Chapter 5 Experimental

5.1. Chemicals and analysis techniques

Chemicals and materials

All chemicals were purchased from Sigma-Aldrich and used as received without further purification unless stated otherwise. Bisnitroxide disulfide 'DIS3' was synthesized by lab colleagues. TEMPO oxoammonium chloride was obtained from Nufarm Ltd. The gas-permeable Teflon tube was obtained from Zeus Industrial Products. Distilled organic monomers (styrene, acrylic acid, methyl methacrylate and acrylonitrile) were supplied by Nufarm Ltd.

NMR

^{1}H and ^{13}C NMR spectra were recorded in the deuterated solvent as indicated, on JEOL ECX 270 MHz, ECX 400 MHz or ECS 400 MHz spectrometers. Chemical shifts are reported in parts per million using the deuterated solvent as an internal reference. Spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), qu (quintet), sx (sextet), sept (septet), m (multiplet), br (broad).

IR

Infra-red spectra were recorded on a JASCO FT/IR-400 spectrometer. For the gas-phase IR measurements, gas samples were obtained from a sealed system. The systems were degassed using vacuum and freeze-thaw method and re-filled with N_2. Gas formation can be observed from the readings of an injected gas syringe. The obtained gas samples were injected into a gas-tight IR cuvette cell (KBr) for measurements.

UV-*vis*

UV-*vis* spectra were recorded on a double beam Hitachi U3000 spectrophotometer. In order to ensure the absorbance falls between 0.1-1 range, typical concentrations used for gold nanoparticles and oxoammonium salt are 5×10-7 M and 0.037 M, respectively.

MS and GC-MS

Mass spectra were recorded on a Bruker micro-TOF with LCQ ion trap with the indicated ion sources. Gas Chromatography was performed on a VARIAN CP-3800 Gas chromatograph prior to MS analysis where indicated. The GC column used is a Zebron ZB-5HT inferno column. The temperature program was held at 60 ℃ for 2 min, and then increased from 60 to 350 ℃ by a 5 ℃ per min increase and finally an isothermal period of 10 min at 350 ℃. Helium was used as carrier gas at a flow rate of 2.2 ml/min.

EPR

Electron Paramagnetic Resonance spectra were recorded on Bruker EMX_{micro} and ESP300E X-band CW-EPR spectrometers. The parameters applied for the EPR measurements vary for different samples. Typical parameters used for organic samples are: modulation amplitude = 1.0 G, microwave power = 5.00 mW, acquisition time = 160 s. For aqueous samples, due to high polarity of the solvent and relatively small linewidth, typical parameters applied are: modulation amplitude = 0.5 G, microwave power = 1.00 mW, acquisition time = 160 s.

Stopped-flow EPR experiments were performed on a home-made triple-mixing stopped-flow apparatus with a standard setup. The mixing cuvette was connected to a quartz EPR flat cell which was placed in the EPR cavity. A time scan on the appropriate magnetic field is performed immediately following mixing of the components.

TGA

Thermogravimetric analysis was performed on a PL Thermal Sciences STA-625 analyzer.

CHN

Elemental analysis was carried out on CE-440 Exeter Analytical Inc. C, H, N, S machine. Standard for calibration used was acetanilide and internal standard check is *S*-benzyl thiouronium chloride.

TEM

Transmission Electron Microscopy of AuNPs was carried out on a FEI Tecnai G2 electron microscope. Samples were obtained by evaporating dissolved nanoparticles onto a copper grid. Core size was calculated using free software 'ImageJ'.

GPC

Gel Permeation Chromatography was performed in methylbenzene or dichloromethane using 200-400 mesh Bio beads-S-XI, purchased from BioRad Laboratories.

TLC

Thin Layer Chromatography analysis was performed on Merck Silica Gel 60 F245 aluminium backed silica plates and visualised by ultraviolet light (254 nm).

GC-FID

Detection of gas phase hydrocarbons was performed[1] on a FGAM dual channel gas chromatograph equipped with flame ionization detector (Perkin-Elmer). Commercial software (Totalchrom) is used for integrating chromatograms. Gas samples were acquired using a gas-syringe from the headspace of the sealed reaction mixture. With the help of a gas valve, sample is passed through a series of adsorbent traps (Carboxen 1000+Carbopack B) which is Peltier cooled (-23 ℃). After acquiring sufficient sample volume, the trap is flushed with helium for 2 min so as to elute methane from the system which cannot be trapped quantitatively. Then analytes are thermally desorbed at 320 ℃ and flushed into the columns. The sample is split in a 50:50 ratio between a Al_2O_3 PLOT and CP LOWOX column in GC oven for their separation. Oven is programmed at 40 ℃ for 16.5 min then heated at a rate of

13 °C·min^{-1} to 110 ℃ and then heated at a rate of 8 °C·min^{-1} to 200^{o}C where it remains for 20 minutes. Eluents from each column are analyzed using separate flame ionization detectors (FID). Each run takes one hour to complete. The detection limit for most of the compounds for 1 litre sample is between 2-9 ppt (v/v).

