CATALYTIC ACTIVITY OF ELECTRODE MATERIALS BASED ON POLYPYRROLE, MULTI-WALL CARBON NANOTUBES AND COBALT PHTHALOCYANINE FOR THE ELECTROOXIDATION OF GLUTATHIONE AND L-CYSTEINE

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ABSTRACT

Electrodes prepared from overoxidized pyrrole, multi-walled carbon nanotubes (MWCNT) and cobalt phthalocyanine (CoPc) exhibit a clear catalytic activity for the electrooxidation of L-cysteine and reduced glutathione. The electrodes are more stable than the ones modified by simple adsorption of CoPc and/or MWCNT. Therefore, these materials offer a good alternative for the development of electrocatalytic applications and thiols detection in biological interest.

Keywords: Electrocatalysis, thiols, modified electrodes, carbon nanotubes

INTRODUCTION

The activation and decomposition of molecules with thiol groups and their derivatives via their electrochemical oxidation is an important topic in industrial and biological research, since these compounds are present as pollutants in fuels [1], and they are used as physiological indicators in biological fluids [2] and as markers of food deterioration [3]. It is known that cobalt phthalocyanines (CoPc) adsorbed on electrode surface or incorporated into polymeric matrices exhibit a pronounced catalytic effect for the electrochemically assisted oxidation of thiols such as cysteine (CySH) and glutathione (GSH) [4-24]. In particular, it has been demonstrated that electrodes modified with electropolymerized CoPc films show superior electrocatalytic activity and greater stability than adsorbed CoPc films [20,25].

When using of phthalocyanine dispersions in polymer matrices [26,27] it is possible to design electrode materials of various features, with improved control of the deposit formed. For example, 4b(SO₃⁻)CoPc (tetrasulfonated cobalt phthalocyanine) was incorporated into polypyrrole matrix (PPy) during the polymer oxidation process and a three-dimensional 4b(SO₃⁻)CoPc arrangement was thus generated, without altering the electrocatalytic properties of the complex. Moreover, Ozoemena et al. [28] studied the electrosorption of 4b(NH₂)CoPc (cobalt tetra-aminophthalocyanine) on single wall carbon nanotubes (SWCNT), generating films that are dense and organized with excellent catalytic properties for the oxidation of thiols. Also, Silva et al. [29] reported that electrodes modified with SWCNT and adsorbed CoPc exhibit electrocatalytic activity for the electro-oxidation of 2-ME.

Therefore, it is expected that the combination of CoPc, PPy and multi-walled carbon nanotubes (MWCNT) should generate materials with higher electrocatalytic activity than that corresponding to each of the individual components. The main goal of this study is the preparation and characterization of electrode materials based on glassy carbon electrodes (GCE), CoPc, MWCNT and PPy, with enhanced electrocatalytic activity for the oxidation of L-cysteine and reduced glutathione.

EXPERIMENTAL

All experiments were performed in a conventional three-electrode electrochemical cell using a GCE disc of 3 mm diameter (imbedded in Teflon) as the working electrode, a platinum wire as counter-electrode and a Ag/AgCl_{sat} wire as the pseudo-reference electrode. The working electrode was polished mechanically using three different sizes of alumina: 1, 0.3 and 0.05 microns, followed by successive rinsing with Milli-Q ultrapure water before each ex-

periment. Pyrrole was freshly distilled prior to experiments. MWCNTs were pre-oxidized in a mixture of H₂SO₄/HNO₃ as described in the literature [30]. All other chemical products were analytical reagent grade from Sigma-Aldrich and were used without prior purification.

The electropolymerization of pyrrole (0.1 M) was conducted in a solution of 0.1 M Bu₄NBF₄ in CH₃CN, applying 10 successive potential sweep cycles between -0.8 and 1.0 V at 25 mV s⁻¹ [31]. The same electropolymerization conditions were used in the presence of MWCNT (0.01 mg/mL) and CoPc (2 mM) to prepare the GCE/PPy/MWCNT, GCE/PPy/CoPc and GCE/PPy/MWCNT/CoPc electrodes. Once the deposit was generated, the electrode was rinsed several times with CH₃CN and characterized in a monomer-free supporting electrolyte (0.1 M Bu₄NBF₄ in CH₃CN) in a potential window of -0.8 to 1.0 V at 50 mVs⁻¹. The modified electrodes were all submitted to an overoxidation treatment of the polypyrrole to reduce the large capacitive current due to the electrodeposited polypyrrole. This consisted of applying 25 potential sweep cycles in the -0.2 to 1.0 V potential window at a rate of 50 mV s⁻¹ in a 0.5 M NaOH solution.

