

Electrocatalytic studies of covalently immobilized metal tetra-amino phthalocyanines onto derivatized screen-printed gold electrodes

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Abstract Metal tetra-amino phthalocyanine complexes (MTAPc; where M is Co or Mn) were immobilized on screen-printed gold electrodes pre-modified with monolayers of benzylamino groups. The functionalized electrodes were then activated using benzene-1,4-dicarbaldehyde as a linker before MTAPc complexes were immobilized. The surface coverages for the modified electrodes confirmed the perpendicular orientation of the MTAPcs. The apparent electron transfer constant (k_{app}) for the electrodes is $2.2 \times 10^{-5} \text{ cm.s}^{-1}$ for both CoTAPc and MnTAPc modified electrodes as calculated with data from impedance measurements. The k_{app} values for the bare and benzylamino modified electrodes were found to be $1.2 \times 10^{-4} \text{ cm.s}^{-1}$ and $4.9 \times 10^{-6} \text{ cm.s}^{-1}$, respectively. The electrocatalysis of the modified electrodes towards detection of H_2O_2 gave significant peak current densities and electrocatalytic potentials at -0.28 V and -0.31 V for the MnTAPc and CoTAPc modified electrodes, respectively.

Keywords Screen-printed gold electrode · Metal tetra-amino phthalocyanine · Electrocatalysis · Hydrogen peroxide · Covalent attachment

Introduction

Chemical modification of electrodes, especially gold and carbon, with materials that exhibit good electrocatalytic properties towards various analytes is an area of interest in the construction of electrochemical sensors or biosensors. Metallophthalocyanines, containing electroactive metal ions such as cobalt, iron and manganese, have shown good electrocatalytic properties towards various analytes if used as modifiers onto gold electrode surfaces [1–3]. The covalent attachment or immobilization of these catalytic complexes onto electrode surfaces may result in the formation of stable and robust systems that may be used for the detection of various analytes in extreme environmental and chemical conditions. Various methods of immobilizing metallophthalocyanines onto gold electrodes have been investigated and these methods include electropolymerization [1, 4] and self-assembled monolayer (SAM) [3, 5]. The advantage of using the SAM method is that it allows for the controllable surface functionalization. However, SAMs possess major disadvantages such as (a) chemical and thermal instability, (b) limited potential window and (c) mobility of the formed monolayer. The investigation of methods that will address the limitations of the SAM method while giving control of surface functionality is desirable.

In the recent years, there has been improvement on the monolayer fabrication method that has the same advantage as SAM method (i.e. control of surface functionality) and also addresses the disadvantages of the SAM method (i.e.

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stability and mobility of the monolayers). This method involves chemical modification of gold electrodes via electrode grafting. This method uses diazonium salts for the controlled and monolayer functionalization of the electrodes and the formed thin films are stable and strong enough for applications at the extreme conditions [6, 7]. Grafting method involves the generation of aryl radicals from aryl diazonium salts which attacks the electrode surface and forms a stable monolayer coating [8, 9]. Diazonium salts are commercially available and different functionalities such a carboxylic acid and amino group can be incorporated onto a monolayer by using a carboxylic acid or amino terminated diazonium salts. These diazonium salts can also be synthesized following a reported method [10] using aryl amines or functionalized aryl amines for specific applications. This method has not been thoroughly studied for immobilization of metallophthalocyanine (MPc) complexes with only one report available in literature [8]. The formed MPc thin films are expected to be stronger and stable on the electrode surfaces. These MPc modified surfaces are desirable for the long term use of MPc-based electrochemical systems or devices. The majority of the work studied using diazonium electrode functionalization has been conducted on the carbon electrodes [8, 9, 11–13] with few reports on gold electrodes [7, 14, 15]. In this work, we report on the new method of covalently immobilizing metal tetra-amino phthalocyanines (MTAPc), via Schiff-base reaction onto screen-printed gold electrodes pre-modified with benzylamino (BA) groups, Scheme 1. The ease of use of screen-printed electrodes and their portability makes them ideal for the design of field testing devices while requiring small volumes for analysis. The electrocatalytic properties of the modified electrodes were investigated towards the detection and monitoring of hydrogen peroxide (H_2O_2).

Experimental

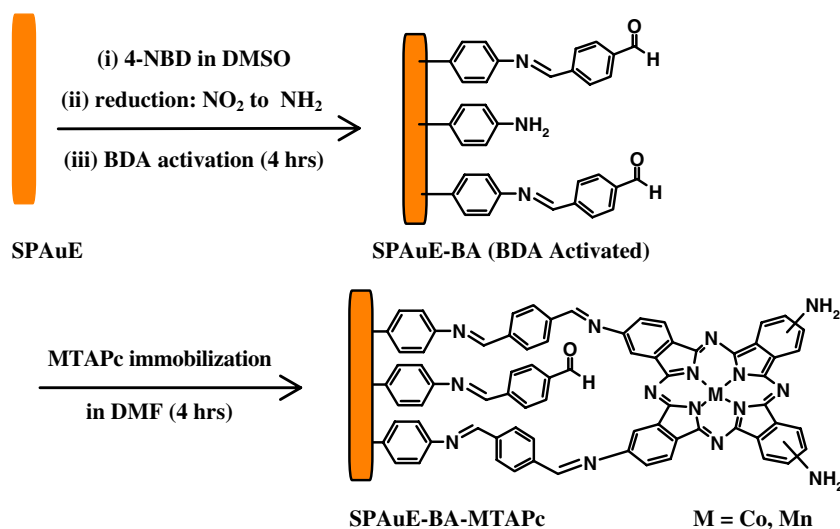
Materials and suppliers

Dimethylsulfoxide (DMSO), dimethylformamide (DMF), tetrabutylammonium tetrafluoro-borate (TBABF_4), 4-nitrobenzene diazonium tetrafluoro borate (4-NBD), benzene-1,4-dicarbaldehyde (BDA), ammonium iron (III) sulphate and absolute ethanol were purchased from Sigma-Aldrich (www.sigmaaldrich.com) and used as received. H_2O_2 (30%) was purchased from SAARChem (www.merck.co.za). Ultra-pure water was obtained from a Milli-Q water system (Millipore Corp. Bedford, MA, USA) and was used throughout the experiments. Acetonitrile (ACN), phosphate salts (NaH_2PO_4 and Na_2HPO_4), ferricyanide $\{\text{K}_3[\text{Fe}(\text{CN})_6]\}$ and ferrocyanide $\{\text{K}_4[\text{Fe}(\text{CN})_6]\}$ were purchase from ACE Chemicals (www.acechem.co.za). The phosphate salts were used to prepare phosphate buffer solutions. Solutions in the electrochemical cell were de-aerated by bubbling argon prior to every experiment and the electrochemical cell was kept under argon atmosphere. Manganese and cobalt tetra-amino phthalocyanine ($(\text{OH})\text{Mn}^{\text{III}}\text{TAPc}$ and CoTAPc) complexes were synthesized according to the published method [16, 17].

