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Electrohemical properties of carbon nanotube paste electrodes modified with redox cationic dyes

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ABSTRACT. The present work describes the electrochemical behavior of methylene blue and toluidine blue as electron mediators adsorbed in the multiwall carbon nanotubes paste. Based on midpoint potential and separation of cathodic and anodic peaks (ΔE), it was not observed interaction of different eletrolytes with the cationic dyes by an ion exchange reaction and, as a consequence, absence of leaching of cationic dyes to the solution phase. The kinetics of electron transfer on the surface electrode was not sufficiently fast showing a fairly resistence of carbon nanotube paste modified with the mediators. The midpoint potential and ΔE also were insentive to the pH range (4-8), confirming the protective effect of carbon nanotubes matrix, owing to strong interaction of between the latter and the nitrogen of nitrogen of cationic dyes with carbon nanotube matrix, minimizing the proton interaction under cationic dye. This result is very important for sensor/biosensor preparation, because the eletrooxidation behavior of the analyte will be only affected by its formal potencial shifting. Carbon nanotubes proved to be an efficient solid matrix for the adsorption of mediator electron in comparison to the electrochemical behavior of free cationic dyes in solution phase.

Keywords: carbon nanotubes, electron mediator, cationic dyes, cyclic voltammetry.

Propriedades eletroquímicas de eletrodos a base de pasta de nanotubo de carbono modificados com corantes redox catiônicos

RESUMO. O presente trabalho descreve o comportamento eletroquímico de azul de metileno e azul de toluidina como mediadores de elétrons adsorvidos em pasta de nanotubo de carbono multiparede. Com base no potencial médio e na separação de pico catódico e anódico (ΔE), não foi observada interação de diferentes eletrólitos com os corantes catiônicos por meio de reação de troca iônica e, como consequência, ausência de lixiviação dos corantes para solução. A cinética de transferência de elétron na superfície do eletrodo não foi suficientemente rápida, mostrando razoável resistência da pasta de nanotubo de carbono modificada com os mediadores. O potencial médio e ΔE também foram insensíveis a variação de pH (4-8), confirmando o efeito protetivo da matriz de nanotubo de carbono, devido a forte interação do nitrogênio no corante catiônico com a matriz de nanotubo de carbono, minimizando a interação dos prótons sobre os corantes. Este resultado é bastante importante para o preparo de sensor/biosensor, porque o comportamento da eletrooxidação do analito será afetado apenas pelo deslocamento de seu potencial formal. Os nanotubos de carbono corfirmaram ser uma eficiente matriz sólida para adsorção de mediadores de elétrons em comparação com o comportamento eletroquímico dos corantes catiônicos em solução.

Palavras-chave: nanotubo de carbono, mediador de elétron, corantes catiônicos, voltametria cíclica.

