgam and dental casting alloys treated with water and increasing HP concentration during bleaching.

7. Conclusions

There is some debate as to whether employing greater concentrations of HP as the active agent in tooth bleaching preparations is desirable (even though current UK laws limits the peroxide content to 0.1%). The availability of reliable data in this field, such as those reported here, is vital for informing both current discussion and scientific debate regarding the safety and efficacy of tooth bleaching agents. The ICP-MS instrumention used for metal ion release proved highly satisfactory in terms of sensitivity and provision of data on all elements present in any given sample. From the discussion of the results presented, the following conclusions can be drawn:

1. Tests on bovine teeth detected statistically significant loss of mineral from enamel and dentine with bleaching, which increased with increasing HP concentration. More calcium was released than phosphorous, thereby reducing Ca/P ratio in the bleached samples. These experiments also showed a significant decrease in enamel microhardness following bleaching. Corresponding data for dentine did not show a significant change in microhardness values. Bleaching bovine enamel and dentine for 24 hours at 30% HP resulted

in a colour change (increased whiteness) ΔE = 10 units in enamel and ΔE = 7 units in dentine.

2. Bleaching amalgam discs with HP showed a statistically significant increase in metal ion release for Hg, Ag, Cu and Sn when compared with control (0% HP). The metal ion release from amalgam discs also increased with increasing HP concentration (0-30% w/v). This is the most clear association yet between HP concentration and damage to a dental material. The change in surface roughness of the amalgam discs was not statistically significant before and after bleaching at all HP concentrations (0-30%). SEM images did not show any morphological differences in amalgam disc surface before and after bleaching with 10% CP.

3. Bleaching casting alloys (Ni–Cr and Pd–Cu–Ga) significantly increased metal ion release from the two alloys compared with control (0% HP). Metal ion release from the two casting alloys significantly increased with increasing HP concentrations. However, the amount of ion release from all elements from Ni-Cr alloy were higher than those released from the elements in Pd-Cu-Ga alloy. Clinically, therefore,

the data favours the use of noble casting alloys. SEM images of cultured cells seemed to indicate more cell growth on the Pd-Cu-Ga alloy. Alamar blue assay showed that there was no difference in *in vitro* biocompatibility between casting alloy samples treated with hydrogen peroxide (0% and 30% w/v).

In general, the level of metal ion release from dental amalgam and casting alloys following treatment with 30% HP can be large enough to have adverse local and/or systemic effects. Mineral loss from tooth tissue and microhardness degradation of enamel was also significant at 30% HP. Extreme caution should, therefore, be exercised when bleaching teeth at relatively high HP concentrations especially, if applied over long periods.

8. Future Work

The work presented in this thesis may be extended in a number of ways. Of these;

1. Metal ion release from amalgam and casting alloy discs were measured at different HP concentrations but only over a 24 h period. It would be clinically relevant to obtain time-release data as well. The ion release data can be measured at different HP concentrations over various bleaching times.

2. Conduct *in vivo* tests on human teeth to measure and compare changes in tooth colour at different HP concentrations and period of application.

3. The metal ion release data presented here was measured using ICP-MS. This technique proved very effective and provided ion release data of all elements in the periodic table present in a particular sample test. The surface chemistry of amalgam discs, for example, can also be studied using X-ray photoelectron spectroscopy (XPS). As well as elemental, this technique will also provide chemical information of the amalgam surface.

4. The biocompatibility work maybe extended to study local or systemic effects including carcinogenicity arising from the application of dental bleaching agents.

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Appendix I

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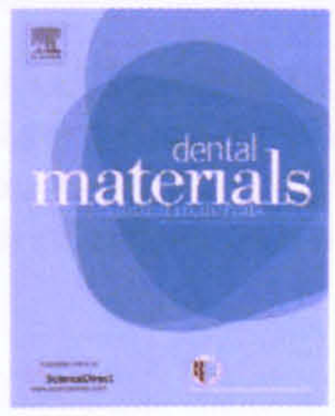

The effect of carbamide peroxide treatment on metal ion release from dental amalgam

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ABSTRACT

Objectives. There is concern that hydrogen peroxide generated by tooth bleaching agents may cause enhanced metal ion release (including mercury) from dental amalgam following contact. The aim of this in vitro study was therefore to investigate the effect of a carbamide peroxide (CP) based tooth bleaching gel on metal ion release from dental amalgam.

Methods. Dental amalgam discs were prepared according to the manufacturers' instructions. These were treated with either a 10% carbamide peroxide (CP) gel or a 0% CP gel for 24 h. Discs were carefully wiped with cotton wool before immersion in distilled water (20 ml) for 24 h at 37 °C. Following immersion, water samples were taken for metal ion release determination (Ag, Cu, Hg and Sn) using inductively coupled plasma mass spectrometry methods. The specimens were further evaluated for surface changes using scanning electron microscopy (SEM) and Talysurf surface roughness measurements.

Results. The differences in concentration of metal ions released after treatment with the 10% CP gel and a placebo gel treatment were not statistically significant ($p > 0.05$). For example, mercury release following treatment with the 10% CP gel and the 0% CP gel was found to be 1.17(0.5) and 0.57(0.1) $\mu g\,cm^{-2}$, respectively. Roughness measurements for samples treated with the 10% CP gel and 0% CP gel were 2.23(0.47) and 1.74(0.16) μm, respectively, again showing no significant difference between groups ($p > 0.05$). SEM images of the amalgam surfaces showed no apparent differences between treatments.

Significance. Treatment with a 10% CP gel did not significantly enhance subsequent metal ion release from dental amalgams compared to a control gel, contradicting previously published studies.

1. Introduction

In recent years there has been an increased demand for tooth bleaching in order to improve the whiteness and perceived aesthetic appearance of tooth tissue [1–4]. This process commonly uses hydrogen peroxide, either directly or via its generation in a carbamide peroxide (CP) gel. The effects of peroxide on enamel and dentin have been extensively studied and there are numerous studies that report peroxide-containing products do not adversely affect enamel and dentin [5–10]. In contrast, there have been studies that provided evidence that high concentrations of peroxide could alter the chemical and

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morphological structures of tooth tissues [11–14]. While work is ongoing to determine the optimum, safe and effective concentration of peroxide for tooth whitening procedures, it is important to also consider other potential interactions that may occur in the oral cavity.

One potential interaction is that between peroxide and dental materials. Various reports have described the effects of bleaching agents on dental materials including glass-ionomer cements, ceramics and gold [8,15–18]. These reports generally concluded that there was little evidence for the bleaching systems causing significant changes to the materials. In the case of dental amalgam, however, several in vitro studies have reported a significant increase in mercury release as a result of treatment with peroxides compared to control treatments [19–21]. Robertello et al. [19] compared the effects of three peroxide-containing commercial tooth whitening products and saline control on a zinc-free, palladium-enriched high copper amalgam. After 80 h of bleaching, 0.98 mg m^{-3} of mercury was detected for one of the products. Hummert et al. [20] studied the effects of two tooth whitening products and saline on mercury release from four different amalgams. After 8 h treatment, the level of mercury released was between 109 and 158 ng ml^{-1} for the tooth whitening products and 5 ng ml^{-1} for saline. Rotstein et al. [21] studied the effects of 10% carabamide peroxide and phosphate buffer (both at pH 6.5) on four different amalgams. After 48 h, the level of mercury released was reported to be in the range 23–161 μg ml^{-1}.

Consideration of published studies shows that a wide variety of methods have been used to model the effects of bleaching agents on tooth tissue and dental materials. This lack of standardisation is reflected by the data generated, and this in turn is the most likely explanation for the very different conclusions reached by authors. In addition to this broad criticism, it was also noted that relatively little attention has been directed at understanding the mechanisms responsible for metal ion release from dental amalgam following exposure to peroxides. The aim of this study was therefore to investigate metal ion release from amalgam discs which were prepared and finished to simulate clinical preparation.

2. Materials and methods

2.1. Materials

The amalgam selected for this study was Sybraloy® (Kerr UK Ltd., Peterborough, Lot 71062). This is a typical restorative material based on a high copper, unicompositional spherical alloy. The composition (% w/w) of this alloy is reported as 41.8 Ag, 29.3 Sn, 28.2 Cu and 0.03 Zn. It is mixed at an alloy to mercury ratio of 1–0.92 (w/w). The Kerr data sheet gives the final mercury concentration as 45%, while the US material safety data sheet gives 44.5% (w/w). Discs (10 mm diameter × 2 mm thickness) were prepared according to the manufacturer's instructions and aged for 7 days at 37 °C in air. They were then polished using standard dental equipment (silicone polishers) and left overnight in air at 37 °C. The disc specimens were treated with either a 10% carbamide peroxide (CP) gel, the 0% CP control gel (2.0 g), a control gel based on lutrol polymer containing no peroxide (2.0 g) or a carbonated beverage (Sprite Light, The Coca Cola Co., Uxbridge, UK, containing citric and carbonic acids, pH 2.84): (20 ml) for 24 h ($n=5$). After treatment the specimens were wiped clean with cotton wool and placed in distilled water (20 ml) for 24 h at 37 °C.

2.2. Scanning electron microscope

The amalgam samples were studied using SEM to evaluate any changes in surface morphology or features. SEM photomicrographs were taken of the surface of amalgam discs following the experimental treatments.

2.3. Ion release

Following immersion of the discs in the water, samples (2× 10 ml) were taken for analysis of ion release by inductively coupled plasma-mass spectrometry (ICP-MS – Agilent 4500). All ion release samples were acidified with 200 μl of nitric acid (for Ag determination) or hydrochloric acid (for all other ions).

2.4. Surface roughness

Following ion release experiments, one disc from each series was also taken for SEM, along with an untreated control in order to determine any changes in surface morphology or features. The surface roughness of the discs was determined using a Talysurf (Mitutoyo Corporation, Kawasaki, Japan), which was calibrated by setting the appropriate zero reading prior to roughness measurement of the discs. The roughness of the uppermost surface was then measured by moving the stylus across its diameter. This procedure was repeated eight times for each disc and the results averaged.

2.5. Statistical analysis

The data were analysed using one-way ANOVA followed by Bonferroni post hoc analyses.

