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Dedicated to Professor Ionel Haiduc,
President of The Romanian Academy at his 70th anniversary

CARBON PASTE ELECTRODES INCORPORATING SYNTHETIC ZEOLITES AND METHYLENE BLUE FOR AMPEROMETRIC DETECTION OF ASCORBIC ACID

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ABSTRACT. Two new electrodes were obtained by incorporating two synthetic zeolites modified with Methyene Blue (MB) in carbon paste. The electrochemical behavior of the modified electrodes (MB-13X-CPES and MB-4A-CPEs) was investigated using cyclic voltammetry and the independence of formal standard potential *vs.* the pH of the surrounding solution was put on evidence. The modified electrodes were successfully tested for electrocatalytic oxidation of ascorbic acid (AA) in phosphate buffer (pH 7.0), at an applied potential with more than 500 mV lower than that used on unmodified carbon paste electrodes. The amperometric sensors for ascorbic acid were characterized by a linear concentration range of 10^{-5} M - 10^{-3} M AA for MB-13X-CPEs and 10^{-5} M - 10^{-4} M AA for MB-4A-CPEs. The theoretical detection limits, calculated from the slope of the regression equation and standard deviation of the calibration curve, were 4.7×10^{-5} M for MB-13X-CPEs and 1.7×10^{-5} M for MB-4A-CPEs.

Keywords: ascorbic acid, electrocatalytic oxidation; Methylene Blue; modified carbon paste electrode.

Introduction

L-Ascorbic acid (AA), also known as Vitamin C, is an organic compound playing a key role in living bodies, being essential for the formation of bone and connective tissue, helping the absorption of iron and helping burns and wounds heal. Like vitamin E, vitamin C is an antioxidant, protecting cells against damage by free radicals, which are reactive by-products of normal cell activity. This is the reason why L-Ascorbic acid is widely used as a dietary supplement and is also added to manufacture foods as an antioxidant for preservation [1]. Consequently, measuring ascorbic acid content is very important for assessing food product quality.

Satisfactory techniques for the determination of ascorbic acid must ideally be specific, reproducible, rapid, simple and sensitive and that is why electrochemical methods have been proposed for its determination.

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However, electrode fabrication for routine quantitative determination presents difficulties such as low selectivity or difficult preparation [2, 3].

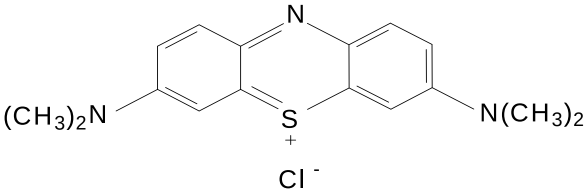
Among the new electrode materials used for ascorbic acid detection, those based on modified zeolites seem to be very attractive, since zeolites offer a number of chemical, physical and structural characteristics of high interest in the design of electroanalytical systems: shape, size and charge selectivities, physical and chemical stabilities, high ion-exchange capacity and hydrophilic character [4-7]. Moreover, zeolites present the ability to immobilize efficiently different redox mediators with electrocatalytic properties towards ascorbic acid oxidation [4, 8]. On the other hand, the well-known advantages of carbon paste (low background current, wide potential window, versatility etc.) can be exploited to obtain efficient entrapping matrices for modified zeolites.

In this context, using a highly efficient technique for zeolite immobilization in a carbon paste matrix [9], two new sensors incorporating synthetic mesoporous zeolites (4A and 13X, Aldrich) modified with Methylene Blue were designed and tested for ascorbic acid amperometric detection. Methylene Blue is a water-soluble cationic dye molecule, with a formal standard potential ($E^{0'}$) of -110 mV *vs.* SCE [8], suitable to catalyze AA oxidation and to prevent fouling of the electrode surface by oxidation products [10].

Experimental part

Chemicals

Methylene Blue (MB) (see Scheme 1), graphite powder and paraffin oil were purchased from Fluka (Buchs, Switzerland).



Scheme 1. Structure of Methylene Blue (MB).