5.2. General procedures of EPR experiments

5.2.1. Preparing EPR samples

EPR is a very sensitive technique and its detection limit can reach in the vicinity of 10^8 spins. For nitroxides, a concentration of 10^{-6} M is detectable, and 10^{-5} M will give a decent signal. In this work, typically samples with concentrations of 10^{-4} to 10^{-3} M were used. Tubes used for EPR measurements were typically a glass Pasteur pipette, with the bottom end flame sealed. The top end is open to air, or sealed with lab film to avoid changing of sample concentration due to solvent evaporation. The volume of sample used in such tubes is around 70 μL. The oximetry studies required a closed system. In this case, melting point capillaries were used for EPR measurements. Typically a melting point capillary was flame sealed on one end, and sample solution was injected through a fine capillary, leaving *ca.* 1/5 of the tube length empty. The capillary thus holds *ca.* 30 μL sample solution. Then the open end was also flame sealed. Oxygen present in such samples is mainly dissolved in solution. The sealed melting point capillary was then placed in a larger tube (*e.g.* an NMR tube) and EPR measurement was carried out. A sketch of the tubes used in this study is shown in Figure 5.1.

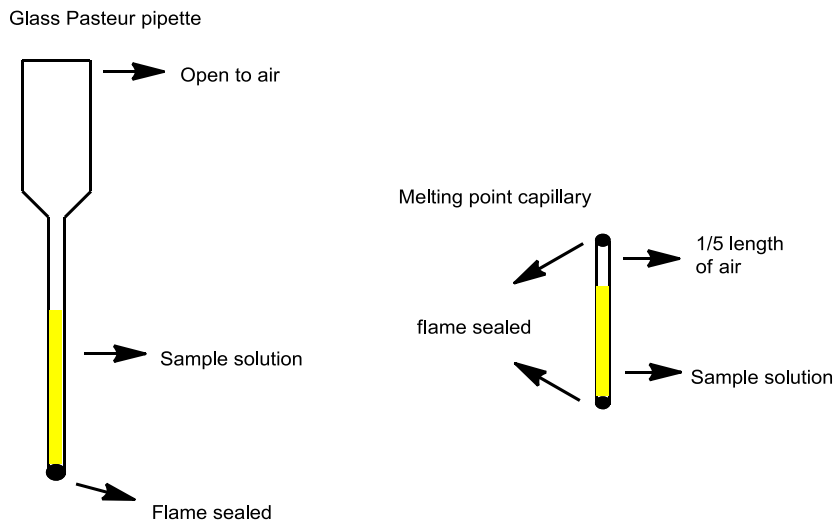


Figure 5. 1. A sketch of tubes used to acquire EPR spectra in normal conditions and in closed systems (*e.g.* for oximetry studies).

5.2.2. Degassing EPR samples

EPR samples occasionally need to be degassed before monitoring, in order to obtain better signal or to monitor reaction in the absence of oxygen. A typical degassing procedure is:

N_2 gas was blown through the liquid EPR sample for 1 minute to remove substantial amount of dissolved oxygen. Oxygen was further removed by a freeze-thaw method. The sample was placed into liquid N_2 and then attached to a Schlenk system, to allow the sample liquid to freeze under vacuum. After the sample was completely frozen (*ca.* 30 s), the sample was detached from the Schlenk line and taken out of liquid N_2, which allowed it to thaw under ambient. The freeze-thaw procedure was repeated five times to completely remove dissolved oxygen.

5.2.3. EPR kinetics

In the kinetic studies, EPR spectra were recorded using a specially written automation program. The program allows automatic multiple EPR measurements with a user defined time interval. The obtained spectra were converted into ASCII format and treated with a spreadsheet in which a Macro was implanted to allow

automatic measurements of the peak height within a user-defined field range. The resulting kinetic profiles were fitted to kinetic models using the procedure described in 'Kinetic studies'.

5.3. Data processing procedures

5.3.1. Kinetic studies

Kinetic data were fitted to the indicated kinetic model using Microsoft Excel add-in 'solver' or with the 'fitting' function of Origin. A fitting curve was generated using equations of the intended kinetic model including adjustable reaction rate constant, initial and end concentrations of the reactants. The fitting was performed by minimizing the sum square of the differences of the data points and the fitting curve via alternation of the kinetic parameters. Errors of the kinetic investigations were obtained from the standard deviations of the simulated rate constants.

5.3.2. Oximetry fitting

A series of EPR spectra obtained at different oxygen concentrations were analyzed using software 'EWVoigt' developed by Smirnov[2]. The Lorentzian broadening was obtained by fitting the spectra against the unbroadened spectrum. For systems with small broadening, the Lorentzian function was obtained by fitting the spectra against a manually simulated sharp spectrum (*e.g.* by manipulating linewidth parameters).