3. Results

3.1. Metal ion release

The ion release data for mercury, silver, tin and copper are shown in Figs. 1–4, respectively. Treatment with Sprite Light did not result in significant release of mercury or silver, but gave the highest release of copper in this study. There were no significant differences in metal ion release between 10% CP and 0% CP gel ($p>0.05$).

3.2. Scanning electron microscopy

The surface of a typical amalgam disc under SEM, before and after treatment with the 10% carbamide peroxide gel, is shown in Fig. 5(a+b respectively). All of the micrographs showed the typical structure of the set amalgam where the spherical nature of the alloy was obvious, with no major differences in the surface before and after treatment.

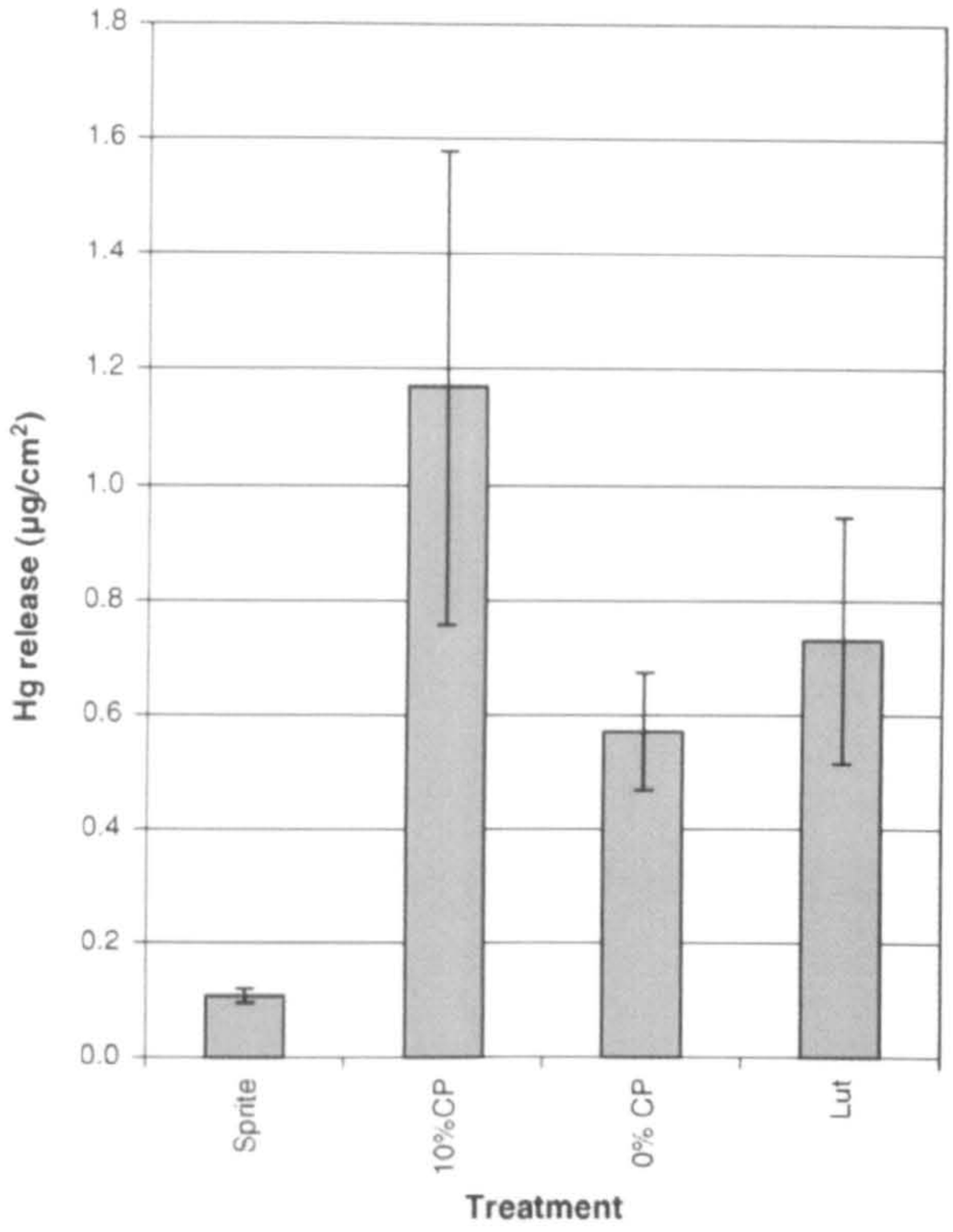


Fig. 1 – Mercury ion release ($\mu g\,cm^{-2}$) from amalgam discs following treatment with experimental and control materials. Results are the arithmetic mean of one analysis of each of five samples (error bars shows standard error of mean).

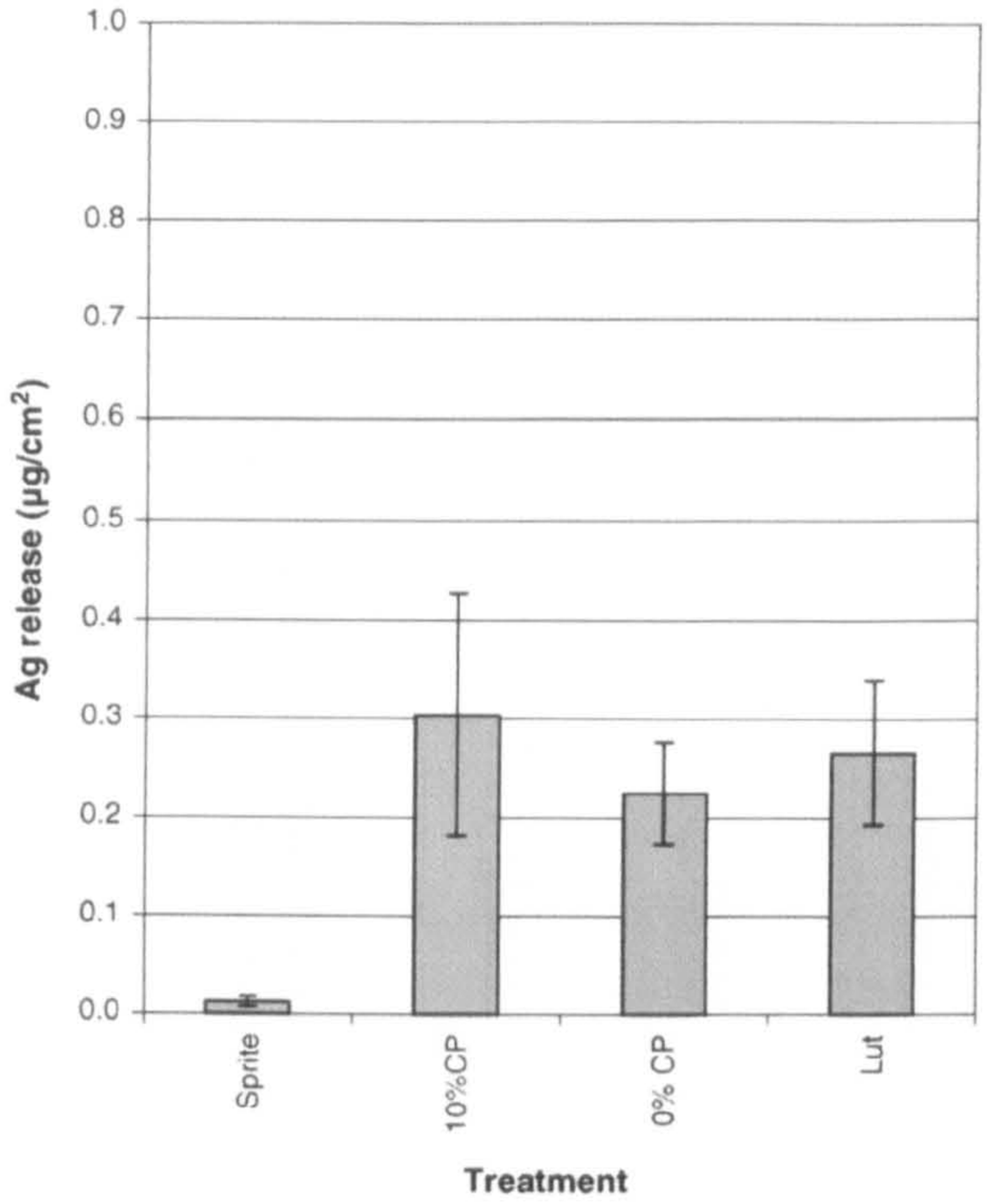


Fig. 2 – Silver ion release ($\mu g\,cm^{-2}$) from amalgam discs following treatment with experimental and control materials. Results are the arithmetic mean of one analysis of each of five samples (error bars shows standard error of mean).

3.3. *Surface roughness*

The average roughness for each specimen after treatment is shown in Table 1. The difference in roughness values for the discs treated with the 10% CP gel and the 0% CP gel was not of statistical significance ($p > 0.05$).

3.4. *Statistical analysis*

The data were analysed using one-way ANOVA followed by Bonferroni post hoc analyses.

4. Discussion

Metal ions, including mercury, were detected in water samples following all of the treatments including controls (Figs. 1–4). The greatest mercury release followed treatment with the 10% CP gel. However, this was not significantly greater than the 0% CP gel treated group. This observation suggested that the physical removal of gel from the surface of the amalgam disc itself contributed to subsequent metal ion release. Treatment with Sprite-Light® (pH 2.84) resulted in relatively low levels of mercury release compared to 10% CP gel, although copper ion release was elevated. Sprite-Light® was selected as an additional control solution because it has been commonly employed in dental studies [22] and has been compared to tooth bleaching agents [10,23]. Tin ion release was consistently higher than silver, and of a similar order to that detected for mercury. However, it was noted that tin release was higher than mercury following treatment with Sprite Light. The ion release profiles for acidic Sprite-Light® and the gel treatments were consistent with the operation of different mechanisms. The carbonated beverage acted on the amalgam by acidic

Table 1 – Surface roughness measurements

	Sample			
	Lutrol Gel	Sprite	10% CP	0% CP
Roughness (µm) (S.E. of mean)	1.23 (0.12)	1.91 (1.23)	2.23 (0.47)	1.74 (0.16)

Surface roughness (µm) determination of discs exposed to different treatments.