Concentrations ranging from 1 to 5 mM of L-CySH and GSH were used in oxygen-free 0.5 M NaOH aqueous solutions. These were deoxygenated for 15 minutes with pure N₂ before electrochemical experiments. Fresh analyte solutions were prepared before each experiment.

RESULTS

The GCE/PPy, GCE/PPy/MWCNT and GCE/PPy/CoPc modified electrodes show the typical potentiodynamic features of PPy, nanotubes (MWCNT) and CoPc in 0.1 M of tetrabutylammonium tetrafluoroborate (Bu₄NBF₄) in acetonitrile (CH₃CN) (Figure 1) [31].

The capacitive current of the modified electrodes is however significant, probably attributed to a larger porosity of the films and increases after the insertion of the various elements into the final material. The enhanced capacitive currents can mask the oxidation signals of the different thiols. However, these enhanced capacitive currents can be diminished if the polypyrrole matrix is overoxidized.

Figure 2 shows the potentiodynamic response of the GCE/PPy/MWCNT/CoPc electrode in 0.1 MBu₄NBF₄ /CH₃CN before (curve a) and after (curve b) the overoxidation of the film in 0.5 M NaOH in aqueous solution. The capacitive current is drastically reduced and this can be attributed to a loss of the electroactivity of the PPy film [32,33]. Nevertheless, overoxi-

zed films of GCE/PPy/CoPc, GCE/PPy/MWCNT and GCE/PPy/MWCNT/CoPc are still conductive enough to allow the rapid electron transfer of a classical redox couple such as ferri/ferrocyanide. The kinetics of this couple are not affected by overoxidation of the film as shown by cyclic voltammograms compared to bare the GCE, as illustrated on Fig. 1B. It is also noticed from these experiments that the active surface of the modified electrode is almost identical to that of the bare electrode.

Figure 1.- Cyclic voltammograms for the electropolymerization of A) GCE/PPy y B) GCE/PPy/MWNTC/CoPc in 0.1 M Bu_4NBF_4 in CH_3CN , (PPy 0.1 M CoPc 0.002 M and 0.01 mg/mL MWNTC) $v = 25 \text{ mVs}^{-1}$

Figure 2. Characterization voltammograms of GCE/PPy/MWCNT/CoPc (a) before and (b) after the overoxidation process, in 0.1 M Bu_4NBF_4 in CH_3CN , $v = 50 \text{ mVs}^{-1}$. (B) Cyclic voltammogram at (a) bare GC (b) overoxidized GCE/PPy/MWCNT/CoPc in 5 mM ferrocyanide solution in 0.5 M NaOH.

The electrocatalytic activity of the different modified electrodes was tested for the electro-oxidation of L-CySH and GSH. Figure 3 shows the electrochemical oxidation of (A) L-CySH and (B) GSH (5 mM) on GCE/PPy/MWCNT (curve i), GCE/PPy/CoPc (curve ii) and GCE/PPy/MWCNT/CoPc (curve iii) in aqueous 0.5 M NaOH solution. For both L-CySH and GSH, the largest oxidation current is observed with the hybrid electrodes containing CoPc, namely GCE/PPy/CoPc and GCE/PPy/MWCNT/CoPc. This demonstrates that, as expected, CoPc catalyzes the oxidation of the thiols in the composite electrode. The presence of the MWCNTs inside the electrode material favors the access of the reacting molecules to the active sites of CoPc within the polymer matrix, leading then to higher oxidation currents.

Figure 3. Voltammograms of the oxidation of 5 mM (A) L-CySH and (B) GSH in 0.5 M NaOH at: (i) GCE/PPy/MWCNT, (ii) GCE/PPy/CoPc and (iii) GCE/PPy/MWCNT/CoPc. $dE/dt = 50 \text{ mVs}^{-1}$

As previously reported and discussed in the literature, the oxidation of thiols in alkaline media most likely involves the formation of a bond between the metal center in the complex and the sulphur atom in the thiolate [5,8,9,10,14,19]. The complete reaction scheme is:

Where RSH is thiol, MPc is metallophthalocyanine, and "sol" and "film" stand for species in solution or incorporated to the film on the electrode, respectively. Step 4 is fast and irreversible. A crucial step is step 2, which involves the formation of a Co-S bond, with a partial reduction of the metal center in the catalyst and partial oxidation of the bound thiol molecule. There is evidence by using *in-situ* reflectance visible spectroscopic for the formation of Co(I)

upon interaction of sulfonated CoPc with L-cysteine [8] on basal plane graphite electrodes. It has been shown that an optimal Co(II)/(I) redox potential exists so to obtain the highest activity for the oxidations of thiols in general [19] that would corresponds to an interaction of the thiol in step 2 that is not too weak, not too strong so to favor the catalytic process.