5.4. Synthesis

n-Butanethiol AuNPs

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4$ $3H_20$, 1.0 g, 2.5 mmol) was dissolved in deionized water to make a 100 mL solution. This 1% w/w solution (4.0 g, 0.1 mmol) was mixed with tetraoctylammonium bromide (TAOB, 280 mg) and methylbenzene (10 mL). The mixture was stirred for 3 min until all Au(III) precursor was transferred to the organic phase. 10% w/w solution of n-butanethiol in methylbenzene (90 mg, 0.1 mmol) was then added to the reaction mixture. A solution of $NaBH_4$ (40 mg, 1.05 mmol) in deionized water (3 mL) was added under vigorous stirring. $NaBH_4$ needs to be added immediately after adding ligand. After 3 minutes, the organic phase was separated and solvent was evaporated at 35 ℃ under vacuum. Methylbenzene (3 mL) was then used to transfer AuNPs to a 50mL centrifugation tube. Methanol (50 mL) was added to the centrifugation tube and a dark precipitate was formed instantly. This crude precipitate was washed with methanol (3×50 mL aided with sonication) and dried under N_2 flow. Product was a dark powder. Yield: 19.6mg, 73%. Yield was calculated using Equation 5.1 by assuming the composition of AuNPs as $Au_{450}(Ligand)_{150}$, which is obtained from particle size and organic content analysis.

$$Yield = \frac{m(product)}{\frac{m[HAuCl_4(aq)]\times 1\%}{M(HAuCl_4)\times 450}\times M(Au_{450}Ligand_{150})} \quad (5.1)$$

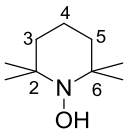
n-Octanethiol AuNPs

1% w/w aqueous hydrogen tetrachloroaurate (5.9 g, 0.15 mmol) was mixed with TOAB (400 mg) and methylbenzene (10 mL). *n*-Octanethiol (22 mg, 0.15 mmol) was added to the reaction mixture followed by immediate addition of $NaBH_4$ (60 mg, 1.57 mmol) aqueous solution (3 mL) with vigorous stirring. The purification process was the same as described for *n*-butanethiol AuNPs. Yield: 29.4mg, 69%.

n-Octadecanethiol AuNPs

1% w/w aqueous hydrogen tetrachloroaurate (12.67 g, 0.32 mmol) was mixed with TOAB (800 mg) and methylbenzene (20 mL) with stirring for 5 minutes. *n*-Octadecanethiol (90 mg, 0.31 mmol) was added as a protecting ligand and then $NaBH_4$ (200 mg, 5.3 mmol) aqueous solution (3 mL) was added immediately with vigorous stirring. After applying the purification procedures described for *n*-butanethiol AuNPs, the product was further purified by Gel Permeation Chromatography using a Bio Beads methylbenzene column. The product was rotary evaporated and dried under N_2 flow.

General procedure for the synthesis of TEMPO hydroxylamine (TEMPOH)



Preparation of TEMPOH was undertaken following literature procedures[3]. TEMPO (1.56g, 0.01mol) was mixed with H_2O (40mL) to form suspension. To this suspension, calcium *L*-ascorbate dihydrate (2.13g, 0.005mol) was added under stirring. The reaction mixture was further stirred for *ca.* 15 min, until the red colour of TEMPO turned to light yellow. The reaction product was extracted by diethyl ether (4×10 mL). The extraction was washed by H_2O (2×10 mL). The combined organic phase was dried with $MgSO_4$. Solvent was dried under vacuum at room temperature. The resulting light orange product was recrystallized from pentane, leading to white needle-crystals. TEMPOH is readily oxidized to TEMPO in air, therefore is stored under N_2 at -18 ℃. Yield: 0.22 g, 14%. ^{1}H NMR (400 MHz, D_2O): δ 1.45-1.19 (m, 4H, H^3 H^5), 1.09 (d, 2H, H^4), 0.97 (s, 12H, *gem*-CH_3). ^{13}C NMR (100.6 MHz, D_2O): δ 68.0 (C^1), 35.8 (C^3), 26.3 (*gem*-C), 18.1 (*gem*-C) 14.2 (C^4). HR(ESI-MS): $[M + H]^{1+}$, calculated 158.1546, found 158.1538.

References:

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2. T. I. Smirnova and A. I. Smirnov, in *ESR Spectroscopy in Membrane Biophysics*, eds. M. A. Hemminger and L. J. Berliner, Springer, Amsterdam, Editon edn., 2007.
3. S. Marque, H. Fischer, E. Baier and A. Studer, *J. Org. Chem.*, 2001, **66**, 1146-1156.