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Article

Methylene Blue Immobilized on Cellulose Acetate with Titanium Dioxide: an Application as Sensor for Ascorbic Acid

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Neste trabalho, o corante azul de metileno foi imobilizado na superfície do acetato de celulose modificado com dióxido de titânio produzindo um material híbrido sólido denominado de CA-TiO$_2$MB. O experimento produziu uma quantidade de 1,8 mmol g^{-1} de TiO$_2$ incorporado no acetato de celulose e uma quantidade de 0,170 ⇓ 0,005 mmol g^{-1} de azul de metileno adsorvido na superfície do CA-TiO$_2$. Um eletrodo de pasta de carbono desse material foi usado para estudar a oxidação eletrocatalítica do ácido ascórbico pelas técnicas de voltametria cíclica e cronoamperometria. O pH da solução não afetou o potencial de pico de anódico nem a corrente de pico anódico ao variar de 3,0 a 7,0. A oxidação do analito ocorreu em 75 mV versus ECS em solução de KCl 1,0 mol L^{-1} e pH 7,0. A intensidade da corrente de pico anódico variou com a concentração de ácido ascórbico na faixa de 5,0 ∂ 10^{-4} mol L^{-1} to 4,5 ∂ 10^{-3} mol L^{-1}, sendo observada uma correlação linear com um limite de detecção de 15 µmol L^{-1}, limite de quantificação de 50 µmol L^{-1} e uma sensibilidade de 7,1 µA L mol^{-1}. A resposta do eletrodo foi muito rápida, com um tempo decorrido de 1,0 s, mostrando potencial para ser utilizado como um sensor eletroquímico para a determinação de ácido ascórbico em produtos comerciais.

In this work, methylene blue dye was immobilized on the surface of cellulose acetate modified with titanium dioxide, producing a solid hybrid material designated as CA-TiO$_2$MB. The experiment yielded an amount of 1.8 mmol g^{-1} of TiO$_2$ incorporated in the cellulose acetate and an amount of 0.170 ⇓ 0.005 mmol g^{-1} of methylene blue adsorbed onto CA-TiO$_2$ surface. A carbon paste electrode of this material was used to study the electrocatalytic oxidation of ascorbic acid by cyclic voltammetric and chronoamperometric techniques. The pH of the solution had no effect on the anodic peak potential and anodic peak current when ranging from 3.0 to 7.0. The oxidation of the analyte occurred at 75 mV *versus* SCE in 1.0 mol L^{-1} KCl solution and pH 7.0. The intensity of the anodic peak current varied with the concentration of ascorbic acid from 5.0 ∂ 10^{-4} mol L^{-1} to 4.5 ∂ 10^{-3} mol L^{-1} and a linear correlation was observed, with a detection limit of 15 µmol L^{-1}, quantification limit of 50 µmol L^{-1} and a sensitivity of 7.1 µA L mol^{-1}. The electrode response was very fast, with an elapsed time of about 1.0 s, showing the potentiality to be utilized as an electrochemical sensor for determination of ascorbic acid in commercial samples.

Keywords: methylene blue, modified cellulose acetate, titanium dioxide, carbon paste electrode, ascorbic acid

Introduction

The development of sensors that allow the measurements of some analyte, which presents analytical or environmental interest, has continued to be of major concern during the past two decades. They use as electrochemical mediator species makes possible the attempt to exert more control over the chemical nature of an electrode and to attain the selectivity of electrochemical measurements. This development has helped the measurement of important analytes in medicine, pharmacy, manufactured food and other areas.[1] The most common material that is usually employed as an inert support for the preparation of chemically modified electrodes (CMEs) is silica gel, that is, silica prepared by the sol-gel process, and zeolites.[2-7]

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Recently, the use of available alternative materials as cellulose and its derivatives, as supports for preparation of electrochemical sensors, has increased.[8] Cellulose acetate (CA) is a polymeric derivative of cellulose particularly useful to prepare modified materials because it can be easily molded into different forms, such as membranes, fibers and spheres. These modified materials are normally made by mixing the cellulose acetate and metal alkoxide solutions followed by a phase inversion process.[9-12] Numerous chemical modifications in cellulosic matrices with highly dispersed metal oxides have been reported in recent years.[8,13-23]