The 13X type zeolite, $1Na_2O:1Al_2O_3:2.8\pm0.2\ SiO_2 \times H_2O$ (particle size, 3-5μ; pore diameter, 10 Å; specific surface area 548.69 m^2/g; bulk density 480.55 kg/m^3; Si/Al ratio 1.5) and the 4A type zeolite, $1Na_2O:1Al_2O_3:2.0\pm0.1\ SiO_2 \times H_2O$ (particle size, 2-3 μ; pore diameter, 4 Å; bulk density 480.55 kg/m^3; Si/Al ratio 1) were purchased from Aldrich (Germany).

Ascorbic acid (AA) was purchased from Sigma (SUA), $K_2HPO_4\cdot2H_2O$ and $KH_2PO_4\cdot H_2O$ were purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade and used as received.

The supporting electrolyte was a 0.1 M phosphate buffer solution. The pH was adjusted in the interval 1-9 using appropriate H_3PO_4 or NaOH solutions.

Electrode preparation

50 ml of a 0.001 % (w/v) MB solution in water were shaken (3 days) with 50 mg 13X type and 4A type zeolite. The modified zeolite was filtered, washed and dried. 25 mg of the modified zeolite were mixed with 25 mg graphite powder and 10 µl paraffin oil in order to obtain the modified carbon paste electrodes (MB-13X-CPEs and MB-4A-CPEs).

The preparation of MB-13X-CPEs and MB-4A-CPEs was reproducible when the experimental conditions and variables were maintained constant during the preparation period. The current response of the electrodes did not change significantly by storing them in air for several months.

Electrochemical measurements

Electrochemical experiments were carried out using a typical three-electrode electrochemical cell. The modified carbon paste electrode was used as working electrode, a platinum ring as counter electrode and an Ag|AgCl/KCl$_{sat}$ as reference electrode.

Cyclic voltammetry experiments were performed on a PC-controlled electrochemical analyzer (Autolab-PGSTAT 10, EcoChemie, Utrecht, The Netherlands).

Batch amperometric measurements at different AA concentrations were carried out at an applied potential with 120 mV more positive than formal standard potential ($E^{0'}$) of the mediator, under magnetic stirring, using 0.1 M phosphate buffer solution (pH 7) as supporting electrolyte. The current-time data were collected using the above-mentioned electrochemical analyzer.

For each electrode, the surface coverage (Γ, mol cm^{-2}) was estimated from the under peak areas, recorded during the cyclic voltammetry (CV) measurements at low potential scan rate ($v \leq 10$ mV s^{-1}) [11] and considering the number of transferred electrons equal to 2 [12, 13].

The experimental results are the average of at least 3 identically prepared electrodes, if not otherwise mentioned.

Results and discussions

Electrochemical characteristics of MB-Z-CPEs

The carbon pastes incorporating unmodified zeolites (4A-CPEs and 13X-CPEs) do not exhibit any redox activity in the potential range from -800 at +410 mV *vs.* Ag|AgCl/KCl$_{sat}$ (results not shown). When MB is immobilized onto the synthetic zeolites, a well-defined peak pair was observed on the cyclic voltammograms, with the formal standard potentials ($E^{0'}$) at -175 mV and -195 mV *vs.* Ag|AgCl/KCl$_{sat}$ for MB-13X-CPEs and MB-4A-CPEs, respectively (Figure 1). The peak pair was attributed to the oxidation / reduction of MB. The small positive shift of $E^{0'}$ for MB-Z-CPEs in comparison with the value observed for MB in solution (-230 mV *vs.* SCE)

[14] gives evidence that the reduced form of the dye interacts much more strongly with the matrix than the oxidized form does [15]. It should be mentioned that a small additional wave around 400 mV *vs.* Ag|AgCl/KCl$_{sat}$ also appears, reflecting the existence of a second form of MB bound to the synthetic zeolites [10]. This wave is more visible when zeolite 4A is used.