Apparatus and methods

All electrochemical and impedance spectroscopy experiments were carried out using a computer-controlled Autolab Potentiostat/Galvanostat PGSTAT 302N purchased from Eco Chemie (Utrecht, The Netherlands, www.ecochemie.nl) driven by the General Purpose Electrochemical Systems data processing software (GPES software for electrochemistry) and FRA for impedance measurements and analysis. The electrochemical data was collected using a screen-

Scheme 1 Schematic representation of covalent immobilization of MTAPc onto benzyl-amino (BA) pre-grafted SPAuE



printed gold electrodes, SPAuE (C223AT purchased from Dropsens, Oviedo, Spain—www.dropsens.com) with three electronic contacts for gold (Au, $r=0.8$ mm) as working electrodes, gold as counter electrode and silver/silver chloride (Ag|AgCl) as reference electrode. Electrochemical impedance spectroscopy (EIS) experiments were recorded in the frequency range between 10 kHz to 100 mHz at the half-wave potential ($E_{1/2}$) of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple for bare and modified SPAuE with an amplitude 5 mV rms sinusoidal modulation. All the experiments were conducted at 25 °C in the jacketed cells. The Scanning Electron Microscopy (SEM) morphology images of the bare and modified gold surfaces were obtained using the gold coated quartz crystal microbalance (QCM) crystals purchased from Stanford Research Systems (SRS, www.thinksrs.com). The SEM images were acquired using Nova NanoSEM 200 from FEI (www.fei.com). The energy dispersive x-ray (EDX) analysis spectra were obtained using the X-ray microanalysis system (www.edax.com) added as a module on the Nova NanoSEM 200.

Electrode modification and pre-treatments

Prior to electrode modification, the gold electrode surfaces were rinsed in ethanol/water (1/9) solution and conditioned in sulfuric acid (0.5 M H_2SO_4) until reproducible scans were observed. The electrode was rinsed with ethanol solution and the modification of SPAuE was followed as illustrated in Scheme 1. Briefly, the electrodes were modified by electrochemical grafting in 1.0 mM 4-NBD in DMSO or ACN solution containing 1 mM TBABF₄ thus forming benzylnitro monolayer (both solvents gave similar results). The formed benzylnitro group was electrochemically reduced to form benzylamino (BA) monolayer by cycling in ethanol/water (1/9) solution containing 0.1 M KCl. The BA functionalized SPAuEs were activated by immersing in the ethanol solution of BDA thus forming the Schiff-base product and exposing the other aldehyde group for further reaction with the amino groups from the CoTAPc and MnTAPc complexes. The gold coated QCM crystals were modified similarly for use in obtaining SEM images and are represented as Au-BA, Au-BA-MTAPc ($M = \text{Co}^{\text{II}}$ or $(\text{OH})\text{Mn}^{\text{III}}$).

Results and discussion

Electrochemical characterization of modified electrodes

The immobilization of MTAPc onto SPAuEs monolayer modified electrodes was investigated following the Schiff-base reaction shown in Scheme 1.

The first step in electrode modification is the functionalization of the electrode surface with a benzylamino group.

This was achieved in two steps; the first step in Scheme 1 is electrochemical grafting of the 4-NBD which is then followed by the reduction of the benzylnitro to form a benzylamino group. The benzylamino group is then activated by the BDA thus forming a Schiff-base product and exposing the other aldehyde group for further reaction with the amino group from the phthalocyanine ring. This reaction of an amino group and an aldehyde is well-known for the immobilization of biomolecules [14] and in most reports glutaraldehyde is used as a cross-linker. The formation of Schiff-base product using BDA as a cross linker has been reported before for the formation of the heterobinuclear porphyrin dimers [18]. The choice of using BDA as a cross-linker in this work is to allow for the extension of the electronic delocalization of the phthalocyanine ring. This extension of electronic delocalization is expected to lower the reduction potential and increase the electrocatalytic activity of the metallophthalocyanine complexes immobilized onto the electrode surface. This lowering of potential, influenced by delocalization, has been reported before for the conjugated porphyrin complexes [19].

Figure 1(a) shows the cyclic voltammograms recorded during the electrochemical reduction of the 4-NBD during the formation of the benzylnitro monolayer. The first scan shows the reduction peak at ~ 0.027 V corresponding to the electrochemical reduction of the diazonium salts and formation of aryl radicals. The reduction peak disappeared during the second and subsequent scans, thus confirming the formation of benzylnitro monolayer.

The surface coverage of the benzylnitro monolayer was calculated by integrating the charge under the well-defined reduction peak (at 0.027 V) and was found to be 2.79×10^{-9} mol.cm⁻². This surface coverage was found to be consistent with the reported values for a closely packed monolayer of the grafted benzylnitro groups onto the electrode surfaces [20]. This method of modifying electrodes is well-known [8, 11, 20], where 4-nitrobenzene diazonium salt is electrochemically reduced in a one electron process leading to the formation of an aryl radical and loss of N₂. The aryl radical then attacks the surface and form a monolayer of benzylnitro on the electrode surface. The controlled formation of the monolayer has also been reported [9, 13, 21], where a small number of cycles (<5) gives the monolayer coverage. In this work, we used 4 cycles in an attempt to form a benzylnitro monolayer functionalized surface. This monolayer was then reduced in an ethanol/water (1/9) solution containing 0.1 M KCl to form benzylamino (BA) monolayer functionalized electrode surface [8, 11]. Figure 1b shows the cyclic voltammograms for the electrochemical reduction of the benzylnitro group to form benzylamino group (1–16 scans). In the first cycle, the well-defined reduction peak at -0.92 V corresponds to the reduction of the benzylnitro group to benzylhydroxylamine

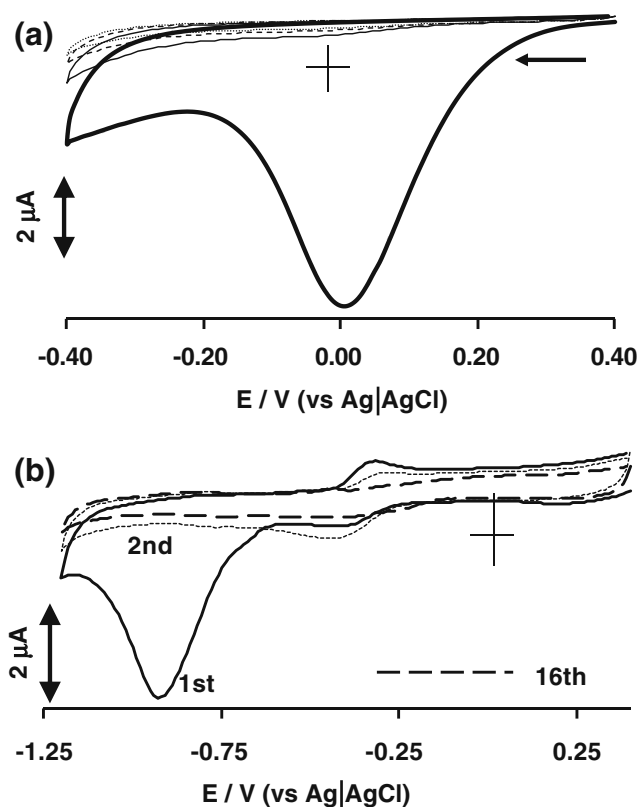


Fig. 1 Cyclic voltammograms of **a** SPAuE recorded for the fabrication of benzylamino monolayer from 1 mM 4-NBD in DMSO containing 1.0 mM TBABF₄ in 4 cycles and **b** reduction of NO₂ to NH₂ in (1:9, ethanol: water) solution containing 0.10 M KCl. Scan rate 50 mV.s⁻¹