The preparation of chemically modified electrodes with films or fibers of polymers and metal oxides has attracted considerable interest for immobilization of electroactives species due to their excellent electron transfer properties. An important class of organic compounds with interesting electrochemical properties, redox behaviour and possibility of immobilization by means of an ion exchange process in these host matrices is constituted by phenothiazines like methylene blue (MB).[24-27]

Methylene blue dye is a cationic molecule discovered by Heinrich Caro in 1876. Cytological dye and redox indicator.[28,29] This cationic compound has been used in preparation of CMEs as redox mediators in reactions between the electrode and substrate of analytical interest. This system has shown high selectivity and sensitivity for ascorbic acid, present in the electrolytic solution.[24,26,27]

Ascorbic acid (AA), which is also known as vitamin C, is one of the most popular nutrients being thoroughly found in fruits and vegetables. It can also be present in enriched industrialized products, such as fruit juices, tablets, candies and morning cereals. This recognition is due to the countless benefits that vitamin C provides to the organism, like antioxidant role, to improve healing processes, to increase iron adsorption from products of vegetable origin and to stimulate the immunological system.[30,31]

In this work, cellulose acetate fiber was chemically modified with titanium oxide, TiO_2, which is widely known for its high ion exchange capacity and conductivity properties.[32,33] This material was used as a matrix to incorporate and retain strongly the methylene blue as an organic electroactive species. Electrodes of this material were obtained by making a paste with carbon. The electrochemical and electrocatalytic properties of this electrode were investigated. In addition, this electrode was used as amperometric sensor for determination of ascorbic acid in aqueous solution.

Experimental

Preparation of the CA-TiO_2 composite

A 10.0 g of cellulose acetate (CA) was added to a reaction flask containing 40 mL of acetic anhydride (Merck) and 50 mL of acetone (Merck). The mixture was allowed to rest for 24 h at room temperature under dry nitrogen atmosphere. After that, viscous syrup was formed. Then, 7.5 mL of titanium (IV) butoxide was added to this syrup (100 g) and magnetically stirred. The resulting syrup was slowly added to a flask containing about 1000 mL of double distilled water, with efficient stirring, in order to regenerate a fibrous material containing TiO_2 immobilized on the cellulose acetate. The fibers were collected by filtration, washed with water and dried under vacuum at room temperature. Finally, the CA-TiO_2 hybrid material was dried at 60 °C.[34,35]

Immobilization of dye on CA-TiO_2

Aqueous solution of the dye methylene blue (Aldrich) was prepared in a concentration of $1.0 \partial 10^{-3}$ mol L^{-1}. An amount of 0.5 g of CA-TiO_2 was added to 50 mL of dye solution. The mixture was shaken for 30 min and the resulting solid was filtered, washed several times with bidistilled water and dried in an oven for 30 min at 60 °C. The material will hereafter be designated as CA-TiO_2MB.

Chemical analyses

The quantity of metal oxide incorporated in the CA-TiO_2 was determined by igniting the material at 900 °C for 3 h and weighing the residue as CA-TiO_2. The amount of methylene blue incorporated in the CA-TiO_2 was determined by nitrogen elemental analysis with a Perkin-Elmer Analyzer 2400 series II CHNS/O apparatus.

EDS analysis

The EDS image was obtained for CA-TiO_2 material dispersed on a double faced conducting tape on an aluminum support and coated with a thin gold film using a Baltec SCD 050 Sputter Coater apparatus. The micrograph was obtained using a Jeol Scanning Electron Microscope, model JSM 5800, connected to a secondary electron detector and X-ray energy dispersive spectrometer (EDS) for elemental mapping in a Noran Instrument. The image was obtained with a magnification of 550 X.

Electrochemical measurements

A carbon paste electrode was prepared by mixing 20 mg of the material CA-TiO_2 with 20 mg of analytical grade graphite (Fluka, 99.9%) and a drop of liquid paraffin as a binder. This paste was placed in a cavity with 1 mm depth, in contact with a 5 mm internal diameter platinum disk which is fused to a glass tube. The carbon paste electrodes made with the CA-TiO_2 material were used as working electrodes, a platinum wire served as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The pH of the solution was adjusted with NaOH or HCl solution. The ascorbic acid solution was freshly prepared before using it.