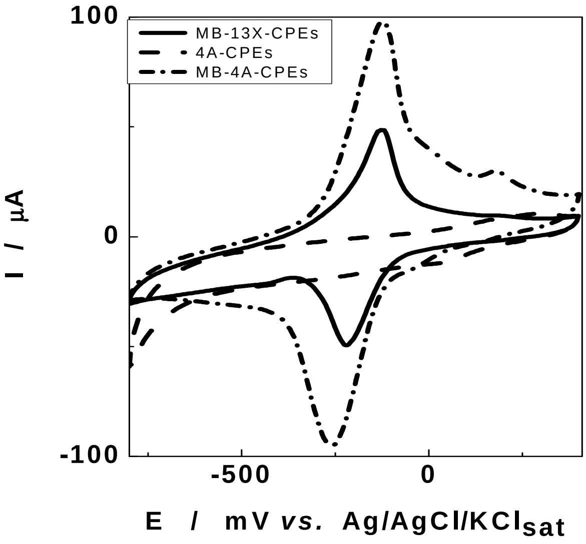


Figure 1. Cyclic voltammograms for 4A-CPEs, MB-13X-CPEs and MB-4A-CPEs. Experimental conditions: starting potential, -800 mV *vs.* Ag|AgCl/KCl$_{sat}$; potential scan rate, 10 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0.

The peak separations for the main peak, ΔE_p, were found to be 90 and 136 mV for MB-13X-CPEs and MB-4A-CPEs, respectively, and increases with the scan rate (results not shown) indicating a quasi-reversible redox process for both modified electrodes. This is an indication that the kinetic of electron transfer on the electrode surface is not very fast, as consequence of the nature of the matrix, whose resistance is considerable [8].

Since it is well known that the redox potential of MB in solution phase is strongly dependent on the pH, the study of pH influence on the electrochemical behavior of MB-Z-CPEs is of great interest [8]. Figure 2 presents the dependence of formal standard potential on the pH of the supporting electrolyte. Surprisingly, the slopes of these dependencies are very small (only 5 mV/ΔpH and 2 mV/ΔpH for MB-13X-CPEs and MB-4A-CPEs, respectively). These results may suggest that the amino groups of

MB at positions 3 and 7 as well as the heterocyclic nitrogen are very strongly involved in the binding to zeolite and as a result, the pH of the solution does not affect $E^{0'}$. The cause for the independence of $E^{0'}$ towards pH variation could be the adsorption of MB into the channels of the synthetic zeolites in a confined position and the acidity of the environment surrounding the dye, which does not change with the solution pH.

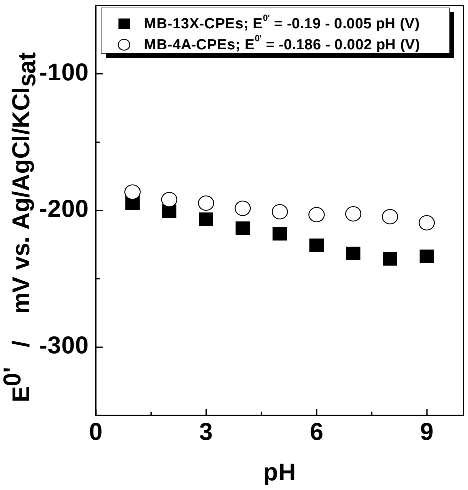


Figure 2. Variation of $E^{0'}$ with pH for MB-13X-CPEs and MB-4A-CPEs. Experimental conditions: starting potential, -800 mV *vs.* Ag|AgCl/KCl$_{sat}$; potential scan rate, 50 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer.

Electrocatalytic oxidation of ascorbic acid

The cyclic voltammograms recorded in 0.1 M phosphate buffer pH 7 at MB-13X-CPEs and MB-4A-CPEs in the presence of different concentrations of AA are depicted in Figure 3. As it can be observed, when AA is present, the anodic peak current of the main voltammetric wave practically does not change, while for the smallest, but more positive wave (at around 250 mV *vs.* Ag|AgCl/KCl$_{sat,}$) the current strongly increases, reflecting the influence of the thermodynamic driving force on the efficiency of the electrocatalytic reaction [10]. Moreover, a new peak appears at about 0 mV *vs.* Ag|AgCl/KCl$_{sat,}$ which could be ascribed to the AA electro-oxidation on the zeolite modified electrodes. This is supported by the fact that the peak is also present, even if much less developed, on 4A-CPEs and 13X-CPEs (see inset in Figure 3). The anodic peak current increases significantly and the oxidation potential is much lower than that recorded on unmodified carbon paste (+490 mV *vs.* SCE [10]).

