CARBON PASTE ELECTRODES MODIFIED WITH METHYLENE BLUE IMMOBILIZED ON A SYNTHETIC ZEOLITE

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A new composite electrode material consisting of Methylene Blue (as redox mediator), a synthetic NaX zeolite (as immobilization support) and carbon paste (as conductive material) was obtained and characterized. The electrochemical behavior of carbon paste electrodes incorporating Methylene Blue adsorbed on zeolite (**MB-Z-CPEs)** has been investigated at various pH values and potential scan rates. From cyclic voltammetry (CV) measurements, the rate constant (k_s) of the heterogeneous electron transfer and the transfer coefficients (α) were estimated, using Laviron's treatment. Finally, the electrocatalytic activity of the modified electrodes toward H_2O_2 reduction was investigated, using both CV and amperometric measurements. At a fixed potential (-400 mV *vs.* Ag|AgCl/KCl$_{sat}$), under magnetic stirring in phosphate buffer solution (pH 6), the calibration plot was linear over the H_2O_2 concentration range $8*10^{-5}$ M to 10^{-3} M and the detection limit was $1.3*10^{-4}$ M.

INTRODUCTION

The incorporation of complex species in the structure of nanoporous molecular sieves enables the design of a new kind of advanced electrode materials, with interesting applications in energy storage, electroanalysis, molecular recognition etc.[1] The channels and the cavities of a zeolite framework offer ideal space for incorporation, stabilization and organization of these complex compounds, which often can play the role of redox mediators in different electrode processes.[2]

In particular, neutral, as well as cationic organic dye molecules were successfully incorporated into various zeolites such as mordenite,[3] clinoptilolyte,[4] faujasite etc.,[5-7] especially in order to obtain electrochemical sensing devices. The zeolitic matrix protects the dye from the surrounding environment and provides signal enhancement due to an increase in the number of dye molecules per nanoparticle.[8] Most works in this field have been performed using zeolite-modified carbon paste electrodes,[9] since their surfaces are easily renewed and present low background current and a wide range of working potentials.[10]

Methylene Blue, a water-soluble cationic phenothiazine dye has high affinity for zeolitic surfaces and is readily adsorbed when added to zeolite suspensions. Due to its redox properties, and to its formal potential very close to the most of biomolecules redox potential,[9] the modified electrodes based on this dye as electron mediator system may be of great interest in electroanalytical chemistry, *e.g.* for the design of amperometric sensors for H_2O_2,[11-16] ascorbic acid,[9,17,18] NADH etc.[19-21] Some results have already been reported about MB-modified electrodes used as amperometric transducers.[11,12,14,15,22,23]

In this context, the aim of the present paper is to obtain and to characterize a new composite electrode material consisting of Methylene Blue (as redox mediator), a synthetic NaX zeolite (as immobilization support) and carbon paste (as conductive material) (**MB-Z-CPE**). A detailed kinetic study was carried out in order to characterize the electrochemical activity of the new electrodes and their electrocatalytic properties toward H_2O_2 electroreduction.

EXPERIMENTAL

Chemicals

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

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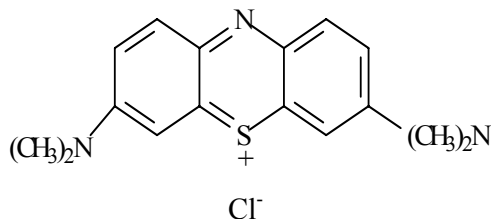
Scheme 1 – Structure of Methylene Blue (MB).

The 13X type zeolite, $1Na_2O:1Al_2O_3:2.8\pm0.2\ SiO_2 \times H_2O$ (particle size, 3 - 5 μ; pore diameter, 10 Å; specific surface area 548.69 m^2/g) was purchased from Aldrich (Germany). Its regeneration temperature lies between 200 and 315 ^{0}C and the bulk density is 480.55 kg/m^3. The Si/Al ratio is 1.5.

Hydrogen peroxide, $K_2HPO_4 \cdot 2H_2O$ 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 solution.