Cyclic voltammetry studies were carried out in an Autolab PGSTAT 30 potentiostat-galvanostat apparatus.

The electrocatalytic oxidation of AA by the CA-TiO_2MB carbon paste electrode was investigated by cyclic voltammetric and amperometric techniques, adding freshly prepared ascorbic acid solutions into the electrochemical cell containing 20 mL of the supporting electrolyte.

Results and Discussion

Characteristics of the material

The elemental EDS analysis shows high titanium content over the cellulose acetate surface. The Ti/C atomic ratio found was 0.40. Figure 1 shows the SEM and EDS images of the CA-TiO_2 material. The material seems to have homogeneous surface, since agglomerated titanium particles were not detected (Figure 1A). From the energy dispersive image (Figure 1B) it was possible to observe that the titanium dispersion was very uniform. The bright points observed in Figure 1B are due to the titanium atoms (Ti K_γ = 4.5 keV). With the magnification used, the EDS image is indicating that the metal is homogeneously dispersed over the cellulose acetate fiber.

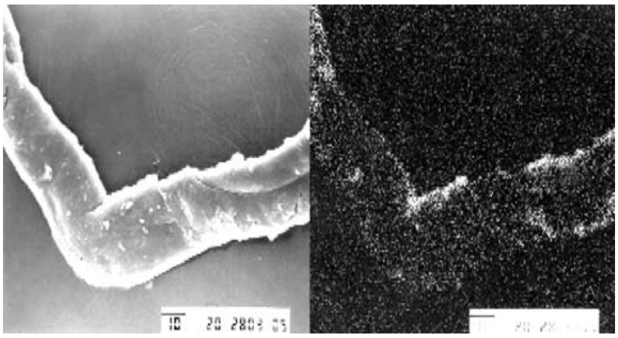

Figure 1. CA-TiO_2 micrographs. (A) SEM image; (B) EDS image.

Cyclic voltammetric studies

The incorporation of titanium oxide on a cellulose acetate can be described by the following reaction:

$$n\ CAOH + Ti(OC_4H_9)_4 \longrightarrow (CAO)_nTi(OC_4H_9)_{4-n} + n\ C_4H_9OH$$

$$(CAO)_nTi(OC_4H_9)_{4-n} + (4-n)\ H_2O \longrightarrow (CAO)_nTi(OH)_{4-n} + (4-n)\ C_4H_9OH$$

The content of TiO_2 incorporated in the cellulose acetate matrix resulted 10.1 wt.% (1.8 mmol g^{-1}).

The immobilization of methylene blue dye on the CA-TiO_2MB was made at pH 6.0, therefore, the immobilized form of the organic compound is MB^+. Figure 2 shows the structures of the organic dye at various pH values. The immobilization of methylene blue dye on the CA-TiO_2 surfaces occurs by an ion exchange reaction, represented by the following reaction:

$$CA\text{-}TiOH + MB^+ \leftrightarrows CA\text{-}TiOMB + H^+$$

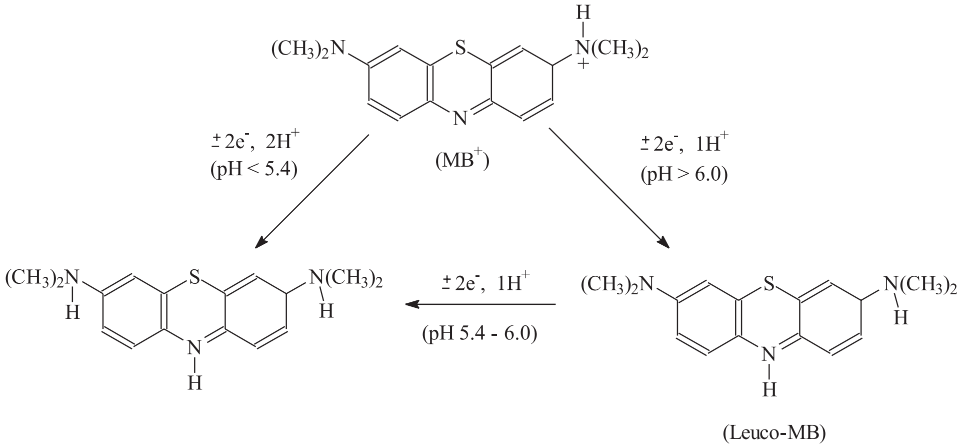

Figure 2. Structural formula of methylene blue in function of pH.