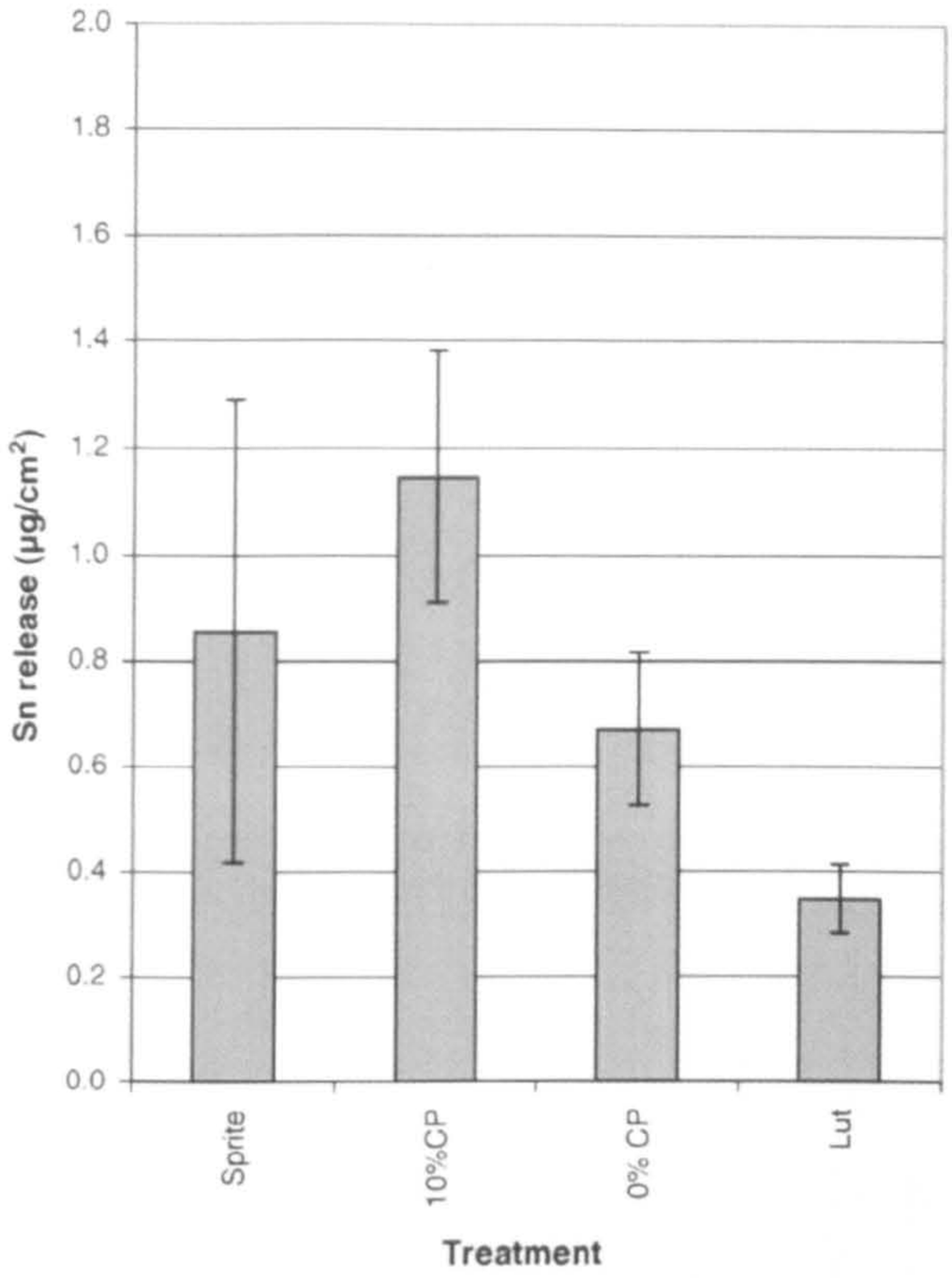


Fig. 3 – Tin ion release ($\mu g\,cm^{-2}$) from amalgam discs following treatment with experimental and control materials. Results are the arithmetic mean of one analysis of each of five samples (error bars shows standard error of mean).

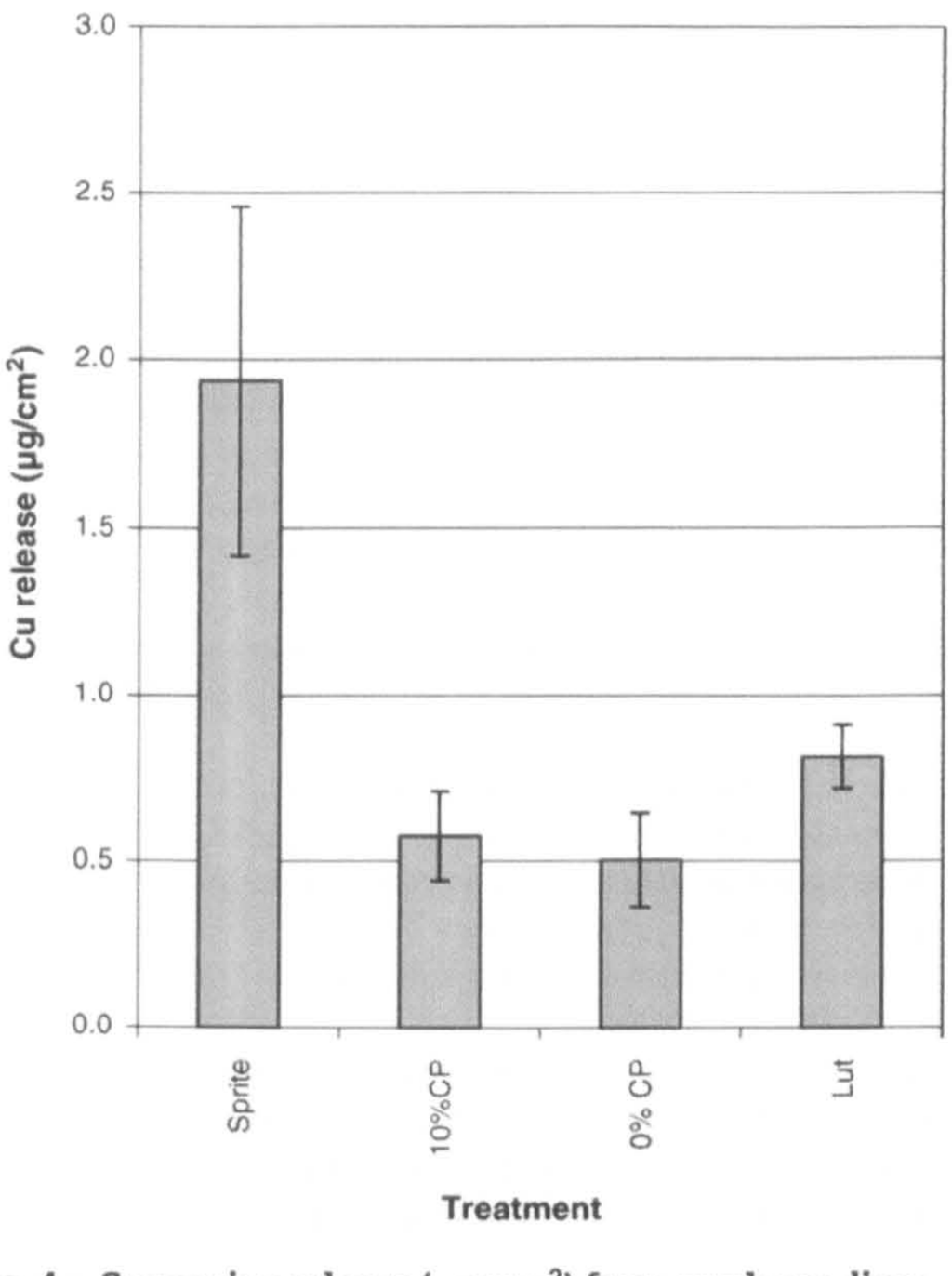


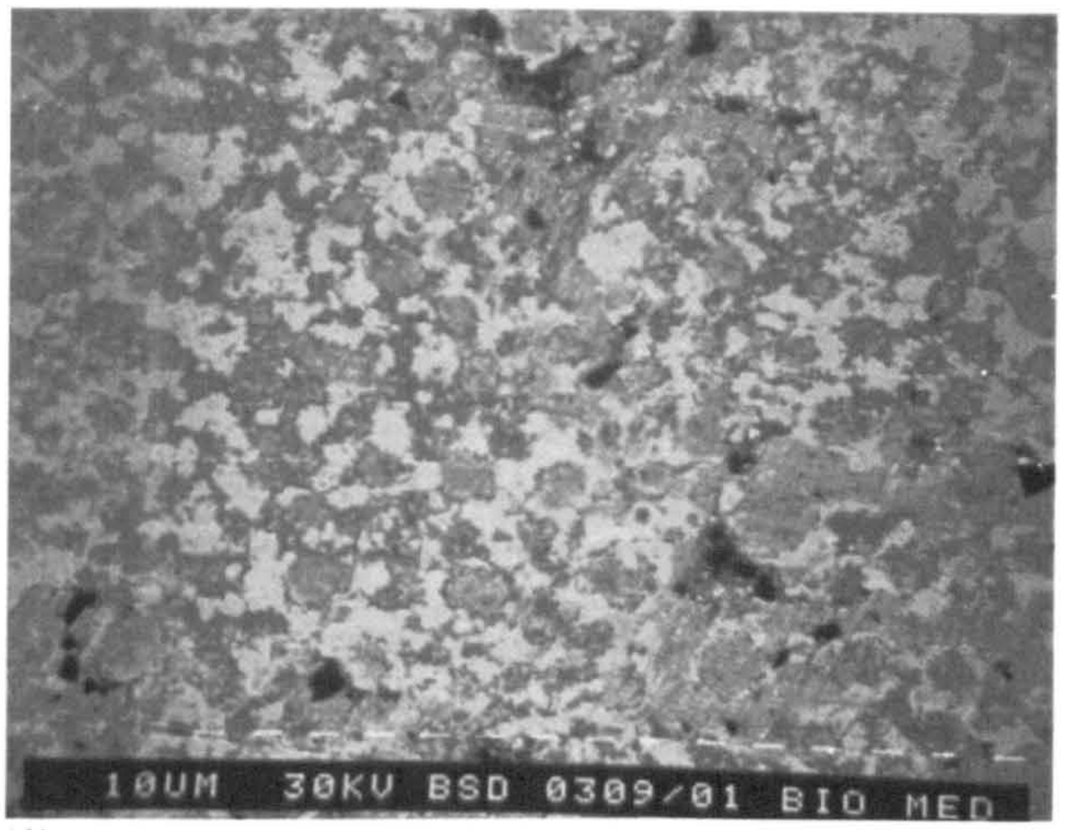
Fig. 4 – Copper ion release ($\mu g\,cm^{-2}$) from amalgam discs following treatment with experimental and control materials. Results are the arithmetic mean of one analysis of each of five samples (error bars shows standard error of mean).

attack, while the active gel oxidised the surface of the material. This did not however account for the increased ion release following placebo gel treatment.