Electrode preparation

50 ml of a 0.001 % (w/v) MB solution in water were shaken (3 days) with 50 mg 13X 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-Z-CPEs**).

The presence of adsorbed MB on 13X zeolite was clearly put on evidence by EDS measurements (Figs. 1A and 1C) which indicate the presence of N, S and Cl belonging to MB. As can be observed from the SEM micrographs (Figs. 1B and 1D), the modification of zeolite with MB also determines an agglomeration of zeolites particles.

The preparation of **MB-Z-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 H_2O_2 concentrations were carried out at an applied potential with 120 mV more negative than formal standard potential ($E^{0'}$) of the mediator, under magnetic stirring, using 0.1 M phosphate buffer solution (pH 6 and 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})[24] and considering the number of transferred electrons equal to 2.[11,12]

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

RESULTS AND DISCUSSION

Electrochemical behavior

Cyclic voltammetric experiments were carried out using carbon paste electrodes modified with the synthetic 13X zeolite (**Z-CPE**) and with Methylene Blue adsorbed on this zeolite (**MB-Z-CPEs**). In Fig. 2, a pair of well-defined redox waves in the case of **MB-Z-CPEs**, can be observed. They were assigned to the oxidation and reduction of MB adsorbed on the 13X zeolite.

The value of the formal standard potential, $E^{0'}$, for **MB-Z-CPEs** was -175 mV *vs*. Ag|AgCl/KCl$_{sat}$, at 10 mV s^{-1} in phosphate buffer pH 7. This result is in agreement with the $E^{0'}$ values reported previously by different authors for MB adsorbed on other supports (Table 1), which cover a wide potential range between -210 V *vs*. SCE and + 154 mV *vs*. SCE, due to the different type of interactions occurring between the dye and the adsorbent surface.