The use of the modified electrodes for the detection of thiols by profiting from the high catalytic activity of CoPc is exemplified in the case of L-CySH. Figure 4 shows the calibration curves obtained from cyclic voltammetry data for concentrations ranging from 1 to 5 mM. A linear correlation is observed with all the modified electrodes examined. The highest sensitivity is obtained with GCE/PPy/MWCNT/CoPc. It is interesting that very low sensitivity is obtained for GCE/PPy/MWCNT, without CoPc. However, the sensitivity of the modified electrode resulting from the simple adsorption of CoPc on the MWCNTs is higher than that of all the electrodes. But the analysis of the standard deviation values RSD shows that the electrode modified with adsorbed CoPc shows lower reproducibility. Indeed, RSD values of 10% and 2% were calculated for GCE-adsorbed CoPc and GCE/PPy/MWCNT/CoPc, respectively. These electrodes show high reproducibility.

Figure 4. Calibration curves for L-CySH on (i) GCE/PPy/MWCNT, (ii) GCE/PPy/CoPc and (iii) GCE/PPy/MWCNT/CoPc electrodes (from cyclic voltammetry, at $E = 0$ V)

Table 1 summarizes the analytical performances obtained for the oxidation of L-CySH and Table 2 describes the data for the oxidation of GSH on different electrode materials: sensitivity and limit of detection (LD) and limit of quantification (LQ).

For L-CySH, electrodes prepared by electropolymerization, despite of showing lower sensitivity, offer lower limits of detection and quantification than the electrodes modified by adsorption, with the GCE/PPy/MWCNT/CoPc electrode presenting the lowest LD and LQ, with values of 0.03 mM and 0.10 mM respectively. Since L-cysteine is present in cellular concentrations of 200–300 μ M, the GCE/PPy/GCE/PPy/MWCNT, GCE/PPy/CoPc and GCE/PPy/MWCNT/CoPc.

Table 1. Sensitivity, detection limit and quantification limit values for the detection of L-CySH with the different hybrid electrodes. Data deduced from cyclic voltammetry experiments for L-CySH concentrations ranging from 1 to 5 mM.

	Sensitivity, μ A/mM	LD, mM	LQ, mM
MWCNT _{ads}	1.01 ± 0.70	0.23 ± 0.03	0.77 ± 0.28
PPy/MWCNT	0.320 ± 0.001	0.190 ± 0.002	0.65 ± 0.02
CoPc _{ads}	6.74 ± 0.40	0.140 ± 0.001	0.470 ± 0.002
PPy/CoPc	2.330 ± 0.002	0.100 ± 0.001	0.340 ± 0.004
MWCNT/CoPc _{ads}	4.00 ± 0.16	0.040 ± 0.001	0.130 ± 0.001
PPy/MWCNT/CoPc	2.930 ± 0.001	0.030 ± 0.001	0.10 ± 0.02

For GSH, GCE/PPy/MWCNT/CoPc electrode presenting the lowest LD and LQ, with values 0.02 mM and 0.66 mM respectively. These electrodes are good candidates as voltammetric sensors for biological samples

Table 2. Sensitivity, detection limit and quantification limit values for the detection of GSH with the different hybrid electrodes. Data deduced from cyclic voltammetry experiments for GSH concentrations ranging from 1 to 5 mM.

	Sensitivity, μ A/mM	LD, mM	LQ, mM
MWNTC _{ads}	0.63 ± 0.30	0.24 ± 0.02	0.82 ± 0.24
PPy/MWNTC	0.037	1.66	5.55
CoPc _{ads}	3.25 ± 0.83	0.25 ± 0.04	0.83 ± 0.48
PPy/CoPc	$0.89 \pm 5 \times 10^{-3}$	$0.22 \pm 5 \times 10^{-3}$	0.75 ± 0.04
MWNTC/CoPc _{ads}	2.22 ± 0.48	0.21 ± 0.02	0.70 ± 0.21
PPy/MWNTC/CoPc	$1.15 \pm 4 \times 10^{-3}$	$0.20 \pm 1 \times 10^{-4}$	$0.66 \pm 2 \times 10^{-3}$

CONCLUSION

It can be concluded that modified electrodes prepared from overoxidized pyrrole, MWCNT and CoPc exhibit a clear catalytic activity for the electro-oxidation of L-cysteine and reduced glutathione. These electrodes exhibit lower detection limits and are more stable than the electrodes modified by simple adsorption of CoPc on the MWCNT. Therefore, these materials could be a good alternative for the development of electrochemical sensors for thiols of biological interest.

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