group. The benzylhydroxylamine group undergoes oxidation at -0.33 V to form benzylnitroso group, which is then finally reduced at -0.42 V to form the benzylamino group. This process of reducing benzylamino groups to form benzylamino groups is known [8, 11] to involve six electrons and six protons. The reduction of benzylamino group to benzylhydroxylamine group is a complete one-step reaction. The reversible couple at $E_{1/2} = -0.38$ V decreased in current as the modified electrode was continuously cycled. These cyclic voltammetric changes confirmed the formation of the benzylamino groups on the electrode surfaces.

The electrode functionalized with the benzylamino group (SPAuE-BA) was then further activated with the BDA for the immobilization of MTAPc molecules, following the procedure in Scheme 1. The activation of the benzylamino modified electrode results in the formation of the Schiff-base product between the amino group of the functionalized electrode and the aldehyde group of BDA to yield an aldehyde activated monolayer. The unreacted aldehyde of BDA was used to further immobilize MTAPc complexes (CoTAPc and MnTAPc) onto the electrode thus resulting in the formation of MTAPc monolayers (SPAuE-BA-MTAPc).

Figure 2 shows the cyclic voltammograms of (a) (i) bare SPAuE, (ii) SPAuE-BA and (iii) SPAuE-BA-CoTAPc and (b) (i) SPAuE, (ii) SPAuE-BA, (iii) SPAuE-BA-MnTAPc in 0.10 M KOH solution and (c) (i) SPAuE-BA-CoTAPc and (ii) SPAuE-BA-MnTAPc in 0.01 M pH 7.4 phosphate buffer solution.

The characterization of the electrode modification clearly shows the presence of the MTAPc monolayer in Fig. 2(a) (iii) and (b) (iii) for SPAuE-BA-CoTAPc and SPAuE-BA-MnTAPc, respectively, in basic media (0.10 M KOH). At unmodified (i) and benzyl-amino (ii) modified electrodes, there were no peaks observed both in Fig. 2(a, b). After modifying the activated electrode with CoTAPc or MnTAPc molecules, we observed peaks due to either metal reduction or oxidation. In Fig. 2(a)(iii), we observed an

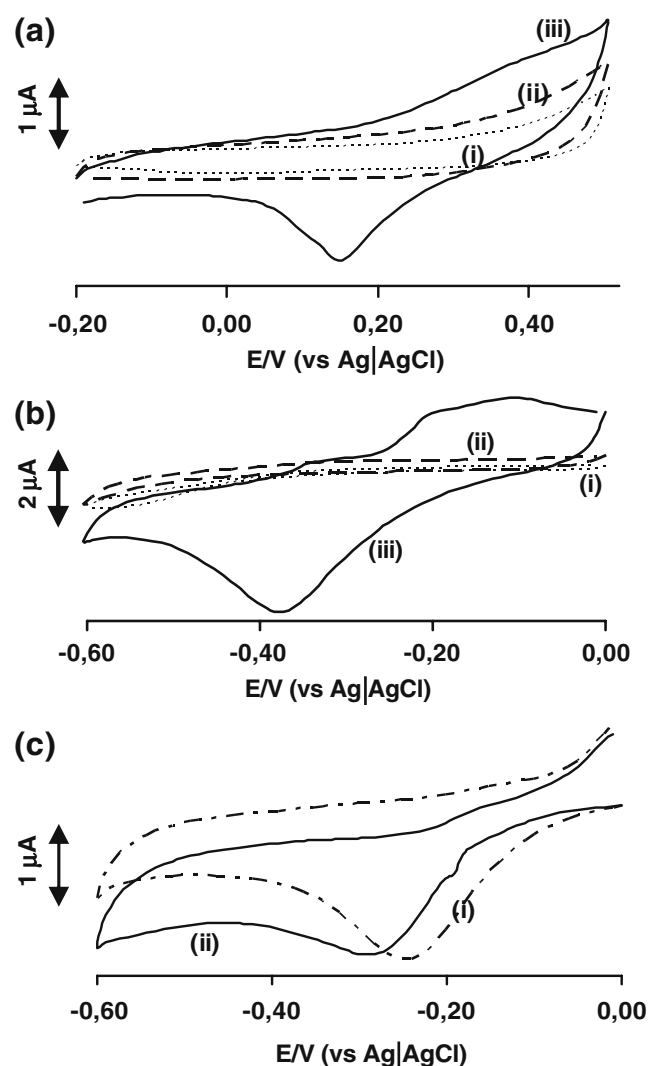


Fig. 2 Cyclic voltammograms of **a** (i) SPAuE, (ii) SPAuE-BA and (iii) SPAuE-BA-CoTAPc; **b** (i) SPAuE, (ii) SPAuE-BA and (iii) SPAuE-BA-MnTAPc in 0.10 M KOH and **c** (i) SPAuE-BA-CoTAPc and (ii) SPAuE-BA-MnTAPc in pH 7.4 phosphate buffer solution. Scan rate = 50 mV.s⁻¹

oxidation peak at $\sim +0.37$ V for SPAuE-BA-CoTAPc, this peak occurred at potentials close to $\text{Co}^{\text{III}}/\text{Co}^{\text{II}}$ oxidation and is similar to that reported previously [22] and the reduction peak was observed at 0.15 V. The $\text{Co}^{\text{III}}/\text{Co}^{\text{II}}$ process is known to be irreversible [23] and notoriously difficult to observe for adsorbed/immobilized CoPc complexes, hence its irreversibility is not surprising. The cathodic peak at -0.38 V, in Fig. 2(b)(iii) is due to the $\text{Mn}^{\text{III}}/\text{Mn}^{\text{II}}$ in comparison with literature for polymerized MnTAPc complexes [24] in alkaline conditions (pH 11) and has a split anodic component at -0.20 V. The split nature of the peak at -0.20 V could be related to both the $\text{Mn}^{\text{III}}/\text{Mn}^{\text{II}}$ and $\text{Mn}^{\text{IV}}/\text{Mn}^{\text{III}}$ at more positive potentials. When pH 7.4 was employed, Fig. 2(c), we observed the reduction peaks which were assigned to (i) $\text{Co}^{\text{II}}/\text{Co}^{\text{I}}$ at -0.24 V and (iii) $\text{Mn}^{\text{III}}/\text{Mn}^{\text{II}}$ at -0.30 V. The latter pH was employed since the detection of the analyte of interest (H_2O_2) will be determined at this pH. These peaks due to metal ions appear at less negative potentials than those obtained on the polymer modified electrodes [25] which were observed at -0.42 V and -0.35 V for Au-polyCoTAPc and Au-polyMnTAPc electrodes, respectively, at the same pH, but not grafted onto the electrode.