Introduction

Some low molecular weight enzymes such as horseradish peroxidase (HRP), cytocrome c peroxidase and fungal peroxidase, commonly used for building amperometric biosensors, present a kinetic barrier for the direct electron transfer between the active sites of redox enzymes and the electrode surface (KUBOTA et al., 2000). This drawback could be overcome by using electrochemical mediators - substances that facilitate the electron transfer between enzymes and the electrode surface (DIAS et al., 2006). Several types of electron mediators considered are as follows: ferrocene, hexacyanoferrate, tetrathiafulvalene, o-phenylenediamine, quinones, flavins, quinoic type compounds, such as phenoxazines and organic dyes, including methylene blue (MB), Prussin blue, phenazines, toluidine blue, methylene green, meldola's blue, and tionin (REZAEI-ZARCHI et al., 2009; YANG et al., 1998). The electrochemical properties of organic dyes are very well known for the solution phase; they represent the formal potential, $E^{o\prime}$, between 0.08 and -0.25 V (versus SCE), very close to those redox reactions of several enzymes over a large pH range. In spite of these features, the use of solution-phase mediators is not preferable for preparing a biosensor, because large amounts of dyes are required and the drawbacks associated with the reference electrode contamination have been noticed (KUBOTA et al., 2000). Various studies were performed by immobilizing organic dyes onto a graphite electrode surface, but a loss of signal and stability through the use of a sensor device due to leaching of the mediator from the enzyme sensor was observed (YANG et. al., 1998). In this way, some researches focused on preparation of a stable biosensor containing water-soluble organic dyes attached to it were carried out. The literature review shows that these devices are constituted of carbon paste electrodes, where the immobilization of the mediator takes place onto a solid matrix, such as cellulose surfaces modified with TiO_2 and $Ti(H_2PO_4)$ (DIAS et al., 2002), porous $SiO_2/ZrO_2/Sb_2O_5$ (ZAITSEVA et al., 2002), SiO_2/Sb_2O_3 binary oxide (RIBEIRO et al., 2003). In the present work, carbon nanotubes were investigated as a solid matrix and efficient conductor material for the adsorption of methylene blue and toluidine blue as electron mediators. Undoubtedly, over the last two decades, no other chemical structure has aroused so much attention other than carbon nanotubes, which can be conceptually considered as originating from single-layer sheets of graphite, rolled up into perfectly seamless tubes with nanometer dimensions. The sp^2 carbon-carbon covalent bond in graphite is among the strongest known bonds in chemistry, and carbon nanotubes are therefore mechanically robust. These tubes also have unique electronic properties, different from graphite, and present an exciting potential for nanoscale electronic devices. Overlap of the unhybridized p_z orbitals yields a π complex both above and below the plane containing the atoms, which is related to the high electron mobility and high electrical conductivity of graphene (ROTKIN; SUBRAMONEY, 2005). Based on these comments, carbon nanotubes have received significant attention for the preparation of electrochemical sensors (SANTOS et al., 2005) due to their intrinsic properties, such as high surface area and high chemical and electrical properties (TARLEY et al., 2009). The present work describes the electrochemical behavior of methylene blue and toluidine blue as electron mediators adsorbed in the multiwall carbon nanotubes paste.

Material and methods

Reagents and instruments

Aqueous standard solutions were prepared using water obtained from a Milli-Q® purification system (MILLIPORE, Bedford, MA, USA). Multiwall carbon nanotubes (MWCNTs) - > 95% purity, diameters of 10-40 nm, lengths of 5-20 μm -were supplied without further purification by CNTs Co., Ltd. (Yeonsu-Gu, Incheon, Korea), and mineral oil used was obtained from Aldrich (Schering-Plough). Methylene blue (MB) and toluidine blue (TB) were acquired from Synth-Brazil and Dinâmica-Brazil, respectively, and utilized without further purification. Graphite powder (purity 99.9%) was supplied by Sigma-Aldrich. Electrolyte solutions were of analytical grade and prepared from their respective salts (Darmstadt, Merck) without further purification.

All electrochemical measurements were performed in a Potenciostat/Galvanostat Autolab PGS TAT12 (Eco Chemie BV, Utrecht, The Netherlands) with a conventional three-electrode cell comprising a platinum wire as auxiliary electrode, a saturated calomelane (SCE) as reference electrode, and a carbon nanotube paste working electrode modified with organic dyes. The pH values were checked with a Metrohm, Model 827 pH Lab pH-meter (Herisau, Switzerland). The working electrode was prepared by mixing 10 mg of MWCNT modified with MB or TB and 40 μL of mineral oil until a paste consistent was obtained. After this step, the resulting paste was carefully forced into a cavity (diameter of 4.0 mm; depth of 1 mm) at the end of a glass tube. The electrical connection was provided with a copper wire connected to the paste in the inner hole of the tube. The paste electrode surface was smoothed with a foil paper prior to use. Before each experiment, the solution was purged with nitrogen gas, and the gas flow through the solution was maintained during measurements.

Adsorption of methylene blue and toluidine blue on MWCNT

The adsorption of MB and TB on MWCNT was carried out by mixing 200 mg of MWCNT with 20 mL of a 1.0×10^{-4} mol L^{-1} solution of each organic dye. Then, the mixture was stirred for 3h. After that, the material was rinsed with deionized water and dried at room temperature. The resulting materials will hereafter be designated as MWCNT-MB and MWCNT-TB.