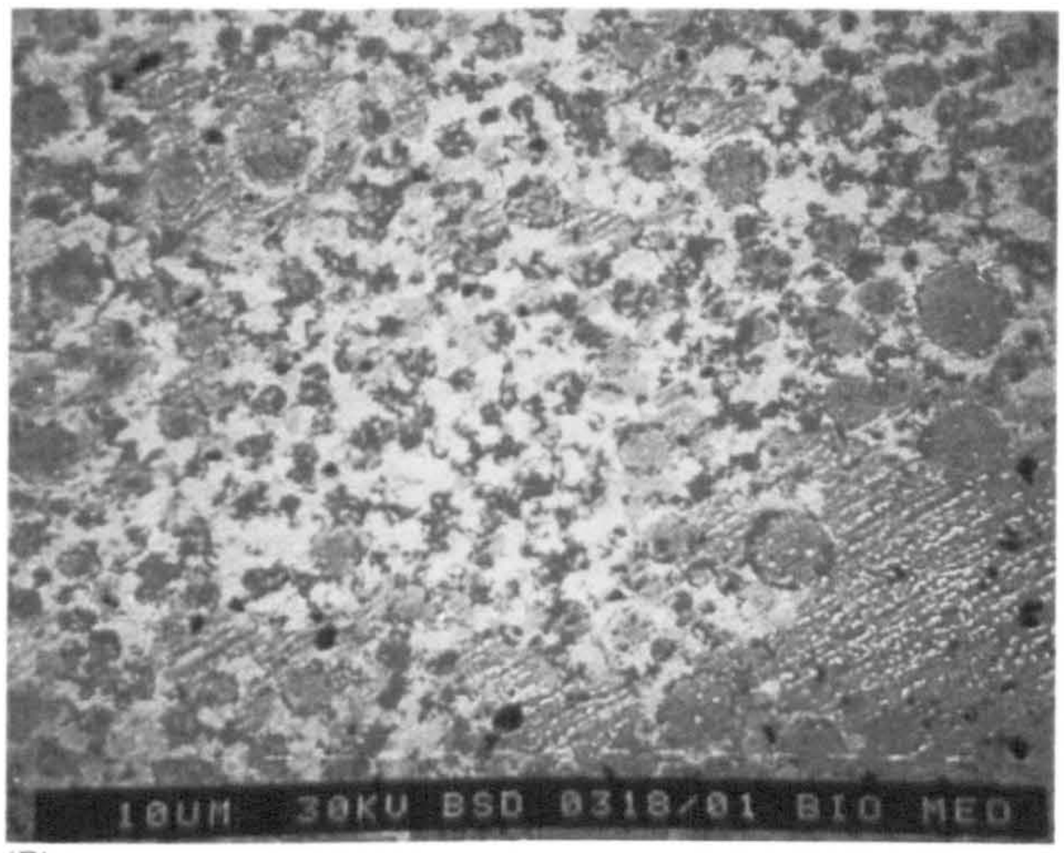
SEM did not show any differences between samples. All of the micrographs showed the structure of the set amalgam, and the spherical nature of the alloy was apparent. Differences in ion release mechanisms were not detected using SEM, even though ion release profiles suggested differences between acidic and oxidative systems. These observations were supported by the study of surface roughness of treated materials. Statistical analysis of surface roughness results showed no significant difference ($p > 0.05$) between the surface of amalgam discs treated with 0% and 10% CP gel. Similar results were reported by Potocnik et al. [23] on the effects of 10% CP on human enamel as substrate.

It is difficult to make direct comparisons between the data from this study and published figures, due to limitations of either method or presentation of data in these papers. Ion release should be expressed in a standard form, ideally as an amount released per unit surface area of specimen. Surprisingly few authors have elected to use this approach to date. Rotstein et al. [21] reported a concentration of between 23 and 161 $\mu g\,ml^{-1}$ of mercury released from their samples after 48 h of bleaching with 10% CP. Their amalgam samples had dimensions of 10 mm × 5 mm × 3 mm giving a total surface area of 1.9 cm^2 for each sample. Their data may be recalculated to give between 60 and 424 $\mu g\,cm^{-2}$ of mercury release directly into CP solutions. This data suggests very high metal ion release, almost certainly due to the use of unpolished specimens along with relatively aggressive test procedures. Hummert et al. [20] used cylindrical amalgam specimens (4 mm × 8 mm diameter). Their published mercury release values can be recalculated in terms of specimen surface area and are found to be in the range 1.42–2.04 $\mu g\,cm^{-2}$ for the bleaching agents and 0.07 $\mu g\,cm^{-2}$ for saline. These values are more similar to the data reported in this study. Recalculation of Robertello et al. [19] data was not possible due to the lack of experimental details given in their paper.

The release of mercury from restorations is time dependent and proportional to the surface area of the restoration [24]. Mackert and Berglund [25] have reported the rate of unstimulated mercury release from amalgam to be on average 0.4 µg per amalgam surface per day, calculated from six different in vivo studies. Assuming an average amalgam surface in vivo is 5 mm × 5 mm, the Mackert and Berglund figure would give a mercury release value from one amalgam of 1.6 $\mu g\,cm^{-2}$ per day. Comparing this value with the values of the current study shows that the bleaching gel system has a similar level of mercury release, thus indicating that bleaching does not unduly accelerate subsequent mercury release. Assuming a surface area of 1 cm^2 is equivalent to four mercury amalgam



(A)



(B)

Fig. 5 – Scanning electron photomicrograph showing typical amalgam surface before (A) and after (B) treatment with a 10% carbamide peroxide gel.

surfaces in vivo, the mercury release value from the current study of approximately 1.0 $\mu g\,cm^{-2}$ in 24 h would mean that four bleached amalgam surfaces would subsequently release only 1.0 μg of mercury into the oral cavity. This is well within the World Health Organisation's maximum acceptable daily intake (ADI) for mercury of 40 μg [26]. Indeed, to exceed the ADI for mercury, a patient would require mercury to be released from approximately 160 amalgam restorations.

In considering the quantity of product applied in these experiments, the amount can be considered to be in excess of in vivo applications since there was no elution of the gel from the amalgam surface. This is in contrast to the in vivo situation where it is known that peroxide levels within bleaching products are depleted during use [27]. Thus the experimental design in the current studies was in excess of what is anticipated under normal use, and can be used as further confirmation of the safety of these types of products. It is therefore highly unlikely that, assuming manufacturers' instructions are followed, metal ion release from amalgam following contact with tooth bleaching gels containing up to 10% CP constitutes a health hazard.

In the current study, no preliminary data were available for power calculations. Based on the current data and using Altman's [28] method of power calculations, a sample of 30 discs would be required to yield an 80% power to find a difference in the magnitude observed for mercury ion release in these data at an alpha of 0.05. In future studies, it may be possible to reduce the sample size by increasing the effect of the bleach (for example by possibly using longer and more exaggerated exposure conditions) and/or by reducing possible variability of the sample preparation.

5. Conclusions

Metal ions, including mercury, silver, copper and tin, were released from amalgam following all treatments. Mercury release from the amalgam samples postbleaching was approximately 1 $\mu g\,cm^{-2}$ over a 24 h period. While metal ion release was found to be above that associated with control treatment (0% CP gel) this was not statistically significant and the levels of metal ion release were not considered sufficiently high to represent a health hazard. It was also concluded that the mechanism for ion release was different for acidic carbonated drinks and tooth bleaching gels based on a carbamide peroxide system.

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Appendix II

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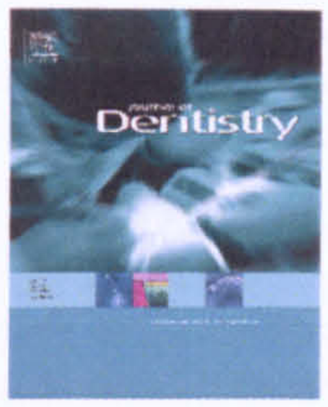

The effect of hydrogen peroxide concentration on metal ion release from dental amalgam

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ABSTRACT

Objectives: The aim of this study was to investigate the effect of hydrogen peroxide (HP) concentration on metal ion release from dental amalgam.
Methods: Dental amalgam discs ($n = 25$) were prepared by packing amalgam into cylindrical plastic moulds (10 mm diameter and 2 mm height). The discs were divided into five equal groups and each group was immersed in 20 ml of either 0%, 1%, 3%, 10% or 30% HP solution for 24 h at 37 °C. Samples were taken for metal ion release determination (Hg, Ag, Sn and Cu) using inductively coupled plasma mass spectrometry (ICP-MS). The surface roughness of each disc was measured before and after bleaching.
Results: The differences in concentration of metal ions released after treatment with 0% (control) and each of 1%, 3%, 10% and 30% HP were statistically significant ($p < 0.05$). Metal ion release for the elements (Hg, Ag, Sn and Cu) increased with exposure to increasing concentrations of HP. Surface roughness measurements of the samples before and after treatments with HP solutions were not significantly different ($p > 0.05$).
Conclusions: Exposure to HP bleaching agent was associated with increased metal ion released from dental amalgams compared to treatment with a control solution. Ion release was in proportion to the peroxide concentration tested, with the highest concentration associated with the greatest metal ion release for all elements investigated.