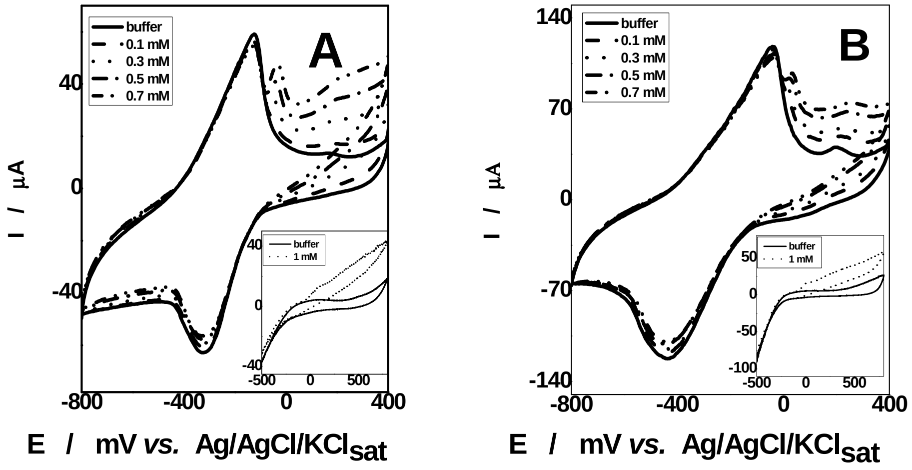
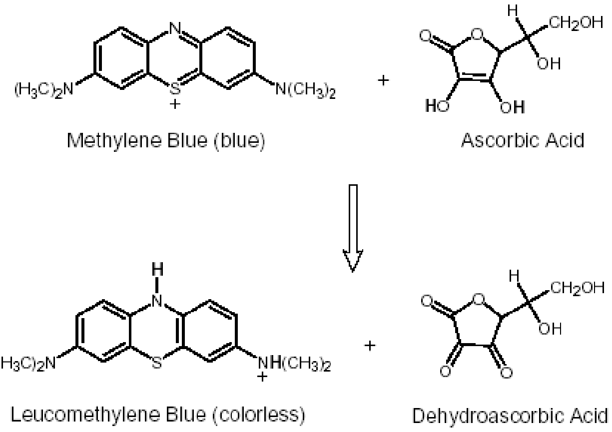


Figure 3. Cyclic voltammograms obtained at MB-13X-CPEs and 13X-CPEs (inset) (A) and MB-4A-CPEs and 4A-CPEs (inset) (B), in the absence and in the presence of different concentrations of AA. Experimental conditions: potential scan rate, 10 mV s^{-1}; starting potential, -800 mV *vs.* Ag|AgCl/KCl$_{sat}$; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0.

The reaction at the electrode-solution interface and into the electrode can be written as follows [16]:



Scheme 2. Methylene blue reduction by ascorbic acid.

Figures 4A and 4B present the calibration curves for AA. The inset of these figures display current-time recordings obtained at MB-13X-CPEs and MB-4A-CPEs for successive injections of 0.1 mM AA when the applied potential was -50 mV *vs.* Ag|AgCl/KCl$_{sat}$. The curve was fitted by the equations: for MB-13X-CPES, $I = 3.1 \times 10^{-4}$ ($\pm 0.2 \times 10^{-4}$)[AA] / [AA]+3.8×10^{-2} ($\pm$ 0.003), with a correlation coefficient R^2 of 0.999 for n = 28, and for MB-4A-CPEs, $I = 1.4 \times 10^{-4}$ ($\pm 1.42 \times 10^{-6}$)[AA] / [AA]+9.17×10^{-3} ($\pm 1.6 \times 10^{-4}$), with a correlation coefficient R^2 of 0.999 for n = 59, where I is the current in A and [AA] is the ascorbic acid concentration in mol l^{-1}. The reproducibility of the electrocatalytic effect of MB-Z-CPEs was confirmed by repetitive amperometric measurements (~ 3 measurements).