Results and discussion

Electrochemical properties of sensors MWCNT-MB and MWCNT-TB

Figure 1 shows the cyclic voltammograms of the unmodified MWCNT (A) and MWCNT-TB (B) sensors. As it could be observed, no cathodic and anodic peaks were found for the unmodified MWCNT sensor within the potential range evaluated, whereas a redox couple of the mediator peak having the midpoint potential E_m = -113 mV vs SCE ($E_m=(E_{ap} + E_{cp})/2$) (MURRAY, 1984), where E_{ap} E_{cp} are the anodic and cathodic peaks, respectively, was verified for the MWCNT-TB sensor. From this result, it could be seen, comparing the midpoint potential of -211 mV *vs* SCE for the free TB in the solution phase (KITANI et al., 1981) at the same pH, that a shift of 98 mV toward more positive values was observed for the dye immobilized onto MWCNT. Moreover, comparing the midpoint potential of -200 mV *vs* SCE for TB directly adsorbed onto carbon paste (MOLINA et al., 1999), there was an increase of 87 mV toward more positive values for the MWCNT-TB sensor. This behavior could be attributed to a strong acid-base interaction with the MWCNT matrix, stabilizing the mediator in its reduced form. As a consequence, in order to promote oxidation of the reduced form of the mediator, a shifting of the formal potential toward more positive values should be applied. The similar behavior was observed for phenoxazines, phenothiazines and phenazines adsorbed onto $Zr(H_2PO_4)$ (MALINAUSKAS et al., 2000) and $Ti(H_2PO_4)$ (KUBOTA et al., 2000).

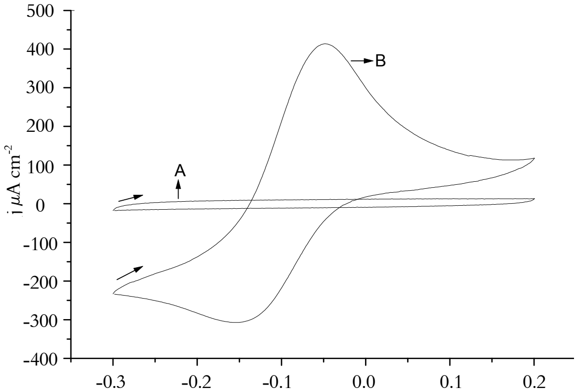

Figure 1. Cyclic voltammograms obtained for unmodified MWCNT (A) and MWCNT-TB (B) in 0.5 mol L^{-1} KCl at pH 7.0 and a scan rate of 10 mV s^{-1}.

The cyclic voltammograms of the unmodified graphite (A), unmodified MWCNT sensor (B), and MWCNT-MB (C) sensors are depicted in Figure 2. In a similar way to the MWCNT-TB sensor, anodic and cathodic peaks were clearly observed for MWCNT-MB with the absence of any redox processes for the unmodified graphite and MWCNT sensor.

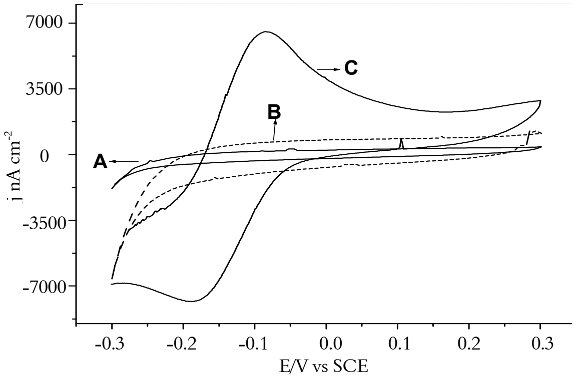

Figure 2. Cyclic voltammograms obtained for unmodified graphite (A), unmodified MWCNT (B) and MWCNT-MB (C) in 0.5 mol L^{-1} KCl at pH 7.0 and a scan rate of 10 mV s^{-1}.

The midpoint potential achieved for the MWCNT-MB sensor was found to be -130 mV (*vs* SCE). Again, comparing this potential with that for methylene blue in the solution phase, a shifting towards more positive values was verified. This finding seems to be very important, because electrochemical measurements could be performed at near-zero potential, thus minimizing interference problems that come from other electroactive substances. Taking into account the separation of cathodic and anodic peaks for the MWCNT-MB sensor (ΔE= 95 mV *vs* SCE), low resistance of the system was evidenced.