1. Introduction

Tooth bleaching is an increasingly popular aesthetic procedure used in dentistry.[1–4] It is relatively simple highly effective, and can often preclude the need for operative intervention.[1,2] There are various agents available for bleaching vital teeth, although they invariably involve the application or generation of hydrogen peroxide (HP), a strong oxidising agent. Despite increased popularity, controversy surrounds the use of peroxide-based bleaching systems to whiten teeth. The situation has not been helped by conflicting reports in the scientific literature and media, further compounded by a lack of standardisation in methodology or presentation of data. Some studies suggested the relatively high concentrations of peroxide used for topical bleaching altered the chemical structure of tooth tissues.[3–6] While vital bleaching does not appear to cause macroscopic changes to the dental hard tissues, microscopic changes have been reported, particularly where peroxide was applied at high concentrations.[7,8]

Reports in the dental literature have suggested that bleaching agents may have adverse effects on the physical properties of dental restorative materials.[9–12] They have also been reported to increase mercury release from dental

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amalgams.[13–16] Data from the latter studies is summarised below, with our own calculation of ion release provided in brackets to assist comparison. Robertello et al.[13] tested three peroxide containing commercial tooth bleaching agents and saline control on a high copper amalgam. After 80 h of bleaching only 0.98 mg m^{-3} (0.98 μg/l) of mercury was detected for one of the products. Hummert et al.[14] tested two tooth bleaching agents and saline on mercury release from four different amalgams. The level of mercury released was reported to be between 109 and 158 ng ml^{-1} (109 and 158 μg/l) for the bleaching agents and 5 ng ml^{-1} (5 μg/l) for saline after 8 h treatment. Mackert and Berglund[15] reported similar levels of mercury release in their tests. Rotstein et al.[16] tested the effects of 10% carbamide peroxide (CP) bleaching agent on four different amalgams and reported mercury release to be in the range 23–161 μg ml^{-1} (23,000–161,000 μg/l) after 48 h treatment. While there are also a few reports that suggested HP has no significant effect on restorative materials[10,17] the weight of evidence to the contrary means that this still remains a source of concern. Several methods have been used to model the effects of bleaching agents on dental amalgam. This lack of standardisation is reflected in the large variation in their reported data which in turn may also explain the very different conclusions reached by some authors.[18,19]

Several bleaching methods exist, including in office bleaching with or without a light source,[20,21] mouth guard bleaching under supervision of a dentist, and bleaching kits that are sold over the counter (where individuals apply the bleaching agent without the supervision of the dentist). We reported previously on metal ion release from dental amalgam following exposure to CP at a single concentration.[17] Active HP concentrations may, however, vary enormously and can be as high as 35% (v/v)[9] even though current UK law limits the peroxide content to 0.1%. Despite widespread debate, there is currently a trend towards employing greater concentrations of HP as the active agent in tooth bleaching preparations.[22,23] The aim of the present study was therefore to investigate the effect of increasing HP concentrations (0 to 30% v/v) on ion release from amalgam. This research will provide data of value in informing current discussion and scientific debate regarding the safety and efficacy of tooth bleaching agents.

2. Materials and methods

2.1. Materials

The amalgam selected for this study was Tytin (Kerr UK, Peterborough, UK). This is a typical restorative material based on a high copper, unicompositional spherical alloy. The Kerr data sheet gives the composition (%, w/w) as 59% Ag, 28% Sn, 13% Cu and final mercury concentration as 42.5% (w/w).

Preparation of amalgam discs ($n = 25$) for testing followed a strict protocol. Plastic moulds (10 mm diameter by 2 mm height) were designed and manufactured. Amalgam was packed into the moulds wearing polythene gloves. The amalgam discs were allowed to fully set in the moulds for 24 h. The discs were then removed, individually numbered for identification purposes and polished on the uppermost surface. Polishing was first performed using silicon carbide paper (Grit number 800 and then 1200) followed by polishing on a felt wheel (matted wool cloth) with a 1 μm diamond suspension to obtain as near a mirror finish as possible. The surface roughness of the polished surface of each disc was determined using a Talysurf contact profilometer (Mitutoyo Corporation, Kawasaki, Japan).

To establish the active concentration of HP in test solutions, an iodine thiosulphate titration was performed. Five titrations were carried out using 30% HP solution, and results were presented as the mean and standard deviation. The actual concentration of the HP solution thus calculated was 30.23%. This HP solution was diluted to obtain a 1%, 3%, 10% as well as 30% HP solutions with 0% as the control.

The 25 amalgam discs were randomly divided into 5 equal groups of 5 discs. Each of the 5 discs in a group was individually immersed in 0%, 1%, 3%, 10% and 30% HP solution (20 ml) for 24 h at 37 °C creating 5 samples of each solution. Each disc was placed in a tapered centrifuge tube, with all surfaces exposed to the particular HP concentration in that tube. All the 25 solutions samples were analysed by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 4500).

2.2. Ion release

All ion release samples were acidified with 200 μl of nitric acid (for Ag determination) or hydrochloric acid (for all other ions). For each analysis, the instrument performs five measurements and calculates the mean and relative standard deviation (%) for each element. Therefore, with 5 discs tested in each group, the total number of measurements recorded per element was 25.

2.3. Surface roughness

Following ion release experiments, the surface roughness of the discs was measured again using a Talysurf instrument that was calibrated by setting the appropriate zero setting prior to roughness measurement of the discs. The roughness of the uppermost surface was then measured by moving the

Table 1 – Means and standard deviations for ion release data

[HP] (%)	Mercury (μg/l)		Silver (μg/l)		Copper (μg/l)		Tin (μg/l)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	2.70	0.92	0.02	0.19	9.16	1.18	0.90	0.36
1	360.00	185.34	80.00	40.58	41.60	15.32	5.56	1.24
3	316.00	75.03	168.40	81.50	52.60	15.11	47.20	8.58
10	782.00	438.66	670.00	346.19	82.60	21.28	230.00	35.55
30	1428.00	882.59	1294.00	770.83	194.00	111.49	752.00	73.28

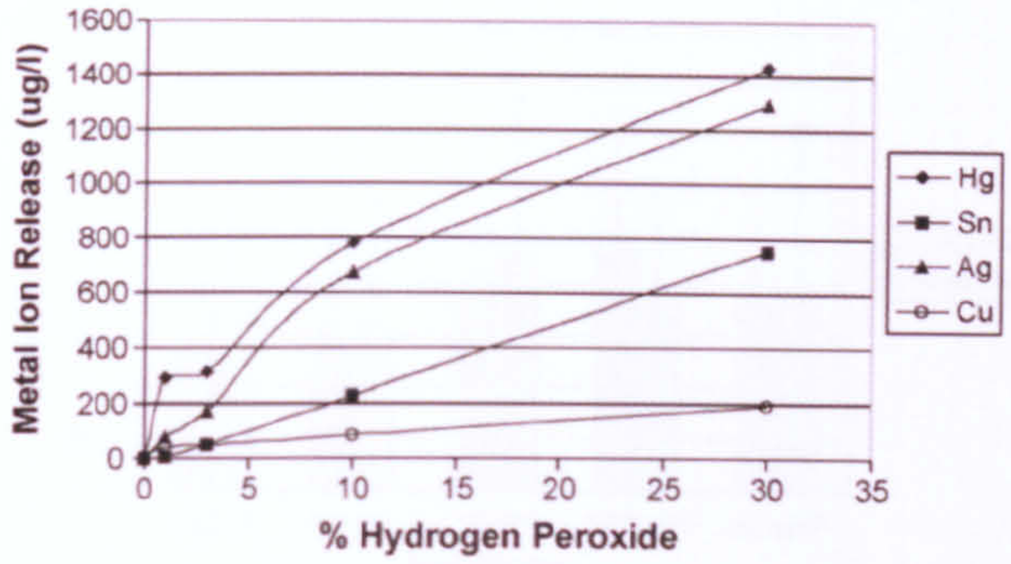

Fig. 1 – The effect of HP concentration on metal ion release.

stylus across its diameter. This procedure was repeated eight times for each disc, altering the orientation each time, and the results averaged.

2.4. *Statistical analysis*

A two-way ANOVA (element by concentration) revealed a significant interaction between concentration and elements ($p < 0.001$) indicating that differences between solutions were different across elements. The two-way ANOVA was followed by a one-way ANOVA and Dunnett Post Hoc test for multiple comparisons between solutions for each element. The roughness measurements were analysed using a paired t-test.

3. Results

The relationships between ion release and HP concentration is shown in Fig. 1. Values for the mean and standard deviation of ion release data for mercury, silver, copper and tin are shown in Table 1, at 1%, 3%, 10% and 30% HP concentrations.

Metal ion release increased with increasing hydrogen peroxide concentration for all elements. The highest ion releases were those for mercury followed closely by silver then tin and finally copper. The distribution of the recorded ion release data, not shown here, was positively skewed for all elements. Accordingly, it was decided to analyse the data using a natural logarithmic transformation. The transformed distributions were normal thus justifying the decision to use such a transformation. The p-values for the one-way ANOVA and Dunnets Post Hoc test are shown in Table 2. The difference in metal ion release between 0% HP (control) and all other concentrations (1%, 3%, 10% and 30%) was statistically significant ($p < 0.05$) for all elements. For tin, there was a significant change in ion release data every time the HP concentration changed. For mercury, silver and copper there was a significant difference in ion release between 30% and each of 1% and 3% but, no significant difference between 30% and 10% HP concentration.

3.1. *Surface roughness*

The average roughness values for each group before and after treatment is shown in Fig. 2. A paired t-test showed no

Table 2 – Multiple comparisons—p-values (transformed data)

Transformed p-values (unequal variance)

	Mercury			Silver			Copper			Tin		
	1	3	10	1	3	10	1	3	10	3	10	30
0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1	–	1.000	0.623	–	0.279	0.005	–	0.868	0.036	<0.001	<0.001	<0.001
3	1.000	–	0.437	0.279	–	0.049	0.868	–	0.212	–	<0.001	<0.001
10	0.623	0.437	–	0.005	0.049	–	0.036	0.212	–	<0.001	–	<0.001
30	<0.001	<0.001	<0.001	<0.001	0.279	0.005	0.047	0.868	0.036	<0.001	<0.001	–

0, 1, 3, 10 and 30 demote the values of [HP] (%).