On the other hand, by comparing $E^{0'}$ for **MB-Z-CPEs** with that obtained for MB dissolved in aqueous solution (-223 mV *vs*. Ag|AgCl/KCl$_{sat}$, pH 7, v = 10 mV s^{-1}) a small shift toward more positive potentials is observed. This behavior suggests an interaction between MB and the matrix, stabilizing the reduced form of MB.[30-32] A similar shift of $E^{0'}$ towards the positive direction in neutral pH solutions was also observed for MB adsorbed on Zr, Ti, Ba and Ca phosphates and was attributed to the matrix acidity or to an interaction of the N atom of the mediator with the phosphate matrix.[20,25,28-30]
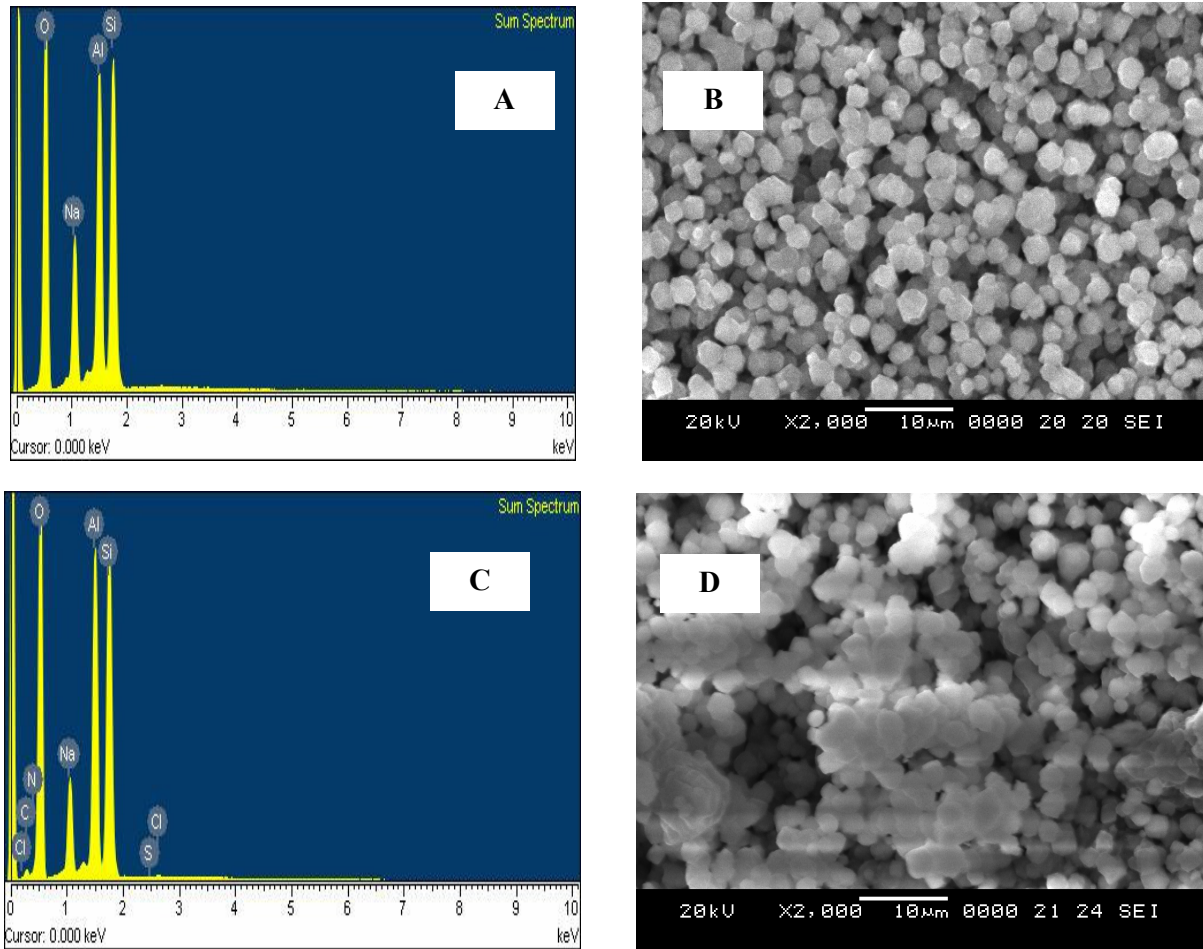
Fig. 1 – EDS spectra (A, C) and SEM images (B, D) for 13X type zeolite (A, B) and 13X type zeolite adsorbed with MB (C, D).

Table 1

Literature data referring to the formal standard potentials values for MB immobilized on different supports

$E^{0'}$(mV) *vs* ref. electrode	Electrode material	Electrolyte pH / v(mV/s)	Reference
-210 *vs.* SCE	zirconium phosphate exposed to gas phase *n*-butyl amine / carbon paste	phosphate buffer, pH 7 / 25	[17]
-150 *vs.* SCE	barium phosphate / carbon paste	0.5 M KCl / 20	[25]
-135 *vs* Ag/AgCl	glassy carbon	6.5	[18]
-131 *vs.* SCE	silica surfaces modified with titanium phosphate / carbon paste	0.5 M LiCl / 10	[26]
-118 *vs.* SCE	silica surfaces modified with titanium dioxide / carbon paste	0.5 M LiCl / 10	[22]
-38 *vs.* SCE	Nafion modified carbon fiber microcylinder electrode	phosphate buffer pH 7.2 / 100	[27]
-30 *vs.* SCE	titanium phosphate / carbon paste	0.1 M phosphate buffer, pH 7 / 20	[28]
+40 *vs.* SCE	non-neutralized zirconium phosphate / carbon paste	0.1 M phosphate buffer pH7 / 20	[29]
+150 *vs.* SCE	zirconium phosphate / carbon paste	0.1 M KCl, pH 6	[30]
+154 *vs* SCE	mordenite type zeolite / carbon paste	0.5 M KCl pH 6.2 / 20	[9]

The peak separation ΔE_p ($\Delta E_p = E_{pa} - E_{pc}$) for **MB-Z-CPEs** was found to be 90 mV in phosphate buffer, pH 7 (10 mV s^{-1}), indicating a quasi-reversible redox process. The fact that the electron transfer is not fully reversible, even at low scan rates, is especially due to the ohmic drop of the carbon paste.[32]