A shift of MnTAPc peak potential to more negative potential with increase in pH was reported before [24] and in this work we observed a 0.08 V shift from -0.30 V peak in pH 7.4 to -0.38 V in 0.10 M KOH solution (pH 13). The observed changes in the cyclic voltammograms (Fig. 2) in the presence of MTAPc complexes clearly confirmed the successful modification of the electrodes using the method in Scheme 1. Furthermore, the use of strong alkaline (0.10 M KOH) solution for the characterization of these electrodes and the persistence of the metal redox peaks during multiple scans indicates the stability of the electrodes modified using the method in Scheme 1. The thiolated self-assembled monolayer modified gold electrodes undergoes reductive or oxidative desorption in these high alkaline conditions. The surface coverages (Γ) of the MTAPc modified electrodes were estimated by integrating the charge under the metal reduction or oxidation peaks in 0.10 M KOH solutions. Equation 1 was used for the estimation of surface coverage.

$$\Gamma_{BA-MPc} = \frac{Q}{nFA} \quad (1)$$

where Q is the background corrected charge under the metal reduction or oxidation peak (Coulombs), n is the number of electron transferred ($=1$), F is the Faraday's constant (96485 Coulombs/mol) and A is the real (experimental) area of the bare gold electrode (0.0352 cm^2). This was calculated using the conventional method [26] applying Eq. 2 (Randles-Sevcik equation).

$$I_{pa} = (2.69 \times 10^5) n^{\frac{3}{2}} D^{\frac{1}{2}} v^{\frac{1}{2}} AC \quad (2)$$

where n is the number of electrons transferred ($=1$), D diffusion coefficient of the redox active species $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ($7.6 \times 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$), v is the scan rate ($0.05 \text{ V} \cdot \text{s}^{-1}$), C (2 mM) is the bulk concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The geometric area (0.0201 cm^2) of the electrode was calculated from the diameter of 1.6 mm ($r=0.8$ mm). The surface roughness factor of the electrode (1.75) was obtained from the ratio of real electrode surface area to electrode geometric area. The real surface area of the electrode was found to be higher than the geometric area and this is due to the fact that the active surface area is rough and uneven, this contributes to the roughness of the electrode. The surface coverage values were then calculated using Eq. 1 and the real area of the electrode (0.0352 cm^2). The surface coverage values were found to be 1.55×10^{-9} and $1.03 \times 10^{-9} \text{ mol} \cdot \text{cm}^{-2}$ for SPAuE-BA-CoTAPc and SPAuE-BA-MnTAPc, respectively. Also in pH 7.4 phosphate buffer solution these electrodes exhibited reduction peaks due to the metal ion and by integrating the charges under these peaks we were able to get surface coverage values of $1.66 \times 10^{-9} \text{ mol} \cdot \text{cm}^{-2}$ and $1.31 \times 10^{-9} \text{ mol} \cdot \text{cm}^{-2}$ for CoTAPc and MnTAPc modified electrodes, respectively. These surface coverage values were within the experimental errors similar for both MTAPc complexes in 0.10 M KOH and 0.01 M pH 7.4 phosphate buffer solutions. Since the real surface area of the electrodes was used to calculate the surface coverages of the immobilized complexes, these values represent the actual concentration of the complexes onto electrode surfaces. The plots of peak currents versus increasing scan rates from Fig. 2(a) and (b) (iii) were linear for both complexes, typical of the adsorbed MTAPc species. The surface coverage values of the CoTAPc modified electrode were comparable to the reported values where cobalt tetra-carboxy phthalocyanine (CoTCPC) complex was immobilized onto an aryl radical modified glassy carbon electrode [8]. The surface coverage of the phthalocyanine molecule lying flat on the surface is approximately $1.0 \times 10^{-10} \text{ mol} \cdot \text{cm}^{-2}$ [2]. In this work, the surface coverages for the immobilized MTAPc molecules are much higher than $1.0 \times 10^{-10} \text{ mol} \cdot \text{cm}^{-2}$. The higher surface coverages obtained confirms the perpendicular orientation of the MTAPc complexes onto the gold surface as illustrated in Scheme 1.

HRSEM characterization of the bare and modified gold electrodes

The HRSEM characterization of the electrode modified with benzylamino group and MTAPc complexes was carried out. Figure 3 shows the morphology of the (a) bare gold (Au) surface, (b) Au-BA-MnTAPc and (c) Au-BA-CoTAPc electrodes and their corresponding energy dispersive x-ray (EDX) spectra.

The HRSEM images show the morphology of the bare and modified electrode surfaces following the method in