The amount of methylene blue (MB^+) adsorbed onto CA-TiO_2 was determined by CHN elemental analysis, resulting in 0.170 ⇓ 0.005 mmol g^{-1}.

Cyclic voltammograms of the carbon paste electrode prepared with modified CA-TiO_2 revealed no Faradaic waves, as observed in Figure 3A, whereas the one prepared with modified CA-TiO_2MB displayed evident voltammetric waves (Figure 3B). The midpoint potential, Em ($E_m = E_{pa} + E_{pc}$)/2 where E_{pa} is the anodic peak potential and E_{pc} is the cathodic peak potential) was 1225 mV *vs.* SCE in 1.0 mol L^{-1} KCl solution at pH 7.0. This modification in E_m of about 425 mV towards more positive potentials, compared to those obtained by organic dye in aqueous solution,[36] suggests that the reduced form of the organic dye interacts more strongly with CA-TiO_2 solid support than with the oxidized form. By integrating the area under the anodic or cathodic curves and considering that the geometric area of the electrode is 0.28 cm^2, the estimated quantity of methylene blue was 3.1 ∂ 10^{-8} mol cm^{-2}.

Cyclic voltammetry curves obtained at different scan rates indicated that the separation of the peak potential, $\pm E = E_{pa} - E_{pc}$, in the sweeping rate from 2 to 50 mV s^{-1}, increased according to the increment of the scan rate (Figure 4), indicating that the velocity of the electron transfer is not high enough. This can be a consequence of the difficulty of diffusion of the species of the electrolyte through the interface electrode-solution in order to keep the electroneutrality. Plotting the peak current, I_p, against the square root of the scan rates, $v^{1/2}$, a linear correlation was obtained (inserted in Figure 4) similar to the one observed for a process of controlled diffusion of the electroative species on the surface of the electrode.[37,38]

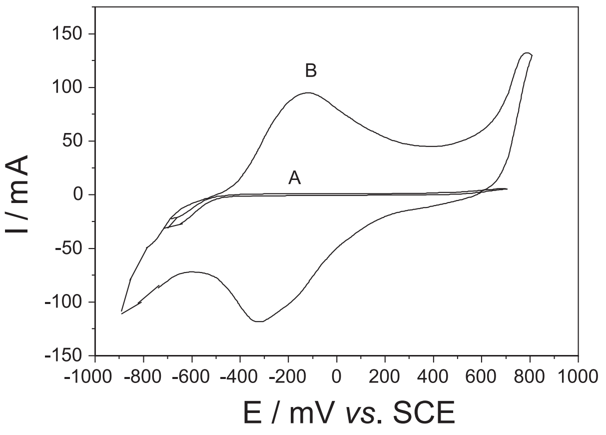

Figure 3. Cyclic voltammograms of the CA-TiO_2 electrode (A) and of the CA-TiO_2MB modified electrode (B) in scan rate at 20 mV s^{-1}, pH 7.0 and 1.0 mol L^{-1} KCl solution.