The electrochemical stability of **MB-Z-CPEs** was tested by cycling the potential in the potential window which covers its redox activity. The procedure was carried out for 50 cycles with a scan rate of 50 mV s^{-1} (results not shown). The peak current intensities remained almost constant, indicating that the dye adheres to the zeolite and it is not released to the solution phase under the operating conditions. This behavior suggests a relatively strong interaction between MB and the matrix. Taking into account that the molecular size of MB$^+$ based on van der Waals radii (7.0 × 16 Å)[3,9] is close to that of pore diameter of 13X type zeolite (10 Å) it is supposed that the dye molecule fits tightly into the zeolite holes and thus does not leach from the electrode surface.

The influence of the scan rate on the electrochemical behavior was also studied. Cyclic voltammograms obtained at different scan rates indicated that, as expected, ΔE_p increased at the higher scan rates (results not shown). This is an indication that the kinetic of electron transfer on the electrode surface is not sufficiently fast as consequence of the nature of the matrix whose internal resistance is considerable, the same behavior being observed for MB adsorbed on zirconium phosphate[17] and mordenite.[9]

Another aspect that should be emphasized is the correlation between the peak current and scan rates. The slope of the log I *vs.* log v dependence, as shown in Table 2, is close to unity, suggesting the existence of a surface-confined redox couple.

Using the treatment proposed by Laviron,[33] from the variation of the peak potentials with the potential scan rate (Fig. 3), the heterogeneous electron transfer rate constant (k_s, s^{-1}) and the transfer coefficient (α) were estimated at different pH values (Table 3). Within the experimental error, as expected for an N-unsubstituted phenothiazine derivative, the heterogeneous electron transfer rate constant is not significantly affected by the pH of supporting electrolyte (Table 3), remaining close to 5.5 s^{-1} (pH 7). On the other hand, the α values indicate that the redox processes are not fully reversible for the different pHs. However, these results show that immobilized MB can act as a good electron mediator, presenting high stability due to its adsorption when employing 13X zeolite as adsorption matrix.

Another interesting point is the dependence of the formal potential of MB adsorbed on 13X zeolite on the solution pH between 2 and 9. Previous reports[20,34,35] clearly mention that, for MB in solution or adsorbed on graphite, the formal standard potential depends on the pH of solution due to the fact that the protonation / deprotonation of MB is involved in the redox conversion (Scheme 2). According to the acidity of the solution, different protonated species appear, which can affect the $E^{0'}$ value of the redox process.
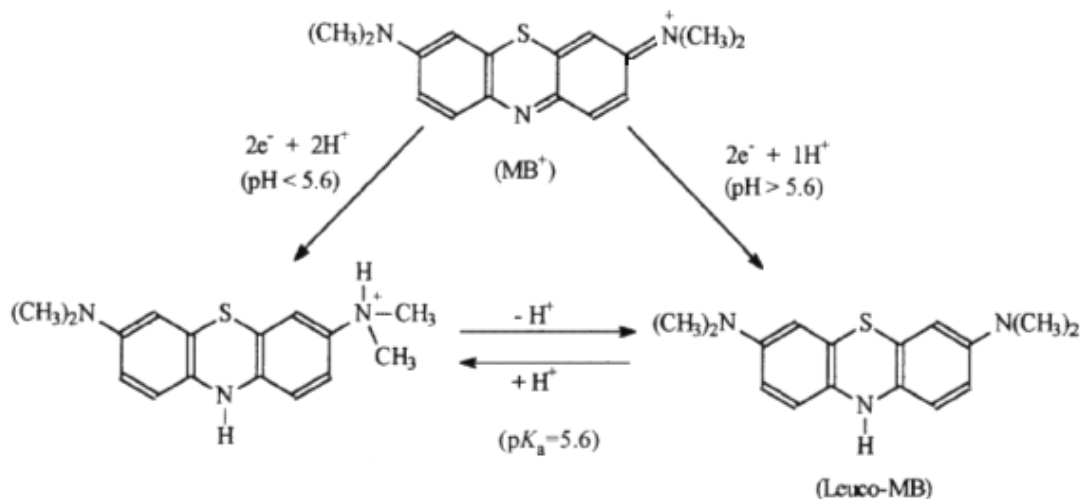
Scheme 2 – Scheme of the Methylene Blue behavior at various pH values.[34]