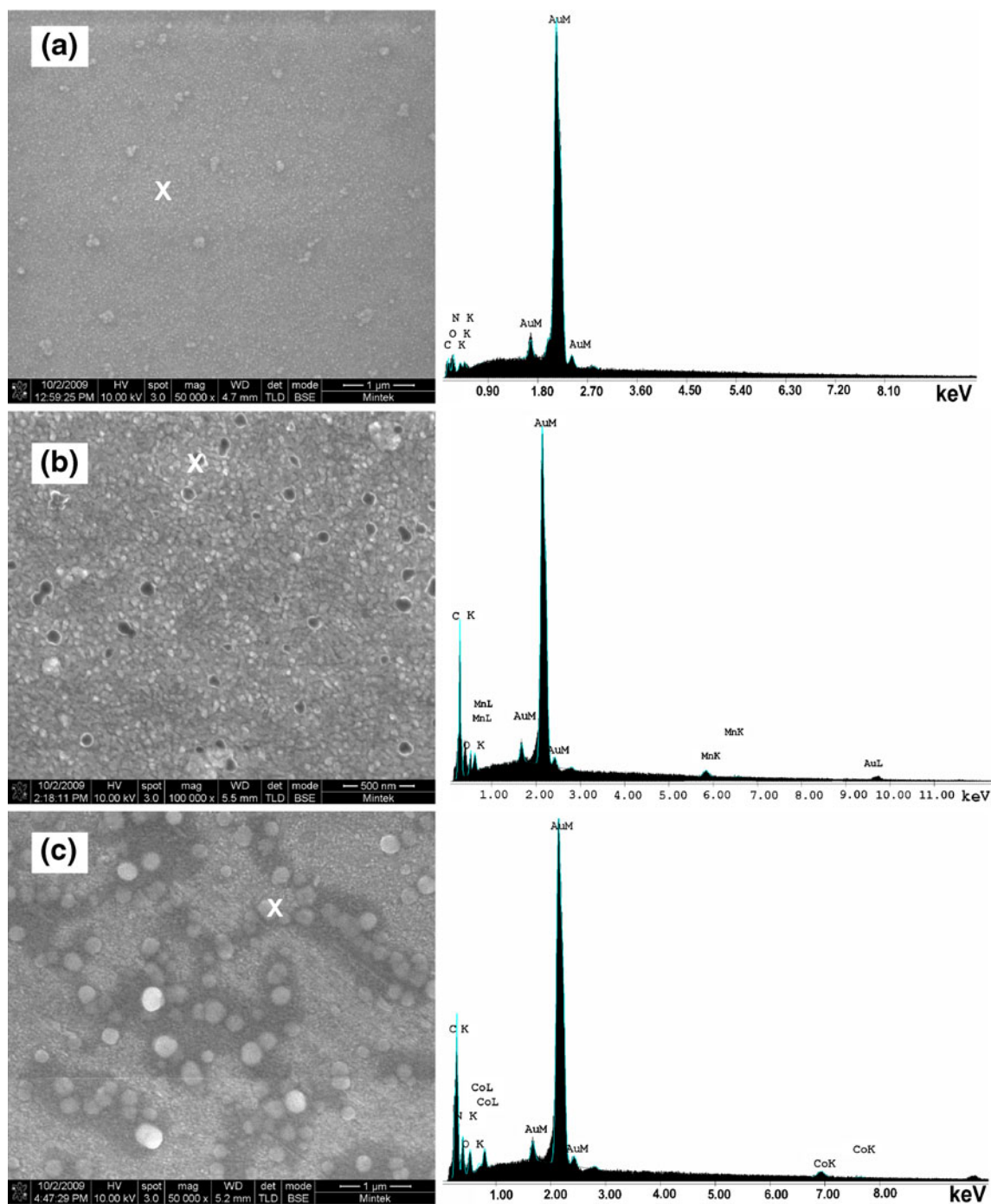


Fig. 3 HRSEM images and their corresponding EDX spectra for **a** bare Au, **b** Au-BA-MnTAPc and **c** Au-BA-CoTAPc modified electrodes. X mark is the spot used for obtaining EDX spectra

Scheme 1. It is discernible that electrode modification indeed took place as the morphology of these images was different. The morphologies of the unmodified, in Fig. 3(a), and the benzylamino modified (data not shown) gold electrodes were similar. The thin film of benzylamino monolayer takes on the underlying gold surface features and hence no difference in surface morphologies of the benzylamino film and the bare electrode. The morpholog-

ical comparison becomes clearer when the bare and benzylamino modified electrode surfaces are compared to the Au-BA-MnTAPc and Au-BA-CoTAPc modified surfaces in Fig. 3(b) and (c), respectively. The differences in morphologies between the Au-BA-CoTAPc and Au-BA-MnTAPc modified electrodes were observed and these could be attributed to the difference in the individual bare QCM electrodes or to the aggregation of these molecules.

The latter could be a logical reason to explain the observed morphological differences because there were no differences (compared to QCM electrode used for Fig. 3a) in surface morphologies of the individual bare QCM electrodes before the immobilization of MTAPc complexes. The aggregation is more evidenced in Au-BA-CoTAPc than in Au-BA-MnTAPc. The latter contains an OH axial ligand which may reduce aggregation [16, 17]. The EDX surface analysis was also performed since it provides information on materials present on the electrode surface. The energy dispersive x-ray spectrum of bare gold electrode showed the dominating gold (Au M) peaks and this was expected as the QCM gold surfaces used were Au coated. The residues due to carbon (C K), oxygen (O K) and nitrogen (N K) were observed and these are due to the impurities and are always present in the spectrum in small quantities. The Au-BA modified surface energy dispersive spectrum (data not shown) exhibited the presence of the dominating gold (Au M) and residual carbon (C K), nitrogen (NK) and oxygen (O K) materials. The residual peaks due to carbon, nitrogen and oxygen at the Au-BA monolayer modified electrodes could not be attributed to the presence of the BA monolayer because the electron beam is too strong and will penetrate this monolayer. The EDX spectra for the Au-BA-CoTAPc and Au-BA-MnTAPc modified electrode exhibited an increase in the carbon (C K) peak intensities with the presence of the peaks due to Co and Mn (L and K) orbitals from the Au-BA-CoTAPc and Au-BA-MnTAPc, respectively. The EDX measurements for the Au-BA-MnTAPc were obtained from the dark spots (as marked by X) observed after modifying the electrodes. Similarly with the Au-BA-CoTAPc, the EDX measurements were obtained from the globular-like or spherical morphologies observed after modifying the electrodes with CoTAPc. The increase in the carbon (C K) materials in the Au-BA-MTAPc (M = Co, Mn) modified electrodes is attributed to the presence of a more bulky BA-MTAPc monolayer compared to the BA monolayer. The presence of the Co and Mn (L and K) orbital peaks from the CoTAPc and MnTAPc modified gold electrodes shows the presence and the successful immobilization of these complexes. Even though the EDX measurements showed the presence of the monolayer as in Scheme 1, we did not attempt to quantify the material compositions on each surface as the residuals due to carbon, oxygen and nitrogen will differ from surface to surface hence giving inaccurate amounts.

Electrochemical properties of bare and modified SPAuEs

The electrochemical properties of bare and modified electrodes were investigated using cyclic voltammetry and electrochemical impedance spectroscopy measurements. These experiments were also studied to further explore the

electrocatalytic properties of the monolayer modified electrode surfaces using ferricyanide $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probing molecule in solution. Figure 4 shows the cyclic voltammograms of (i) bare and modified electrodes with (ii) benzylamino (BA), (iii) CoTAPc and (iv) MnTAPc monolayers in (a) 1.0 mM $\text{NH}_4\text{Fe}(\text{SO}_4)_2$ in 1.0 mM HClO_4 and (b) (1:1) 2.0 mM $\text{K}_4/\text{K}_3\text{Fe}(\text{CN})_6$ in 0.10 M KCl.