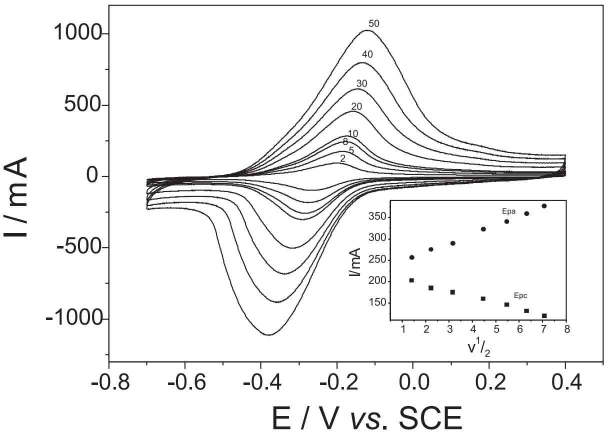

Figure 4. Cyclic voltammograms obtained at different scan rates (2, 5, 8, 10, 20, 30, 40, 50 mV s^{-1}). Supporting electrolyte: 1.0 mol L^{-1} KCl solution. Plot of the cathodic and anodic peak currents, I_{pc} and I_{pa}, against the square root of the scan rates, $v^{1/2}$, (inset on Figure 4).

The in-operation stability of the electrode was also tested by cycling the potential several times. The peak current intensities, Ipa and Ipc, measured between the potentials –0.7 and 0.4 V at scan rate of 20 mV s^{-1} in 1.0 mol L^{-1} KCl as the supporting electrolyte solution, decreased only by 2% after 100 cycles, indicating that the organic dye is strongly adhered onto the matrix surfaces and neither leached out from the material nor decomposed. The studies of chemical stability indicated that the electrode presented good performance when operating for two days, used 8 h/day and maintained overnight in 0.01 mol L^{-1} KCl solution. Under this condition, it was observed a decrease of 7% in the current intensities. The electrode can be considered as having good chemical stability, allowing its use for reasonable time without significant variation in the response. In addition, modified carbon paste electrodes are easily prepared and can be obtained in a short period of time.

The pH effect on the electrode response (Figure 5) was studied because it can affect both midpoint potential, E_m, anodic and cathodic peak current intensities. The CA-TiO_2MB modified electrode response was investigated in the pH range between 3.0 and 7.0, using the 1.0 mol L^{-1} KCl as the supporting electrolyte solution. In this pH range, E_m, I_{pa} and I_{pc} are not affected, remaining practically constant, as opposed to a different behavior that is presented in aqueous solution by the methylene blue dye, whose redox process becomes more efficient with the decrease of the pH.[24] The results suggest that the MB organic dye is immobilized on the in CA-TiO_2 solid matrix as a protonated form, held by an electrostatic interaction and protected against the change of the external pH solution. Additionally, it was observed that when the organic dye is present in aqueous solution or adsorbed on the graphite electrode the E_m is dependent on the pH of the solution due to the protonation and deprotonation process of amino functional groups during the conversion from oxidized to reduced forms and vice versa. This behavior in relation to the pH of the solution suggests that the methylene blue compound is very strongly involved in the binding to CA-TiO_2 through the two amino groups of methylene blue at positions 3.0 and 7.0 as well as the heterocyclic nitrogen.[39]

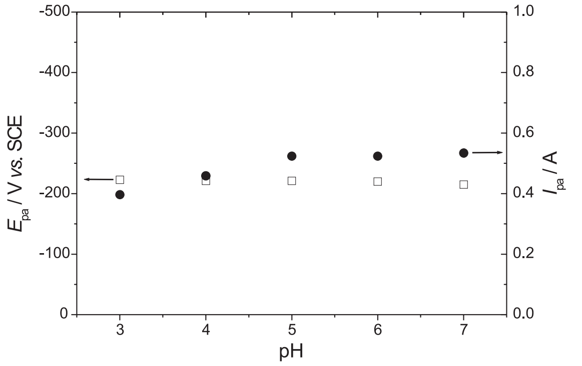

Figure 5. Plot of the solution pH against E_m, I_{pa} and I_{pc}. Supporting electrolyte: 1.0 mol L^{-1} KCl solution and in scan rate at 20 mV s^{-1}.

Oxidation of AA

Figure 6 shows the ascorbic acid oxidation on the CA-TiO_2MB electrode surface, immersed in 1.0 mol L^{-1} KCl solution at pH 7.0 in the absence (Figure 6, curve A) and in the presence of different concentrations of ascorbic acid (Figure 6, curves B-E). An increase in intensity of the anodic current peak, as the ascorbic acid concentration was increased stepwise from $1.0 \partial 10^{-3}$ mol L^{-1} to $40.0 \partial 10^{-3}$ mol L^{-1}, is an indication of catalytic oxidation of ascorbic acid mediated by CA-TiO_2MB electrode.