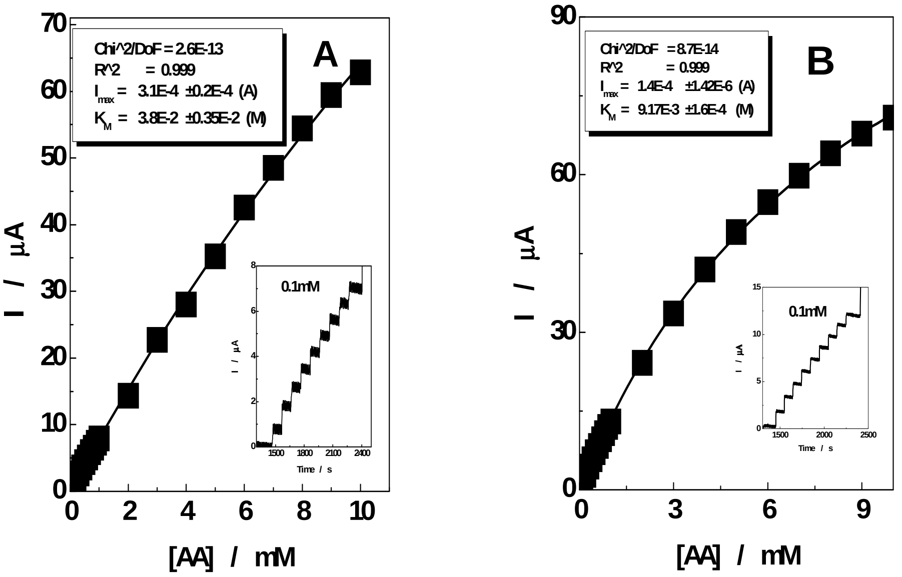


Figure 4. Calibration curves at MB-13X-CPEs (A) and MB-4A-CPE (B) and amperometric response to successive increments of 0.1 mM AA (inset). Experimental conditions: applied potential, -50 mV *vs.* Ag|AgCl/KCl$_{sat}$; rotation speed, 500 rpm; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0.

Both modified electrodes exhibit a short current time response (5s) and can act as AA sensors. As can be observed, the relationship between the steady-state current and the concentration of AA was linear in the range from 10^{-5} M to 10^{-3} M AA for MB-13X-CPEs and 10^{-5} M to 10^{-4} M AA for MB-

4A-CPEs. The theoretical detection limits, calculated from the slope of the regression equation and standard deviation of the calibration curves were 4.7x10^{-5} M for MB-13X-CPEs and 1.7x10^{-5} M for MB-4A-CPEs (Table 1).

Table 1.
Analytical parameters corresponding to amperometric sensors for AA.

Electrode	**Detection limit (M)**	**Sensitivity (A / M)**	**Linear concentration range (M)**	**R/N**	**Log I *vs.* Log c**	
					slope	**R/N**
MB-13X-CPEs	4.7x10^{-5}	8x10^{-3}	10^{-5} - 10^{-3}	0.999/20	0.90	0.999/28
MB-4A-CPEs	1.7x10^{-5}	11x10^{-3}	10^{-5} - 10^{-4}	0.987/ 11	0.88	0.997/28

As can be seen, the slope of Log I *vs.* Log c dependence is close to 1, proving the existence of kinetic control.

The analytical parameters of the MB-zeolite modified carbon paste electrodes are in accordance with those obtained for other electrodes used as amperometric sensors for AA reported in the literature (Table 2).

Table 2.
Analytical parameters for different modified electrodes
reported as amperometric sensors for AA.

E_{app} (mV *vs.* Ag|AgCl/KCl$_{sat}$)	**Electrode**	**pH / solution**	**Detection limit (M)**	**Linear range (M)**	**Ref.**
+ 550	Fe^{3+}Y/ZCME	6.8 /PhB	1.5x10^{-6}	1.58x10^{-6}-2.15x10^{-2}	[17]
+ 200	Mordenite-MB-CP/ GC	6.2 /PhB.	1.2x10^{-5}	2x10^{-5}-8x10^{-4}	[8]
+ 100	ZrP-MB-CP	7 /PhB	10^{-6}	10^{-6}-4x10^{-5}	[10]
- 40	GC	10 /AB+ MB (2.5x10^{-5}M)	1.1x10^{-6}	1.2x10^{-6}-1.12x10^{-3}	[18]

PhB = phosphate buffer; AB = ammonia buffer; GC = glassy carbon; ZrP = zirconium phosphate.

Conclusions

Two new electrodes based on synthetic mesoporous zeolites modified with MB incorporated in carbon paste were obtained and characterized. These modified electrodes presented good electrocatalytic effect towards ascorbic acid oxidation in neutral aqueous solution, at an overpotential with more than 500 mV lower than that observed on unmodified electrodes. The MB-Z-CPEs offer the advantages of easy fabrication and cleaning, fast response time, high sensitivity, a low background current and detection limit, which are suitable for routine determinations.

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