CHAPTER TWO

EXPERIMENTAL

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2.1 Materials and Reagents

Single-walled carbon nanotube-*poly* (m-amino benzene sulfonic acid) (SWCNT-PABS, Figure 2.1a), Epinephrine, 2-Dimethylaminoethanethiol hydrochloride ($HS(CH_2)_2N^+H(CH_3)_2Cl^-$ DMAET), Hexadecyltrimethylammonium bromide (CTAB) and N, N-Dimethylformamide (DMF) obtained from Sigma-Aldrich; DMF was distilled and dried before use. 5 nm Colloidal Gold (AuNP) of 0.01% $HAuCl_4$ concentration was purchased from Sigma. Monolayer-protected clusters of gold nanoparticles (MPCAuNP) were supplied by Mintek. Sodium 2-mercaptoethanesulphonate (MES) was obtained from Merck. Iron (II) tetrasulfophtalocyanine (FeTSPc - Figure 2.1b) was synthesised following the well established Weber and Busch strategy [1] and the nanostructured iron (II) phthalocyanine (*nano*FePc), was synthesized as described by Siswana *et al*. [2]. The main principle of the synthesis as depicted in Scheme 2.1 is simply the breaking of the intramolecular forces and protecting the species in CTAB environments. Tetrabutylammonium tetrafluroborate ($TBABF_4$) was Ultra pure water of resistivity 18.2 MΩ.cm was obtained from a Milli-Q Water System (Millipore Corp., Bedford, MA, USA) and was used throughout for the preparation of solutions. Phosphate buffer solutions

(PBS, pH 7.4) were prepared with appropriate amounts of K_2HPO_4 and KH_2PO_4. Potassium hexacyanoferrate (II) was obtained from B. Jones Ltd., SA, potassium hexacyanoferric (III) was purchased from Bio-Zone Chemicals, SA. All electrochemical experiments were performed with nitrogen-saturated phosphate buffer. All other reagents were of analytical grades and were used as received from the suppliers without further purification.
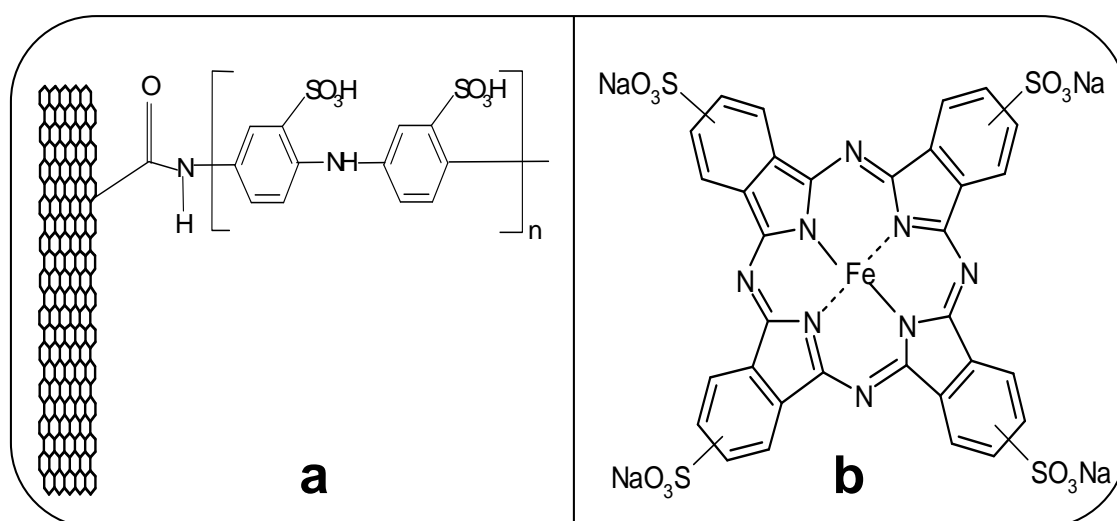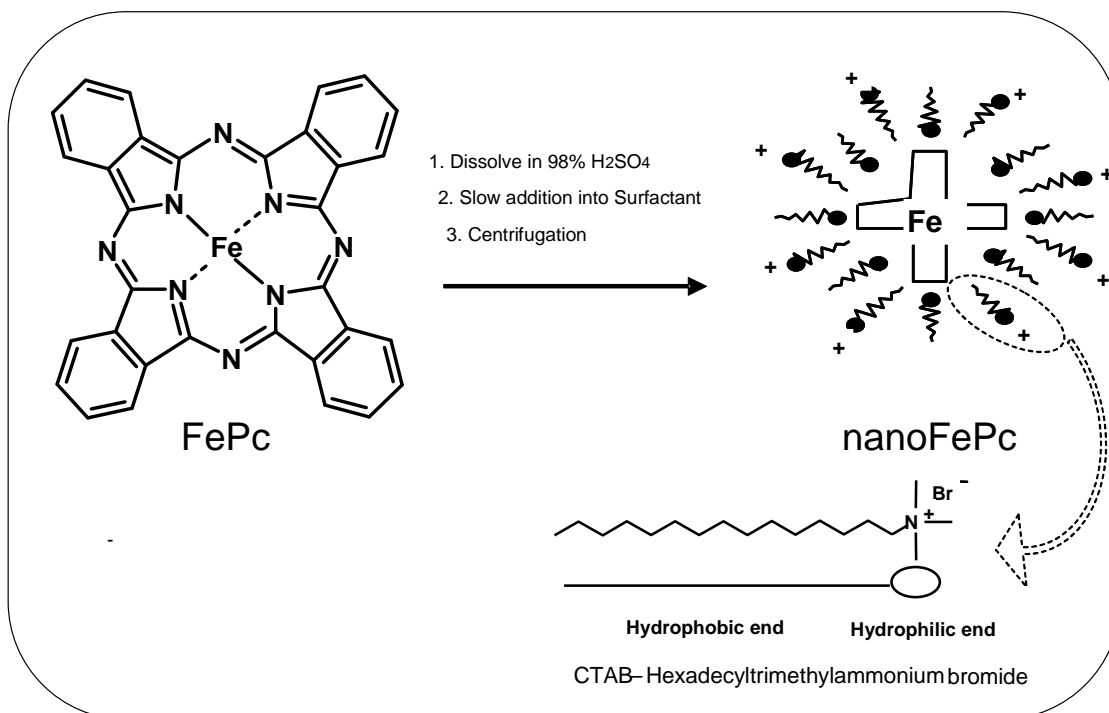