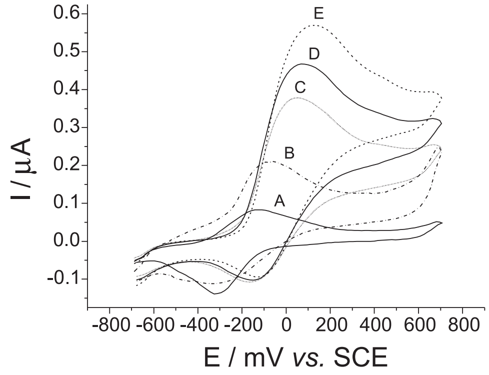

Figure 6. Cyclic voltammograms curves for the CA-TiO_2MB electrode in the absence of ascorbic acid (A) and in the presence of the ascorbic acid in concentrations mol L^{-1}: (B) 1×10^{-3}, (C) 20×10^{-3}, (D) 30×10^{-3}, (E) 40×10^{-3}. Supporting electrolyte: 1.0 mol L^{-1} KCl solution, pH 7.0 and scan rate at 20 mV s^{-1}.

The reaction process occurs by means of an electrochemical-chemical step, involving an electron transfer reaction followed by a hydration process, in which, respectively, the acid is irreversibly oxidized to dehydroascorbic acid followed by a hydration reaction in which the formation of hydrated dehydroascorbic acid takes place.[40] The reaction mechanism at the electrode-solution interface can be written as follows:

$$\text{CA-TiO}_2\text{MB}_{ox} + \text{H}_2\text{AA} \longrightarrow \text{CA-TiO}_2\text{MB}_{red} + \text{HAA}^+ + \text{H}^+$$
$$\text{CA-TiO}_2\text{MB}_{red} \longrightarrow \text{CA-TiO}_2\text{MB}_{ox} + 2e^1$$

The pH of electrolyte solution could present considerable influence in the reaction of electro-oxidation of the ascorbic acid as earlier reported.[41] On the other hand, in this work, the effect of the pH solution on peak currents and peak potentials for AA electro-oxidation were studied using CA-TiO_2MB modified electrode. It was observed that for pH solutions ranging from 3.0 to 7.0 no significant effects on the anodic peak potential as well as on the peak currents were seen, when the AA concentration was fixed at $1.0 \partial 10^{-3}$ mol L^{-1}.

Amperometric detection

Aiming to check the potential of using this electrode made with CA-TiO_2MB as sensor for ascorbic acid, chronoamperometry experiments were undertaken. First, amperometric studies were performed in order to determine the best potential to be applied. The potential was chosen by measuring the intensities of the catalytic currents for a solution containing $1.0 \partial 10^{-3}$ mol L^{-1} AA at 298 K and pH 7.0. The potential was fixed at 0.300 V in the subsequent experiments.

Figure 7 shows the amperometric curve obtained upon addition of the AA into the electrochemical cell keeping fixed E_{pa} = 0.300 V, pH 7.0 in 1.0 mol L^{-1} KCl supporting electrolyte solution. In the concentration range of ascorbic acid between $5.0 \partial 10^{-4}$ mol L^{-1} and $4.5 \partial 10^{-3}$ mol L^{-1}, a linear correlation was observed, as demonstrated by the equation I = (0.96 ⇓ 2.46) + (71.26 ⇓ 1.61) [AA], with a linear correlation coefficient R = 0,999 for n = 9. The detection limit

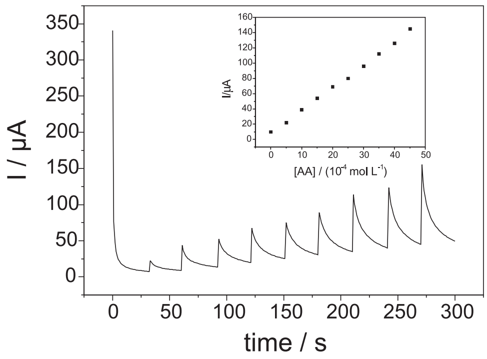

Figure 7. Chronoamperometry study for CA-TiO_2MB in 1.0 mol L^{-1} KCl solution. Chronoamperogram obtained by successive addition of 100.0 μL ascorbic acid 0.1 mol L^{-1} into an electrochemical cell containing 20 mL of 1.0 mol L^{-1} KCl solution at pH 7.0.