The cyclic voltammograms of the bare screen-printed electrode in Fig. 4(a)(i) exhibited a broad anodic peak at ~ 0.15 with a cathodic component at ~ -0.09 V. This redox process is due to $[\text{Fe}(\text{H}_2\text{O})_6]^{2+/3+}$ which shows slow electrode kinetics. Upon modifying the electrode with the benzylamino and MTAPc monolayers the reduction peak disappeared completely while the oxidation peak was still observed at benzylamino monolayer with the decrease in current. The peak observed at ~ 0 V for (OH)MnTAPc is due to metal based redox processes of MTAPc, shifted to more positive potentials (compared to Fig. 2) due to the use of a different electrolyte. The blocking capabilities of the monolayers towards the $[\text{Fe}(\text{H}_2\text{O})_6]^{2+/3+}$ redox couple is the clear confirmation of the successful electrode modification. The blocking ability of the electrode was studied using the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ couple which is a fast electron transfer redox

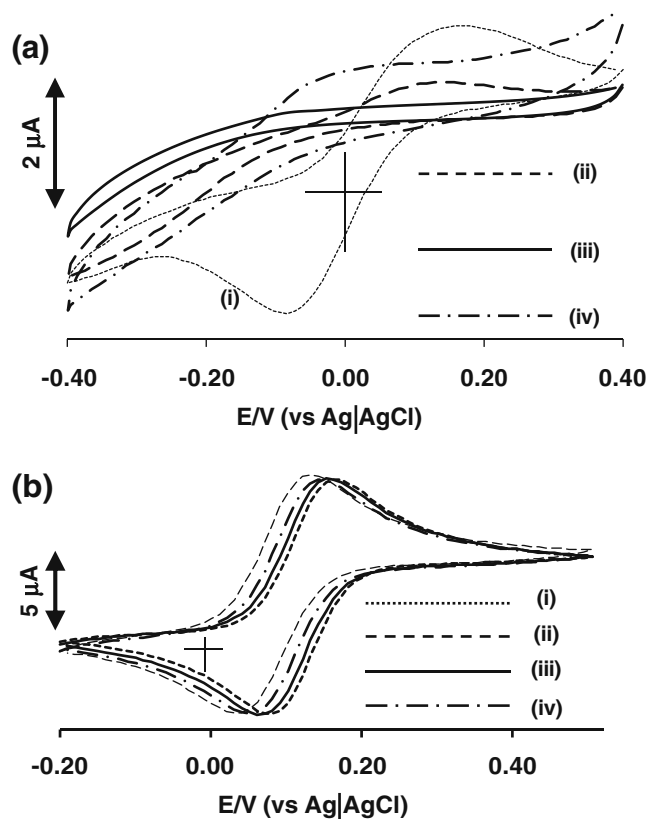


Fig. 4 Cyclic voltammograms of (i) SPAuE, (ii) SPAuE-BA, (iii) SPAuE-BA-CoTAPc and (iv) SPAuE-BA-MnTAPc in (a) 1.0 mM $\text{NH}_4\text{Fe}(\text{SO}_4)_2$ in 1.0 mM HClO_4 and (b) (1:1) 2.0 mM $\text{K}_4/\text{K}_3\text{Fe}(\text{CN})_6$ in 0.10 M KCl

couple. In Fig. 4(b), the cyclic voltammograms of the (i) bare electrodes shows the reversible redox couple due to $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with the peak-to-peak separation (ΔE) of 81 mV. Upon modifying the electrodes with the benzylamino group, in Fig. 4(b)(ii) ΔE increased to about 90 mV and this is attributed to the insulating layer of benzylamino that has formed on the electrode surfaces. However, the redox peak due to $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was not hindered. Furthermore, the modification of the electrodes with the MTAPc in Fig. 4(b)(iii–iv) showed that the reversibility was restored and ΔE was the same as that of the bare (81 mV), but less than that for benzylamino monolayer (90 mV) modified electrodes. It is of interest to note the work by Somashekarappa et al. [27] where the MTAPc complexes were immobilized as SAMs on gold electrode and the electron transfer of the modified electrodes towards $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was not hindered by MTAPc SAMs. In this work, the benzylamino monolayer gave higher ΔE values compared to MTAPc electrodes. The immobilization of MTAPc complexes onto the benzylamino modified electrodes without BDA activation gave a ΔE value of 90 mV. This clearly indicates that in the absence BDA as a cross-linker, the immobilization of MTAPc complexes did not take place. This experiment confirmed that the activation of the benzylamino modified electrode with the aldehyde group from the BDA group is necessary for the immobilization of the MTAPc complexes.

The electrochemical impedance spectroscopy (EIS) measurements in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution were also performed in order to assess the electron transfer properties of the modified electrodes. Figure 5 shows (a) the Nyquist plots, (b) their corresponding Bode plots for the (i) SPAuE, (ii) SPAuE-BA, (iii) SPAuE-BA-CoTAPc and (iv) SPAuE-BA-MnTAPc electrodes and (c) the Randles equivalent circuit used to fit the Nyquist data obtained in (a).

The Nyquist plots in Fig. 5(a) (for bare gold electrode) shows the semi-circle at kinetically controlled regions and this semi-circle increases after modifying the electrodes with the BA and MTAPc monolayers. The data was fitted using the Randles equivalent circuit which shows the connection of various units of the circuit, e.g. $R_s(Q[R_{CT}Z_w])$, where R_s is solutions resistance, Q is capacitance (constant phase element), R_{CT} is charge transfer resistance and Warburg impedance (Z_w). The R_s is connected in series to the parallel combination of Q/CPE and R_{CT} which is in series to the Z_w . The fitted data is shown in Table 1, where R_{CT} exhibited an increase from bare to modified surfaces. For the bare electrode surface, $R_{CT}=1.79 \text{ k}\Omega\cdot\text{cm}^{-2}$ and this value increased to $43.67 \text{ k}\Omega\cdot\text{cm}^{-2}$ for BA, $9.66 \text{ k}\Omega\cdot\text{cm}^{-2}$ for CoTAPc and $9.69 \text{ k}\Omega\cdot\text{cm}^{-2}$ for MnTAPc electrodes. The increase in R_{CT} from a bare to benzylamino modified electrodes is due to the formed insulating layer thus slowing electron transfer kinetics