Figure 2.1: Molecular structure of (a) Single-walled carbon nanotube-*poly* (m-amino benzene sulfonic acid) and (b) Iron (II) tetrasulfophtalocyanine.

Scheme 2.1: Cartoon representation showing the synthesis of nanostructured Iron (II) phthalocyanine from Iron (II) phthalocyanine complex.

2.2 Apparatus and Procedure

All electrochemical experiments were carried out using an Autolab Potentiostat PGSTAT 302 (Eco Chemie, Utrecht, Netherlands) driven by the GPES and FRA softwares version 4.9). Electrochemical impedance spectroscopy measurements were performed using a 5 mV rms sinusoidal modulation in a solution of 1 mM of $K_4Fe(CN)_6$ and 1mM $K_3Fe(CN)_6$ (1:1) mixture containing 0.1 M KCl, and at the $E_{1/2}$ of the $[Fe(CN)_6]^{3-/4-}$ (0.124V vs. Ag|AgCl, sat'd KCl). The FRA software allowed the automatic fitting of the raw EIS data to equivalent circuit

models using a *complex non-linear least squares* (CNLS) method based on the EQUIVCRT programme [3], with *Krammers-Kronig rule check*. Gold electrode (BAS, r = 0.08 cm) was used as the working electrode. Ag|AgCl, sat'd KCl and platinum electrodes were used as pseudo-reference and counter electrodes, respectively. All solutions were de-aerated by bubbling pure nitrogen (Afrox) prior to each electrochemical experiment. All experiments were performed at 25±1°C. All pH measurements were performed using Labotec Orion bench top pH meter model 420A. Solutions were sonicated using a 220V UMC 5th Integral Systems sonicator. Transmission electron microscopy (TEM) studies of the particles were carried out at an accelerating voltage of 197 kV using a Philips CM200 microscope equipped with a LaB_6 source at Mintek Advanced Materials Division. Field emission scanning electron microscopy (FESEM) images were obtained using JEOL JSM 5800 LV (Japan) while the energy dispersive x-ray spectra were obtained from NORAN VANTAGE (USA) at the Microscopy and Microanalysis Laboratory of the University of Pretoria. All the AFM images were obtained using Eco-Chemie SPR gold disks and the AFM experiments were performed using a AFM 5100 System (Agilent Technologies, USA) using a AC contact mode AFM scanner interfaced with a PicoScan controller (scan range 1.25 μm in x–y and 2.322 μm in z). Silicon type PPP-NCH-20 (Nanosensors®) of thickness

4.0±1.0 µm, length 125±10 µm, width 30±7.5 µm, spring constants 10 – 130 N m^{-1}, resonant frequencies of 204 – 497 kHz and tip height of 10-15 µm were used. All images (256 samples/line × 256 lines) were taken in air at room temperature and at scan rates 0.9–1.0 lines s^{-1}.

2.3   Electrode Modification and Pre-treatment

Prior to the experiments, the Polycrystalline gold electrode (BAS) was first cleaned using slurries of aluminum oxide nano-powder (Sigma-Aldrich), mirror finished on a Buehler felt pad and then subjected to ultrasonic vibration in ethanol to remove residual alumina nano-powder at the surface. The gold electrodes were then treated with 'Piranha' solution {1:3 (v/v) 30% H_2O_2 and concentrated H_2SO_4} for about 2 min, this step is necessary in order to remove organic contaminants and was followed by thorough rinsing with distilled water and ethanol. The gold electrode was finally cleaned electrochemically by carrying out CV experiments in 0.5 M H_2SO_4 and scanning the potential between −0.5 and 1.5 V (versus Ag|AgCl, sat'd KCl) at a scan rate of 0.05 V s^{-1} until a reproducible CV scan was obtained. The electrode was again rinsed with absolute ethanol and immediately placed into a nitrogen-saturated absolute ethanol solution of 4.5 mM DMAET for 18 h in the dark to form the base monolayer (Au-DMAET).

The pK_a of DMAET is ~ 10.8 and expected to be positively charged [4]. The newly formed Au-DMAET electrode was rinsed in copious amount of distilled deionised water and ethanol for 2 min to remove weakly adsorbed DMAET molecules. Thereafter, SWCNT-PABS, FeTSPc, SWCNT-PABS/FeTSPc and MPCAuNP were assembled on the base monolayer as follows: to complete their respective self assembly processes.

2.3.1 SWCNT-PABS and AuNP Based Electrodes

The formation of SWCNT-PABS on the Au-DMAET was assembled by dipping the Au-DMAET electrode in a dispersion of SWCNT-PABS (1 mg SWCNT-PABS / 1 ml PBS, pH 7.4) for 3.5 h. The electrode containing SWCNT-PABS is herein referred to as Au-DMAET-SWCNT-PABS. Thereafter, Au-DMAET-SWCNT-PABS was immersed in a dispersion of *nano*FePc (1 mg *nano*FePc / 1 ml PBS, pH 7.4) for 3.5 h. The electrode containing a single layer of SWCNT-PABS and *nano*FePc is herein referred to as Au-DMAET-(SWCNT-PABS-*nano*FePc)$_1$. Layer-by-layer assembly involves the alternating adsorption of SWCNT-PABS and *nano*FePc affording the formation of a multilayer system where each consecutive adsorption of SWCNT-PABS and *nano*FePc is referred to as a bilayer. The multilayered electrode formed by layer-by-layer assembly is herein referred to Au-DMAET-(SWCNT-PABS-*nano*FePc)$_n$,

where n = 1 – 5 bilayers. Au-DMAET-(AuNP-*nano*FePc)$_n$, was fabricated using the above mentioned process where 1 ml AuNP was used instead of 1 mg ml^{-1} SWCNT-PABS.