Table 1. Performance of the CA-TiO_2MB modified electrode in comparison with others amperometric sensors prepared in different solid supports for the determination of AA

Electrode	Solid Support	Linear range/(mmol L^{-1})	Detection limit/(πmol L^{-1})	Sensitivity/(mA cm^{-2})/(mol L^{-1})	Ref.
CA-TiO_2MB	cellulose acetate	0.5-4.5	15	7.1	this work
ZrP/MB	phosphate	0.2-1.7	10	-	24
Si-An^+/CR^-	sol-gel	0.79-6.7	-	-	44
C/Fe(II)NP	carbon paste	1.0-26	1400	18	45
$(C/SiPy^+)_4\{Fe(CN)_6]^4$	graphite bare	0.025-0.25	25	-	46
MBMOR/P	zeolite	0.02-0.8	12.1	-	47
CA/DCPI-CME	cellulose acetate	0.1-6	10	-	48
$[DCPI]_3$-La-SPE	screen-printed	0.01-1	10	-	49
CuP-Poly	polyester resin	0.02-3.2	10	-	50

achieved (3 standard deviations of the blank divided by the slope of calibration curve) was 15 µmol L^{-1}, the quantification limit (10 standard deviations of the blank divided by the slope of calibration curve) was 50 µmol L^{-1}, and the sensitivity was 7.1 µA L mol^{-1}. The average response time, measured after various substrate additions in the electrochemical cell, was 1.0 s.

It was performed an experiment to determine the ascorbic acid concentration in commercial pharmaceutical tablets, using the CA-TiO_2MB electrode. Commercial tablets were dissolved in 25.0 mL of distilled water (solution A). The amperometric response was obtained by adding 100.0 µL aliquots of solution A into a cell filled with 20.0 mL of a 1.0 mol L^{-1} KCl solution at pH 7.0 under nitrogen. The ascorbic acid concentration in the commercial Vitamin C formulation, which prescribed amount is 1 g/tablet was determined by amperometry (1.02 ⇓ 0.01 g/tablet) and further compared with iodometric titration method (0.99 ⇓ 0.03 g/tablet).[42] The presence of other substances in the Vitamin C tablets, such as citric acid, lemon flavor, orange flavor, sodium bicarbonate, sodium cyclamate and quinoline yellow, did not affect the results.[43]

The performance of the CA-TiO_2MB modified electrode in comparison with others amperometric sensors, based on carbon paste electrodes for the determination of AA, were also shown in Table 1. As it can be observed, the results indicated that the carbon paste electrode modified with CA-TiO_2MB presented good performance with low detection limit and high sensitivity, showing potentiality to be utilized as electrochemical sensor for determination of ascorbic acid in commercial tablets.

Conclusions

The elemental EDS analysis shows high titanium content homogeneously dispersed over the cellulose acetate surface. The immobilization of methylene blue dye on the CA-TiO_2 surfaces occurs by ion exchange. The effect of pH on the electrode response was studied in the range of 3.0 to 7.0 and the results suggest that the organic dye is entrapped in the CA-TiO_2 matrix in a protonated form, held by an electrostatic interaction and protected against the change of pH of the external solution. The modified electrode showed chemical stability for various oxidation-reduction cycles. When the concentration of ascorbic acid varies from 5.0 ∂ 10^{-4} to 4.5 ∂ 10^{-3} mol L^{-1}, a linear correlation is observed, with a detection limit of 15 µmol L^{-1}, quantification limit of 50 µmol L^{-1} and a sensitivity of 7.1 µA L mol^{-1}. The electrode response to ascorbic acid is very fast, with an elapsed time of about 1.0 s. The results indicate that carbon paste electrode modified with CA-TiO_2MB shows good potentiality to be utilized as a sensor for determination of ascorbic acid in commercial products.

Acknowledgments

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