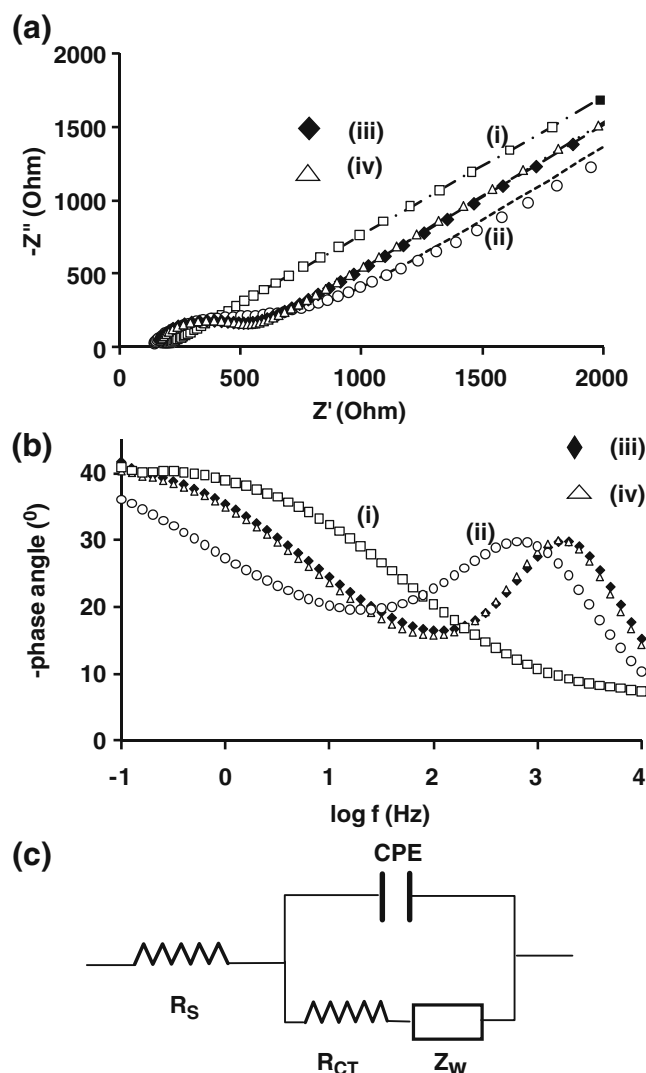


Fig. 5 a Nyquist, b Bode plots of (i) bare SPAuE, (ii) SPAuE-BA, (iii) SPAuE-BA-CoTAPc and (iv) SPAuE-BA-MnTAPc in (1:1) 2 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution with 0.1 M KCl and c Randles equivalent circuit used to fit the Nyquist data in (a)

towards the electrode surfaces. However, upon modifying the electrodes with the MTAPc complexes via Schiff-base reactions, the R_{CT} value decreased to $9.66 \text{ k}\Omega\cdot\text{cm}^{-2}$ and $9.69 \text{ k}\Omega\cdot\text{cm}^{-2}$ for CoTAPc and MnTAPc, respectively. This decrease in R_{CT} after modifying the electrode with MTAPc is attributed to the known catalytic properties of the metallo phthalocyanines and hence the electron transfer is enhanced. MPCs, especially those containing cobalt and manganese metal centers are known [22, 28] to be good electrocatalysts and enhance the electron transfer to the electrode, hence the decrease in R_{CT} . The R_s value did not show any significant changes before and after modifying the electrodes with BA and BA-MTAPc. This was expected as the surface modification was not expected to change the solution resistant. The capacitance values (CPE) are

affected by the electrode modification as the increase in capacitance (CPE values) after modification electrode surfaces with benzylamino monolayer was observed. Upon modifying the electrodes with MTAPc, the CPE decreased and this is attributed to the already observed phenomenon on R_{CT} values.

The bode plots after modifying the electrode in Fig. 5(b) showed a presence of a symmetrical peak which was clearly not observed at the bare electrode. The phase angle maxima of the symmetric peaks were observed to be -29.87° at $\log f=2.8$ Hz (for BA modified electrode), -29.72° at $\log f=3.2$ Hz (for CoTAPc) and $(-30.08^\circ$ at $\log f=3.2$ Hz (for MnTAPc). The shift of the symmetrical peak to lower $\log f$ values (compared to bare gold electrode) confirms the different surface properties introduced on the electrode after modifying with MTAPc. The phase angle values for all the electrodes studied in this work are much less than the ideal -90° for a true capacitor leading to the conclusion that these electrodes have capacitive-like behaviour [29]. From Table 1, it is important to mention the n values (where n is an exponent-related to the depression angle [30]) observed from the fitted data and these values are all ~ 0.9 . The n values for a pure resistor ($n=0$), for Warburg diffusion ($n=0.5$) and for pure capacitor ($n=1$). The n values are close to unity and thus leading to the conclusion that the electrodes have a capacitive-like behaviour. This conclusion confirms the results obtained from the phase angle values which were less than -90° due to the capacitive-like nature of the thin films studied in this work.

The apparent electron-transfer rate constant (k_{app}) was obtained from the conventional Eq. 3 [30, 31]. The redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and the R_{CT} values obtained in Table 1, were used to calculate the k_{app} from Eq. 3. This method investigates the outer sphere kinetic properties of the immobilized thin films and their interaction with the redox probe in solution.

$$k_{app} \approx k^0 = \frac{RT}{n^2 F^2 A R_{CT} C} \quad (3)$$

where C is a concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (2 mM), R_{CT} (charge transfer from fitted data in Table 1), A is the real area of the gold electrode (0.0352 cm^2), $n=1$ (electron

transferred in the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox process), R , T and F have their usual meaning. The bare electrode surface gave the k_{app} value of $1.20 \times 10^{-4} \text{ cm.s}^{-1}$. Upon modifying the electrodes with BA and BA-MTAPc monolayers the k_{app} decreased to $0.049 \times 10^{-4} \text{ cm.s}^{-1}$ and $0.22 \times 10^{-4} \text{ cm.s}^{-1}$ for BA and BA-MTAPc modified electrodes, respectively. The k_{app} values are as shown in Table 1 and are lower compared to the values obtained in literature [33] and this may be due to the real area (0.0352 cm^2) of electrode used in this work. The BA-MTAPc modified electrodes exhibited the conducting properties as the k_{app} values were higher than those of the benzylamino monolayer modified electrodes. The electrode modification clearly affects the k_{app} values and this was also reported earlier by our group [33] where an increase in R_{CT} (hence decrease in k_{app}) was observed with increasing deposition time for the SAMs. The electrode modification with BA and the covalent attachment of the MTAPc complexes onto the benzylamino-aldehyde activated reaction is expected to yield a closely packed monolayer. This closely packed monolayer resulted in the decrease in k_{app} compared to the unmodified electrodes leading to the decrease in the electron-transfer rate constants.

Electrocatalysis of H_2O_2 at SPAuE-BA-MTAPc electrodes

The electrocatalytic properties of the MTAPc modified screen-printed electrodes were investigated for the detection of H_2O_2 in physiological conditions. Figure 6(a) shows the electroreduction of 1.0 mM H_2O_2 in phosphate buffer solution (pH 7.4) for (i) bare and (ii) benzyl amino modified screen-printed electrodes. Clearly there was no peak at the unmodified electrodes in Fig. 6(a)(i) and after modifying with benzylamino monolayer in Fig. 6(a)(ii). The electrocatalytic behavior was observed for the MTAPc modified electrodes. Figure 6(b) and (c) shows the cyclic voltammograms for SPAuE-BA-MnTAPc and SPAuE-BA-CoTAPc in the (i) absence and (ii) presence of 1.0 mM H_2O_2 in pH 7.4 phosphate buffer solution. In the absence of H_2O_2 we observed the metal reduction peaks at -0.24 V ($\text{Co}^{\text{II}}/\text{Co}^{\text{I}}$) for SPAuE-BA-CoTAPc and at -0.30 V ($\text{Mn}^{\text{III}}/\text{Mn}^{\text{II}}$) for SPAuE-BA-MnTAPc electrodes.