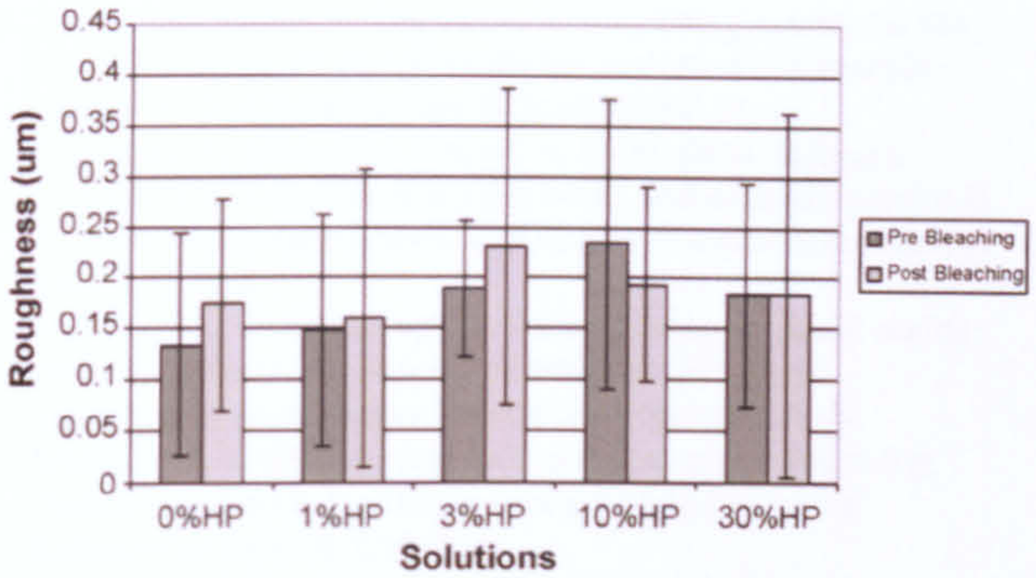

Fig. 2 – Roughness measurements before and after bleaching.

significant difference ($p > 0.05$) in mean surface roughness values before and after bleaching in all the groups.

4. Discussion

Metal ions (Hg, Ag, Cu and Sn) were detected in all samples following experimental treatments. For all the elements, ion release increased with increasing HP concentrations, with mercury release consistently being the highest (Fig. 1). The *p*-values of the transformed data (Table 2) show that there are significant differences between the control and each of the four HP concentrations for all elements ($p < 0.05$). The statistical tests for the surface roughness data showed no statistical significance before and after bleaching at all concentrations ($p > 0.05$).

Of the four elements reported here, tin was the most responsive to changes in HP concentration exhibiting a fairly linear relationship between ion release and HP concentration (Fig. 1). The ion release data for mercury and silver follow a similar trend especially at high HP concentrations.

The quantity of mercury released from dental amalgam as reported in scientific journals varies considerably. The data reported by Rotstein et al.[16] maybe recalculated to show between 0.60 and 4.24 $\mu g/mm^2$ of mercury released directly into CP solutions. In a further paper by Rotstein et al.,[24] the amount of mercury released from amalgams treated with 10% CP was very similar to that released by 10% HP. The data reported for mercury release by Hummert et al.[14] and Mackert and Berglund[15] were similarly recalculated to be between 0.014 and 0.020 $\mu g/mm^2$ and 0.016 $\mu g/mm^2$, respectively. It is obviously difficult to make direct comparisons between our data and published figures due to lack of standardisation of experimental details and the form of data presentation. Recalculation of Robertello et al. data,[13] for example, was not possible due to the lack of experimental details given in their paper.

The release of metal ions from restorations had been reported to be time dependent and proportional to the surface area of the restoration.[19] Exposure to these metal ions, particularly mercury, is a potential hazard and could cause adverse effects. The World Health Organisation (WHO) guidelines for maximum intake of mercury is 40 μg/day. In the present study the average amount of mercury release from a typical sample was 28.6, 15.6, 6.3 and 7.2 μg/day for 30%, 10%, 3% and 1% HP concentrations, respectively. The total surface area of the cylindrical amalgam discs was 220 mm^2. Therefore average mercury release from a sample over a 24 h period was 0.130, 0.071, 0.029 and 0.033 $\mu g/mm^2$. Assuming the area of a typical restoration in the mouth to be 5 mm × 5 mm approximately, based on these data a single restoration will release on average 3.245, 1.775, 0.718 and 0.818 μg/day at 30%, 10%, 3% and 1% HP concentrations (v/v), respectively. Clearly, to exceed WHO's maximum acceptable intake of 40 μg/day would require mercury release from 12, 23, 56 and 49 restorations when treated with 30%, 10%, 3% and 1% HP concentrations, respectively. It is therefore unlikely that metal ion release from amalgam following contact with tooth bleaching agents containing up to 10% HP constitutes a hazard to health. While this is encouraging, it should be noted that some manufacturers advocate the use of even higher concentrations of HP such as the 30% reported here. This consideration, combined with the trend towards direct marketing of tooth bleaching products to the general public, suggests that the situation should be closely monitored by government and the dental profession to ensure that no new hazards present themselves in the future.

The data for mercury release reported here for 10% HP is about 0.07 $\mu g/mm^2$; an order of magnitude less than that reported by Rotstein et al.[16] but about five times higher than those reported by Hummert et al.[14] and Mackert and Berglund.[15] Large fluctuations in the reported values of measured mercury is not uncommon. It is partly due to the difficulties involved in measuring accurately the released mercury ions, and great care has been taken in this study to overcome these problems. The ICP-MS instrument used for ion release measurements was carefully calibrated each time using a known concentration of mercury standard. Additionally, a strict specimen preparation protocol was followed to ensure uniformity in preparation of the amalgam discs.

5. Conclusions

Metal ions (Hg, Ag, Cu and Sn) were released from amalgam following all treatments. The rate of ion release increased with increasing HP concentration and was statistically significant compared with control treatment ($p < 0.05$). Differences in surface roughness values before and after bleaching were not statistically significant ($p > 0.05$). We conclude that the trend towards using increasing concentrations of HP in tooth bleaching is undesirable without further research before these products are made available. The most oxidative bleaches, therefore, should only be available to dental professionals and they should not be incorporated into over the counter or 'home kits'.

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Appendix III

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The effect of hydrogen peroxide concentration on metal ion release from dental casting alloys

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SUMMARY **There are concerns that tooth bleaching agents may adversely affect dental materials. The aim of this study was to test the hypothesis that increasing concentrations of hydrogen peroxide (HP) are more effective than water at increasing metal ion release from two typical dental casting alloys during bleaching. Discs (n = 28 for each alloy) were prepared by casting and heat treated to simulate a typical porcelain-firing cycle. Discs (n = 7) of each alloy were immersed in either 0%, 3%, 10% or 30% (w/v) HP solutions for 24 h at 37 °C. Samples were taken for metal ion release determination using inductively coupled plasma-mass spectrometry and the data analysed using a two-way ANOVA followed by a one-way ANOVA. The surface roughness of each disc was measured using a Talysurf contact profilometer before and after bleaching and the data analysed using a paired t-test. With the exception of gold, the differences in metal ion concentration after treatment with 0% (control) and each of 3%, 10% and 30% HP (w/v) were statistically significant ($P < 0{\cdot}05$). Metal ion release from the two alloys increased with increasing HP concentrations (over 3000% increase in Ni and 1400% increase in Pd ions were recorded when HP concentration increased from 0% to 30%). Surface roughness values of the samples before and after bleaching were not significantly different ($P > 0{\cdot}05$) Exposure of the two dental casting alloys to HP solutions increased metal ion release of all the elements except gold.**

KEYWORDS: **tooth bleaching, metal ceramic alloys, ion release, surface roughness**

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Introduction

There is an increasing demand for aesthetic procedures in dentistry to satisfy high patient expectations. Tooth bleaching, in particular, has become popular in recent years. Hydrogen peroxide (HP) is the most common bleaching agent, used either directly or following generation by carbamide peroxide (CP) (1, 2).

While the effects of bleaching agents on oral tissues are relatively well known (3–5), there has been relatively little study of their effects on dental materials. Bleaching agents may come into contact with restorative materials, particularly when using mouth guard bleaching or home kits (6, 7). Amalgam restorations and casting alloys are known to corrode on contact with a strong oxidizing agent such as HP and metal ions may be released into the oral cavity (8–11). Elemental release from dental alloys is related to known biocompatibility issues because of the interaction of these elements with surrounding or systemic tissues (12–16).

The phase structure of an alloy is critical to its corrosion properties and therefore it is also related to biocompatibility (15, 17, 18). Multiple phase alloys tend to release more elements than single phase alloys (17, 19). Certain metallic elements or ions have a tendency to be released (lability) regardless of the alloy composition (14). Copper, nickel and gallium, for example, are labile elements whereas gold, palladium and platinum have low labilities.

A number of studies have shown that environments around the alloy will influence corrosion (12–14, 20), with acidic environments increasing corrosion in some

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systems (21, 22). This is more relevant to Ni-based alloys, but not the high noble alloys (23, 24). The corrosion of alloys may be investigated using a variety of techniques including visual observation of the alloy surface, electrochemical tests that measure elemental release indirectly through the flow of released electrons, or by direct measurements of the released elements (14).

For direct measurements of ion release, Tufekci *et al.* (25) used inductively coupled plasma-mass spectrometry (ICP-MS) to measure the *in vitro* elemental release from two high palladium alloys into a corrosion medium having a pH-value of 2·24. They reported a significant increase in ion release with increased immersion time. It was also reported that there were significantly more ions released into the solution from the Pd-Cu-Ga alloy compared to the Pd-Ga alloy. The mean measured ion release data for Pd-Cu-Ga alloy at 7 h immersion time was reported to be 0·74 μg cm^{-2} for Pd, 0·57 μg cm^{-2} for Ga, 1·50 μg cm^{-2} for Cu and 0·06 μg cm^{-2} for Sn. Wataha *et al.* (20) also used ICP-MS to investigate the effect of pH on element release from dental casting alloys. High noble, noble and base metal casting alloys were treated in different solutions with pH-values of 1, 4 and 7. High noble and noble alloys were resistant to acidic environments, whereas, Ni-based alloys released large amounts of Ni in solutions of pH-values of 1 and 4. For the Pd-Cu-Ga alloy, they reported ion releases of 0·02 μg cm^{-2} for Pd and 0·01 μg cm^{-2} for Cu at pH-value of 4. For the base metal alloy, they reported 1·25 μg cm^{-2} Ni release.