2.3.2 FeTSPc Based Electrodes

For the FeTSPc based electrodes the freshly prepared bare gold electrode was immersed in a 5 mM DMAET ethanol solution for a period of 36 h. The subsequent FeTSPc assembly on Au-DMAET was formed by immersing the Au-DMAET in 10 ml aqueous solution of 4 mg FeTSPc for 6 h to obtain the electrode herein abbreviated as Au-DMAET-FeTSPc. Also, Au-DMAET was deposited for 6 h into a resultant mixture of an equal volume of SWCNT-PABS solution and FeTSPc solution to obtain the electrode herein abbreviated as Au-DMAET-SWCNT-PABS/FeTSPc. The resultant mixture of the SWCNT-PABS solution and FeTSPc solution was sonicated for 2 h.

2.3.3 MPCAuNP Based Electrodes

Citrate-stabilized gold nanoparticles of 14 nm diameter were prepared using the well known Turkevich-Frens procedure [5,6]. Briefly, an aqueous solution of sodium citrate (10 ml, 17 mM) was added to a boiling solution of HAuCl$_4$ (180 ml, 0.3 mM), and heated under reflux for 30 min. The reaction mixture was allowed to cool to room

temperature, and then continuously stirred for ~ 24 h, and finally filtered using a 0.45 μm Millipore filter paper before use. (1-sulfanylundec-11-yl) tetraethylene glycol (PEG-OH) and (1-sulfanylundec-11-yl) polyethylene glycolic acid (PEG-COOH) were purchased (Prochimia, Poland) or prepared using the established procedure [7,8]. For example, the 50:50 (PEG-COOH/PEG-OH) was prepared as follows. The ethanolic solutions of PEG-OH (1 mg, 0.5 mL) and PEG-COOH (1 mg, 0.5 mL) were mixed and added simultaneously under stirring into the citrate-stabilised gold nanoparticles (20 ml, 2 nM). The reaction mixture was stirred for 3 h and filtered using a 0.45 μm Millipore filter paper. The filtered particles were purified by repeated centrifugation and redispersion in distilled deionized water. This solution is abbreviated as MPCAuNP-COOH$_{50\%}$. Three different % mass ratios of PEG-COOH to PEG-OH (1:99, 50:50 and 99:1) were used. The same procedure was used for the preparation of the other ratios. The ratio of 1:99 (PEG-COOH : PEG-OH) is abbreviated as MPCAuNP-COOH1%, while that of 99:1 (PEG-COOH : PEG-OH) is abbreviated as MPCAuNP-COOH$_{99\%}$. The final concentration of each solution mixture was 1.5 nM (12.5 ml), obtained by using a molar absorption coefficient of 4.2 x 10^8 M^{-1} cm^{-1} (at 526 nm) based on gold nanoparticles of 15±1.2 nm diameter [9].

All the modified electrodes were then thoroughly rinsed with water and dried gently in a weak flowing nitrogen gas. The modified electrodes were stored in nitrogen-saturated phosphate buffer pH 7.4 at room temperature.

The real surface area of the bare gold electrode was determined using the Randles-Ševčík equation (Eq. 1.6) for reversible electrochemistry:

$$i_p = (2.69 \times 10^5) n^{3/2} AC(Dv)^{1/2}$$

where n is the number of electrons involved (n = 1 in the $[Fe(CN)_6]^{3-/4-}$ redox system), A is the geometric area of the electrode (0.020 cm^2), D is the diffusion coefficient of the $[Fe(CN)_6]^{3-/4-}$ = 7.6×10^{-6} cm^2 s^{-1} [10], while C = 1.0×10^{-6} mol cm^{-3} is the bulk concentration of the $[Fe(CN)_6]^{3-/4-}$. From the slope of the plot of the anodic peak current (I_{pa}) versus the scan rate, the experimentally determined surface area (A) was found to be 0.0289 cm^2 giving a surface roughness factor of 1.44 (ratio of real to geometrical surface area).

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