The electrocatalytic peaks due the reduction of H_2O_2 occurred at potentials close to where the metal reduction

Table 1 The summary of the parameters obtained from cyclic voltammetry and electrochemical impedance spectroscopy experiments in (1:1) 2 mM $\text{K}_3/\text{K}_4\text{Fe}(\text{CN})_6$ solution containing 0.1 M KCl

Electrodes	R_s ($\text{k}\Omega.\text{cm}^{-2}$)	R_{CT} ($\text{k}\Omega.\text{cm}^{-2}$)	n	CPE ($\mu\text{F}.\text{cm}^{-2}$)	ΔE (mV)	$k_{app} \times 10^{-4}$ (cm.s^{-1})
SPAuE	4.72	1.79	0.87	56	81	1.20
SPAuE-BA	4.12	43.67	0.88	94	90	0.049
SPAuE-BA-CoTAPc	4.43	9.66	0.92	22	80	0.22
SPAuE-BA-MnTAPc	4.32	9.69	0.94	22	80	0.22

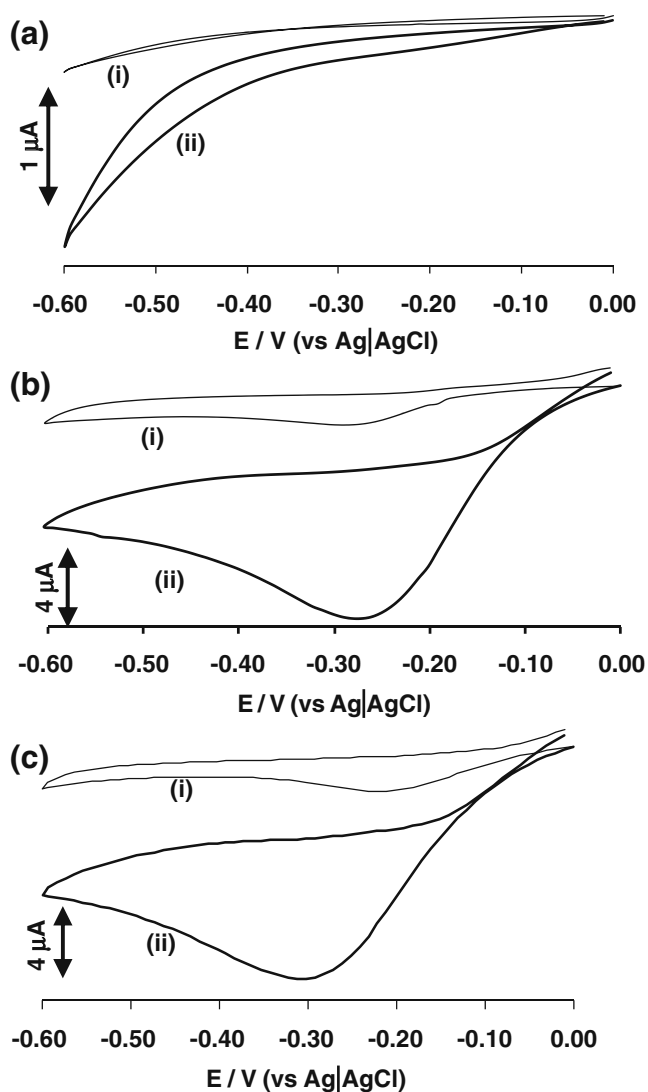


Fig. 6 Cyclic voltammograms of (a) (i) bare and (ii) SPAuE-BA monolayer modified in 1.0 mM H_2O_2 phosphate buffer (pH 7.4) solution. b SPAuE-BA-MnTAPc and c SPAuE-BA-CoTAPc electrodes in pH 7.4 phosphate buffer solution (i) without and (ii) with 1.0 mM H_2O_2

peaks were observed for all the modified electrodes in pH 7.4. The MTAPc modified electrodes exhibited much higher currents in pH 7.4 phosphate buffer solution containing 1.0 mM H_2O_2 and peaked at about -0.31 V and -0.28 V for SPAuE-BA-CoTAPc and SPAuE-BA-MnTAPc electrodes, respectively. These electroreduction peaks due to H_2O_2 occurred at potentials which were less negative compared to the electrode modified with polymers of similar complexes [25]. The appearance of the H_2O_2 electrocatalytic reduction peaks close to the observed metal reduction peaks clearly indicates the involvement of the metal ion in the electrocatalytic process. Therefore, the metal ions mediate the electrocatalytic reduction of H_2O_2 . This is not surprising as from our previous reports [5, 25] the involvement of metal

ions in the electrocatalytic reduction of H_2O_2 was confirmed by spectroscopic methods [5]. The use of screen printed electrodes in this work clearly shows the potential of miniaturizing these systems for field-test applications.

Reproducibility of SPAuE-BA-MTAPc electrodes and their electrocatalysis

The electrodes were further studied for their reproducibility and three electrodes for each complex were modified following the method shown in Scheme 1. The electrocatalytic peaks (Fig. S1 of Supporting Information) were observed at potentials close to where the original peak potentials in Fig. 6 occurred. At SPAuE-BA-MnTAPc modified electrodes, the H_2O_2 peak potential varied slightly from -0.27 to -0.32 V with similar current densities. At SPAuE-BA-CoTAPc modified electrodes, the H_2O_2 peak potentials and current densities varied slightly with the potentials varying from -0.27 to -0.29 V. The obtained results, especially the potential at which the H_2O_2 was electrocatalytically reduced, were comparable to those of electrodes modified with horse-radish peroxidase [34–36]. These results lead to the conclusion that the different electrodes modified using the method reported are reproducible and gave very similar results. The surface coverages estimated from the different electrodes measured in phosphate buffer solution (pH 7.4) were also similar with slight deviation and were found to be $1.31 \pm 0.86 \times 10^{-9}$ mol.cm $^{-2}$ and $1.66 \pm 0.52 \times 10^{-9}$ mol.cm $^{-2}$ for SPAuE-BA-MnTAPc and SPAuE-BA-CoTAPc, respectively. The long term use of these electrodes was also investigated and the surfaces can be regenerated by cycling in phosphate buffer solution (pH 7.4). The regenerated MTAPc modified electrodes gave good electrocatalytic currents of about 87% for SPAuE-BA-CoTAPc and 85% for SPAuE-BA-MnTAPc after 3 weeks of storage.

Conclusions

The covalent immobilization via Schiff-base reactions of MTAPc complexes containing cobalt and manganese as central metal ions has been accomplished. The electron-transfer rate constants of these modified electrodes were studied and clearly showed the effect of immobilizing MTAPc complexes compared to benzylamino monolayer modified electrodes. The MTAPc monolayer modified electrodes gave good results for the electrocatalysis and electroanalysis of H_2O_2 with the bare and benzylamino modified electrodes exhibiting no signal within the studied potential range. The use of the screen-printed electrode was important in demonstrating the possible miniaturization of these electrochemical systems for the detection of H_2O_2 .

The use of these electrodes is continuing for the immobilization of glucose oxidase enzyme and the detection of glucose (as a substrate) will be investigated using MTAPc monolayers as electron mediators, thus forming a second generation of biosensors.

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