In order to determine the maximum adsorption capacity of the MWCNT matrix towards the organic dyes, the surface concentration of electroactive species, Γ (mol cm^{-2}), was defined as $\Gamma = Q/nFA$, where Q is the charge (in Coulombs), n is the number of electrons, F is the Faraday constant (96500 C), and A is the surface area of the sensor (cm^2). Figure 3 shows the curves constructed for the determination of the surface concentration of the electroactive species, where the value of Γ was determined by integration of the anodic or cathodic peaks obtained at a low scan rate (10 mV s^{-1}) (JI et al., 2006). As one can see from Figure 3, starting from 0.4 mmol L^{-1}, the surface concentration is unchanged for both the organic dyes, thus suggesting multilayer formation. Therefore, the experiments for the adsorption of the organic dyes onto the MWCNT matrix were carried out at a concentration of 0.4 mmol L^{-1}.

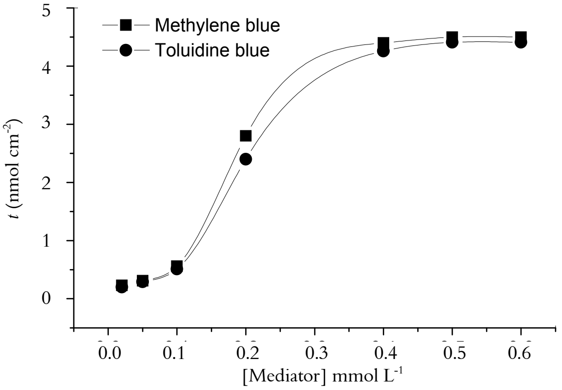

Figure 3. Determination of the organic dye concentrations at the surface of MWCNT-TB and MWCNT-MB. The experiment was carried out in 0.5 mol L^{-1} KCl at pH 7.0 and a scan rate of 10 mV s^{-1}.

The influence of different electrolytes on the MWCNT sensors modified with the organic dyes was also investigated. Table 1 presents the values of the separation of cathodic and anodic peaks (Δ*E*), which clearly demonstrate independence of electrolyte type. In addition, poor reversibility for MWCNT-TB and quasi-reversibility for the oxidation and reduction peaks of MB adsorbed on MWCNT was observed. This result suggests low charge transfer kinetics due to reasonable resistance of the sensor.

Table 1. Separation of cathodic and anodic peaks Δ*E* (mV) obtained with different electrolytes. The experiment was carried out at pH 7.0 and a scan rate of 10 mV s^{-1}.

	LiCl	KCl	$BaCl_2$	$CaCl_2$	NaCl	$NaNO_3$	Na_2SO_4	$NaClO_4$
MWCNT-MB	70	65	65	70	60	55	55	60
MWCNT-TB	120	120	130	130	125	130	130	130

The influence of some supporting electrolytes on the midpoint potential was also studied (Table 2). From the results obtained, the midpoint potential was found to be almost independent of the electrolyte types, except for sodium perchlorate that showed a shifting towards larger potential values. Such result was somewhat expected due to the strong affinity between perchlorate ions and nitrogen atoms present in the molecules of the cationic dyes (SPRINGBORG et al., 1995). According to the aforementioned, KCl was used as supporting electrolyte for further experiments.

Table 2. Midpoint potential (mV) obtained with different electrolytes. The experiment was carried out at pH 7.0 and a scan rate of 10 mV s^{-1}.

	LiCl	KCl	$BaCl_2$	$CaCl_2$	NaCl	$NaNO_3$	Na_2SO_4	$NaClO_4$
MWCNT-MB	-70	-60	-65	-65	-70	-80	-80	-100
MWCNT-TB	-113	-113	-115	-115	-114	-116	-116	-130

The KCl concentration effect on the electrochemical behavior of MWCNT-MB and MWCNT-TB was investigated in the range from 0.1 up to 1.0 mol L^{-1}. The increase of the KCl concentration in the experimental domain investigated did not practically change the values of Δ*E* (mV) and midpoint potential (mV). On the other hand, at concentrations being lower than 0.1 mol L^{-1}, the values of Δ*E* (mV) were drastically diminished. Therefore, a concentration of 0.5 mol L^{-1} was chosen for posterior experiments.