Clearly, there is a dearth of data in the published literature regarding the effect of bleaching agents on ion release because of corrosion of metallic restorations (26, 27). This study was designed to test the hypothesis that HP bleaching agents (3–30% w/v) are more effective than water in increasing metal ion release from Ni-Cr and Pd-Cu-Ga alloys.

Materials and methods

Materials

Two typical metal alloys were evaluated, a Ni-Cr alloy* and a Pd-Cu-Ga alloy†. The composition (% w/w) for the two alloys is presented in Table 1. Based on the % (w/w) of the elements in Table 1, the Ni-Cr is a base metal alloy and the Pd-Cu-Ga is a noble alloy as classified by the American Dental Association (17). Preparation of test discs (n = 28 for each alloy) was carried out using the lost wax technique. The discs were made in wax from silicone moulds (10 mm × 2 mm for the Ni-Cr discs giving a total surface area of 2·2 cm^2 for each disc and 5 mm × 1 mm for the Pd-Cu-Ga discs with a total surface area of 0·55 cm^2; for each disc), identically sprued and invested in phosphate-bonded investment. Melting and casting of the alloys were carried out using an induction casting machine‡. Castings were allowed to bench cool, devested and sprues with excess alloy ground away. Cast discs were subjected to heat treatment for VITA§ porcelain-firing cycles. Both sides of each disc were polished using fine stones, rubber wheels and bristle brushes loaded with universal polish and finally lambs wool mop. The surface roughness of each disc was determined by using a Talysurf contact profilometer¶ and stored in individually labelled polythene bags. Volumetric analysis based on iodine thiosulphate titration was performed to establish the active concentration of HP in test solutions (28). Five titrations were carried out by using nominally a 30% HP (w/v) solution** and the results recorded as the mean and s.d. The HP solution was diluted to obtain 3% (w/v), 10% (w/v) as well as 30% (w/v) HP solutions with high-purity distilled water as the control (0% HP). The pH of the HP solutions was measured with a pH meter††. The pH meter was first calibrated using two standard buffers of pH 4 and 7.

Table 1. Alloy composition

Ni-Cr alloy		Pd-Cu-Ga alloy	
Element	% (w/w)	Element	% (w/w)
Ni	65·0	Au	2·0
Cr	22·5	Pd	78·5
Mo	9·5	Cu	6·9
Si	1·0	Ga	5·5
Nb	1·0	In	4·5
Fe	0·5	Sn	2·0
Ce	0·5	Zn and Ru	1·0 max
C	0·02 max		

*Wiron 99; Bego, Bremen, Germany.
†Cerapall II, Metalor; Technologies SA, CH-2009 Neuchatel, Switzerland.
‡Modular 3S; ASEG Galloni, Italy.
§Vita Zahnfabrik H. Rauter GmbH & Co., Bad Sackingen, Germany.
¶Mituyoto Corporation, Kawasaki, Japan.
**Aristar Hydrogen Peroxide, VWP International Ltd, Poole, UK.
††Checker 1; Hanna Instruments, Leighton Buzzard, UK.

Ion release

The 28 discs, for each alloy, were randomly divided into four equal groups. The discs in a group ($n = 7$ per group) were immersed in either 0%, 3%, 10% and 30% HP solutions (20 mL for Ni-Cr alloy and 10 mL for Pd-Cu-Ga alloy) for 24 h at 37 °C creating seven samples of each solution (2, 29). This was carried out by placing each disc in a tapered centrifuge tube, with all surfaces exposed to the particular HP concentration in that tube. All the solution samples were analysed by ICP-MS, Agilent 4500. ICP-MS detection limits for the target elements in the two alloys are given in Table 2. The surface area of the discs to the volume ratio of bleaching solution was 0·055 $cm^2\ mL^{-1}$ for the Pd-Cu-Ga alloy and 0·11 $cm^2\ mL^{-1}$ for the Ni-Cr alloy which are below the range 0·5–6·0 $cm^2\ mL^{-1}$ recommended by ISO Standard 10933 (30). As no biological studies were being performed, our experimental sample surface area to bleaching solution volume ratios was considered acceptable (25, 30).

For each analysis, the ICP-MS instrument performs five measurements and calculates the mean and relative s.d. (%) for each element. Thus, with the seven discs tested in each group, the total number of measurements recorded per element was 35.

Surface roughness

Following ion release experiments, the surface roughness of the discs was measured again using a Talysurf instrument (with a conical-shaped stylus measuring 0·830 mm at the base and 0·015 mm at the tip) that was calibrated in accordance with manufacturers instructions. For each disc, the roughness of both polished surfaces was then measured by moving the stylus across its diameter. The procedure was repeated several times for each disc altering the orientation after each measurement.

Statistical analysis

A two-way ANOVA (element by concentration) revealed a significant interaction between concentration and elements ($P < 0·001$) for both alloys indicating that differences between solutions were different across elements. The two-way ANOVA was followed by a one-way ANOVA and Dunnet's *post hoc* test for multiple comparisons between solutions for each element. The roughness measurements were analysed by using a paired *t*-test.

Results

The measured pH-values for 0%, 3%, 10% and 30% HP concentrations were 6·41, 5·62, 4·75 and 3·83, respectively. Values for the mean and s.d. of elemental ion release data measured in $\mu g\ L^{-1}$ were converted to units of $\mu g\ cm^{-2}$ and are shown in Tables 3 and 4 for Ni-Cr and Pd-Cu-Ga alloys, respectively. This was obtained by dividing the total amount of ion release in microgram over a 24-h period for each element by the total surface area of the corresponding disc. Tables 3 and 4 show a steady increase in ion release values from all the constituent elements of the two alloys, except gold, with increasing HP concentration. The constituents with percentage weight of 1% or less in the two alloys were not included in the tables. For each sample tested, the ICP-MS recorded ion release values of all the elements in the periodic table. It was noted that the mean ion release value of Pd from the Ni-Cr alloy was 0·00015 $\mu g\ cm^{-2}$ and that of Ni from the Pd-Cu-Ga alloy was 0·002 $\mu g\ cm^{-2}$ at 30% HP concentration. These values are negligibly small which, in turn, adds weight to the accuracy of the recorded data.

The distribution of the recorded ion release data (not shown here) was normal for all elements. The *P*-values for the one-way ANOVA and Dunnet's *post hoc* test are

Table 2. Inductively coupled plasma-mass spectrometry detection limits for elements

Elements	Detection limits ($ng\ L^{-1}$)
Ni, Mo, Pd, Sn and Ga	1
Cr, Cu and In	3
Au	6

Table 3. Mean and s.d. Ni-Cr alloy

	Nickel ($\mu g\ cm^{-2}$)		Chromium ($\mu g\ cm^{-2}$)		Molybdenum ($\mu g\ cm^{-2}$)	
(HP)	Mean	s.d.	Mean	s.d.	Mean	s.d.
0	0·26	0·08	0·01	0·01	0·07	0·03
3	0·90	0·16	0·75	0·11	1·43	0·31
10	5·05	1·50	3·63	1·21	4·11	0·76
30	8·23	0·49	6·65	0·91	4·62	1·07

Table 4. Mean and s.d. for Pd-Cu-Ga alloy

	Palladium ($\mu g\ cm^{-2}$)		Copper ($\mu g\ cm^{-2}$)		Gallium ($\mu g\ cm^{-2}$)		Indium ($\mu g\ cm^{-2}$)		Tin ($\mu g\ cm^{-2}$)		Gold ($\mu g\ cm^{-2}$)	
(HP)	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
0	$9{\cdot}90^{-03}$	$2{\cdot}70^{-03}$	$1{\cdot}70^{-02}$	$2{\cdot}42^{-03}$	$3{\cdot}04^{-03}$	$7{\cdot}27^{-04}$	$2{\cdot}91^{-03}$	$8{\cdot}91^{-04}$	$2{\cdot}24^{-03}$	$3{\cdot}27^{-04}$	0·05	0·02
3	0·06	$7{\cdot}82^{-03}$	$2{\cdot}48^{-02}$	$4{\cdot}91^{-03}$	$8{\cdot}73^{-03}$	$1{\cdot}64^{-03}$	$6{\cdot}55^{-03}$	$1{\cdot}55^{-03}$	$3{\cdot}69^{-03}$	$4{\cdot}55^{-04}$	0·04	$5{\cdot}42^{-03}$
10	0·12	0·01	0·04	$6{\cdot}73^{-03}$	0·01	$3{\cdot}05^{-03}$	0·01	$3{\cdot}55^{-03}$	$8{\cdot}24^{-03}$	$1{\cdot}30^{-03}$	0·03	$5{\cdot}29^{-03}$
30	0·28	0·02	0·09	0·01	0·04	$6{\cdot}55^{-03}$	0·03	0·01	0·26	$2{\cdot}05^{-03}$	0·04	$7{\cdot}38^{-03}$

shown in Tables 5 and 6 for Ni-Cr and Pd-Cu-Ga alloys, respectively. The difference in metal ion release between 0% HP (control) and all other concentrations (3%, 10% and 30%) was statistically significant ($P < 0{\cdot}05$) for all elements except gold (the *P*-values for gold are not included in Table 6). Additionally, there was a generally significant change in ion release data at each increase in HP concentration except for Mo (Table 5) where there was no significant difference ($P > 0{\cdot}05$) when HP concentration was increased from 10% to 30% and indium (Table 6) when HP concentration changed from 3% to 10%. The average roughness values for Ni-Cr and Pd-Cu-Ga alloys before and after treatment is shown in Fig. 1a and b. Paired *t*-tests showed no significant difference ($P > 0{\cdot}05$) in mean surface roughness values before and after bleaching in all the groups.