In order to prove the viability and reliability of the solid-phase matrix for the adsorption of the cationic dyes, studies based on several voltammetric cycles are commonly performed to assess the binding strength of an electron mediator and a solid phase. Table 3 shows clearly that even after carrying out 100 voltammetric cycles at a scan rate of 10 mV s^{-1}, the anodic peak current remains constant, thus demonstrating that the electron mediators are not released from the MWCNT matrix.

Table 3. Anodic peak current (μA cm^{-2}) after performing several voltammetric cycles. The experiment was carried out in 0.5 mol L^{-1} KCl at pH 7.0 and a scan rate of 10 mV s^{-1}.

Cycles	5	10	20	40	60	80	100
MWCNT-MB	50.1	50.1	50.0	50.1	50.1	50.0	50.1
MWCNT-TB	35.8	35.8	35.9	35.8	35.9	36.0	36.0

The linear relationship existing between peak current and the square root of the scan rate over the 5 - 200 mV s^{-1} range (Figure 4) indicates a diffusion-controlled process (BARD; FAULKER, 1980), which could be related to the limitation of the electrolyte diffusion into the MWCNT paste in order to keep the electroneutrality onto the electrode surface. Similar results were observed for the sensor prepared from inorganic materials, such as silicon and metallic phosphates, mixed with carbon paste (PESSOA et al., 1997).

The influence of the solution pH on the electrochemical behavior of the organic dyes was studied in a wide range of pH values (2.0 – 8.0). From this study, the midpoint potential and separation of cathodic and anodic peaks (Δ*E*) were not affected by pH ranging from 4.0 up to 8.0, thus confirming the sensor robustness for this parameter and strong interaction between the cationic dye nitrogen and the carbon nanotube matrix, as well as minimizing the proton interaction in the cationic dyes. Nevertheless, in highly acidic medium (pH 2.0 and 3.0), the shifting of the midpoint potential towards more positive values was observed (Table 4). Such result is very important for the sensor/biosensor preparation, because the electrooxidation behavior of the analyte would be only affected by the shifting of its formal potential.

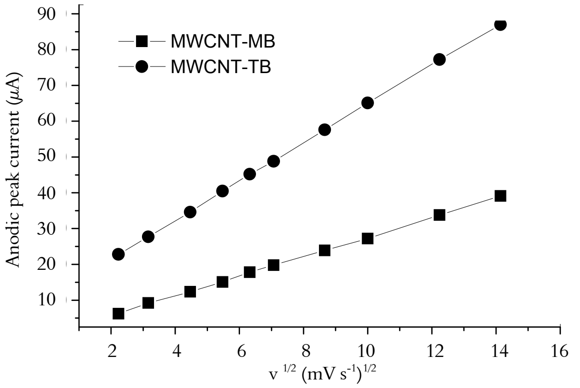

Figure 4. Plot of anodic peak current versus square root of the scan rate at potential scan rates of 5-500 mV s^{-1}. The experiment was carried out in 0.5 mol L^{-1} KCl at pH 7.0.

Table 4. Influence of pH on midpoint potential (mV) and separation of cathodic and anodic peaks ΔE (mV). The experiment was carried out at a scan rate of 10 mV s^{-1}.

	MWCNT-MB		MWCNT-TB	
pH	ΔE	E_m	ΔE	E_m
2	60	-25	90	-80
3	65	-30	90	-85
4	60	-55	100	-100
5	60	-60	120	-110
6	60	-60	120	-110
7	55	-55	120	-113
8	60	-60	120	-113

Conclusion

The aim of this work was to evaluate MWCNT as a solid-phase matrix adsorbent for the electron mediators - methylene blue and toluidine blue. The cationic dyes, confirmed by the absence of their leaching into the solution phase, were very efficiently attracted to the MWCNT matrix, even after carrying out several cyclic voltamograms. In addition, comparing with the electrochemical behavior of the free cationic dyes in the solution, the shifting of the midpoint potential of the adsorbed cationic dyes toward more positive values suggests that MWCNT strongly interacts with the electron mediator in its reduced form. Finally, the data obtained herein demonstrate that multiwall carbon nanotubes can serve as a good support for electrochemical mediators with a focus on the preparation of biosensors.

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