Discussion

With the exception of gold, ion release increased with increasing HP concentration for both alloys. Generally, Pd-Cu-Ga alloys are considered to be more corrosion resistant than non-precious metal-based alloys. In this study, the amount of ion release of all elements from Ni-Cr alloy was higher than those released from the elements in the Pd-Cu-Ga alloy at 3–30% HP concentration. The Ni-Cr alloy used here was a Ni high Cr alloy which is the most resistant of the Ni-based group having a Cr content of 22·5% (w/w). The corrosion of these alloys is far better than the Ni-Cr-Be alloys (as Be lowers the corrosion resistance of these alloys), but not as good as the noble alloy groups (13, 17, 31, 32).

The *P*-values (Tables 5 and 6) showed that there were significant differences ($P < 0{\cdot}05$) between the

Table 5. Multiple comparisons – *P*-values for Ni-Cr alloy

	P-values (unequal variance)								
	Nickel			Chromium			Molybdenum		
(HP)	3	10	30	3	10	30	3	10	30
0	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001
3	–	<0·001	<0·001	–	<0·001	<0·001	–	<0·001	0·001
10	<0·001	–	<0·001	<0·001	–	<0·001	<0·001	–	0·840

Table 6. Multiple comparisons – *P*-values for Pd-Cu-Ga alloy

	P-values (unequal variance)														
	Palladium			Copper			Tin			Indium			Gallium		
(HP)	3	10	30	3	10	30	3	10	30	3	10	30	3	10	30
0	<0·001	<0·001	<0·001	0·026	<0·001	<0·001	<0·001	<0·001	<0·001	0·002	0·003	0·003	<0·001	<0·001	<0·001
3	–	<0·001	<0·001	–	0·005	<0·001	–	<0·001	<0·001	–	0·069	0·005	–	0·011	<0·001
10	<0·001	–	<0·001	0·005	–	<0·001	<0·001	–	<0·001	0·069	–	0·015	0·011	–	<0·001

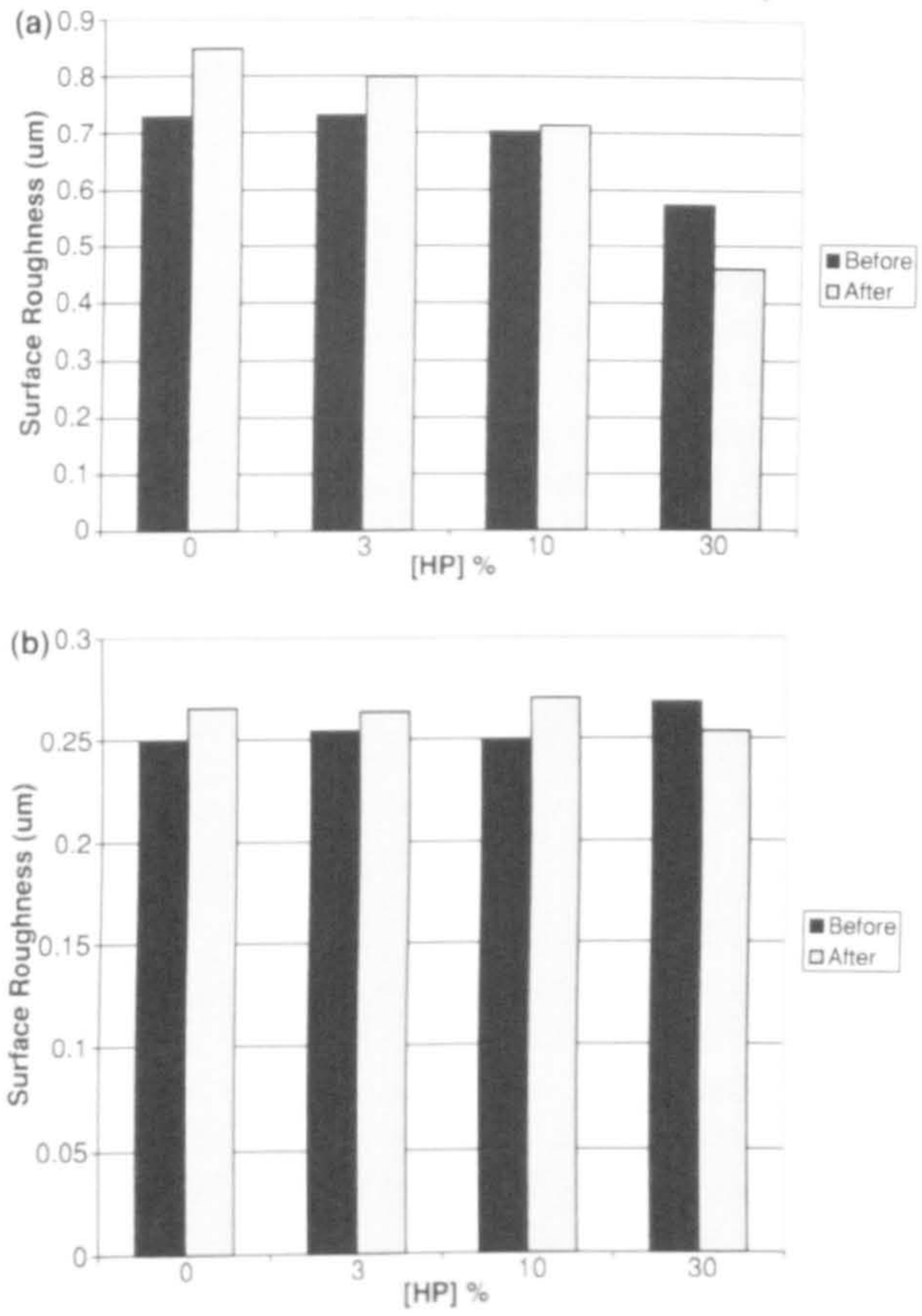

Fig. 1. Mean surface roughness values (1a) Ni-Cr and (1b) Pd-Cu-Ga alloys. Paired *t*-tests showed no statistically significant differences ($P > 0{\cdot}05$) in surface roughness before and after bleaching.

control and each of the three HP concentrations for the elements in the two alloys given in these tables. There was also a significant change in ion release data every time the HP concentration changed (except for molybdenum between 10% and 30% HP and indium between 3% and 10% HP). This indicated an increase in corrosion with increasing HP concentrations. The statistical tests for the surface roughness data showed no statistical significant differences before and after bleaching at all concentrations ($P > 0{\cdot}05$).

It is difficult to compare our data with those in the published literature because of lack of standardization in previously reported testing procedures. There are differences in the units of the data presented, exposure times, pH of the treatment solutions and HP concentration. To facilitate meaningful comparison, it was necessary first to convert the units of our measured ion release data from $\mu g\ L^{-1}$, as recorded by the ICP-MS instrument to $\mu g\ cm^{-2}$ to correspond to the units quoted, for example, in references (20) and (25). Secondly, it was necessary to account for the differences in the exposure times. For this purpose, an attempt was made to calculate the ion release data by Tufekci *et al.* (25) at 24 h exposure time by applying a linear interpolation to their recorded data at 7 and 70 h. This resulted in ion release data of 5·67, 4·50, 4·69, 0·15 and 0·05 $\mu g\ cm^{-2}$ for Pd, Cu, Ga, Sn and In, respectively, when treated with a solution of pH 2·24. Our ion release data expressed in $\mu g\ cm^{-2}$ per surface area at 30% HP (w/v) are much lower than the corresponding values reported by Tufekci *et al.* (25). The differences can be attributed, in part, to differences in pH-values, as Tufekci *et al.* investigated corrosion under more acidic conditions (pH 2·24 compared with 3·83). Wataha *et al.* (20) reported ion release data of about 0·02 $\mu g\ cm^{-2}$ for Pd and 0·01 $\mu g\ cm^{-2}$ for Cu from a Pd-Cu-Ga alloy exposed to a solution of pH-value of 4 for a period of 30 min. Data presented here show much higher release figures than the corresponding ones reported by Wataha *et al.* The differences may be partly because of differences in exposure times and pH-values. Further work is needed in this area, particularly regarding the standardization of the testing procedures.

The surface area for typical restorations fabricated from dental alloys vary from about 0·33 cm^2 for the exposed palatal metal collar of a metal ceramic crown to about 1·50 cm^2 for a full coverage metal crown. Estimates of daily intake in the diet of some of the elements in dental alloys which are of interest to the present work are 400 μg for Ni and Mo, 240 μg for Cr, and 3110 μg for Cu (14). With regard to Ni release, based on our measured data, the daily diet figures equate to ion releases from about 147 metal ceramic crowns or 32 full coverage crowns treated with 30% HP concentration over 24-h period. The corresponding numbers for the other three elements quoted are 262 and 58 crowns for Mo, 109 and 24 crowns for Cr, 104 714 and 23 037 crowns for Cu. There are a large number of other elements taken daily in the diet which are also released from dental alloys. A number of these elements are needed for normal body function (e.g. Zn), whereas others (e.g. Pd), if introduced in excess, could become toxic. The route, however, by which an element gains access to the body is critical to its

biological effects. Elements released from a casting alloy enter the oral cavity rather than the body. Similarly, the elimination of an element from the body also depends on its route of access to the body. Elements released from casting alloys introduced through the oral cavity are believed to be eliminated from the body in a relatively short space of time (14). Whereas the risk of systemic adverse reactions due to elements released from dental alloys though clearly elevated is, however, unlikely to constitute a health hazard (33). A dental crown can, however, extend below the level of the gingival margin, forming a sulcus between the gingiva and the alloy (14). If elements from the alloy are released into the sulcus, they may reach high enough concentration to cause a local adverse reaction (34).

Conclusions

Metal ions were released from both dental alloys following all treatments. The rate of ions released for all elements except Au increased with increasing HP concentrations and was statistically significant ($P < 0{\cdot}05$). Differences in surface roughness values before and after bleaching were not statistically significant ($P > 0{\cdot}05$). An increase in elemental ion release into the oral cavity from dental casting alloys following bleaching can trigger allergic reactions and caution need to be exercised in particular when applying HP at high concentrations.

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