Table 2

Parameters of the log-log linear regression corresponding to the peak current dependence on the potential scan rate for MB-Z-CPEs (0.005 to 0.4 V s^{-1}). Experimental conditions: as in Fig. 3

pH	Slope		R / no. of exp. points		Surface coverage (μmol cm^{-2})
	oxidation	reduction	oxidation	reduction	
3	0.80 ± 0.01	0.92 ± 0.02	0.999 / 12	0.996 / 12	0.028
5	0.81 ± 0.007	0.95 ± 0.02	0.999 / 12	0.997 / 12	0.025
6	0.82 ± 0.006	0.96 ± 0.02	0.999 / 12	0.997 / 12	0.022
7	0.90 ± 0.02	1.04 ± 0.02	0.998 / 12	0.995 / 12	0.014
8	0.81 ± 0.01	0.95 ± 0.02	0.999 / 8	0.998 / 8	0.020
9	0.81 ± 0.006	0.96 ± 0.015	0.999 / 12	0.998 / 12	0.021

Table 3

Kinetic parameters for the heterogeneous electron transfer at MB-Z-CPEs. Experimental conditions: as in Fig. 3

pH	k_s (s^{-1})	α	R / no. of exp. points	
			oxidation	reduction
3	5.6	0.55	0.998 / 5	0.999 / 5
5	5.4	0.61	0.998 / 5	0.998 / 6
5	5.5	0.69	0.992 / 5	0.993 / 6
7	5.5	0.72	0.996 / 5	0.999 / 5
9	4.9	0.79	0.989 / 5	0.999 / 5

Contrarily, in the case of **MB-Z-CPEs**, the potential change was very small, the slope of E$^{0'}$ *vs*. pH dependence being 5 mV/ΔpH (Fig. 4). Moreover, the slopes change of the E$^{0'}$ *vs*. pH representation taking place at the pKa of the mediator[34] is attenuated (Fig. 2) and the pKa value is displaced towards higher pH values than for MB in solution. These results are remarkable and may suggest that MB (guest molecule) is strongly hold by electrostatic interactions, entrapped in the holes of the 13X type zeolite (host matrix) and it is no affected by the external solution pH change. Thus, practically, the zeolitic matrix minimizes the proton effect on the adsorbed dye molecules. Clearly, H$^+$ is not involved in the redox process, however, the mechanism behind this observation is not clear yet.

This set of electrochemical data is very similar to those previously reported for Methylene Blue entrapped in zirconium phosphate,[29,30,36] in titanium phosphate,[28] in barium phosphate,[25] in mordenite,[9] on a SiO$_2$ / Sb$_2$O$_3$ binary oxide matrix obtained by sol-gel processing method[21] and for Methylene Green adsorbed on a synthetic zeolite from Bayer.[4] In all these cases, the acidity of the adsorbent was mainly made responsible for the E$^{0'}$ independence.

It should also be mentioned that in the case of MB adsorbed on other zeolites (a montmorrilonite and a β-type zeolite, using glassy carbon electrode)[11,12], the slope of E$^{0'}$ *vs*. pH was about 30 mV / ΔpH for pH values situated between 5.5 and 9, suggesting a redox process of 1H$^+$ / 2e$^-$.

In this context, the independence of E$^{0'}$ on the pH of the supporting electrolyte in the case of **MB-Z-CPEs** when 13X zeolite was used is very attractive for practical aspects and mechanistic studies of the reactions between MB and other species. The almost constant value for E$^{0'}$ when using 13X zeolite recommend **MB-Z-CPEs** for the obtaining of chemical sensors for real samples, where the pH plays an important role and can not be changed in order to optimize the electrode response.
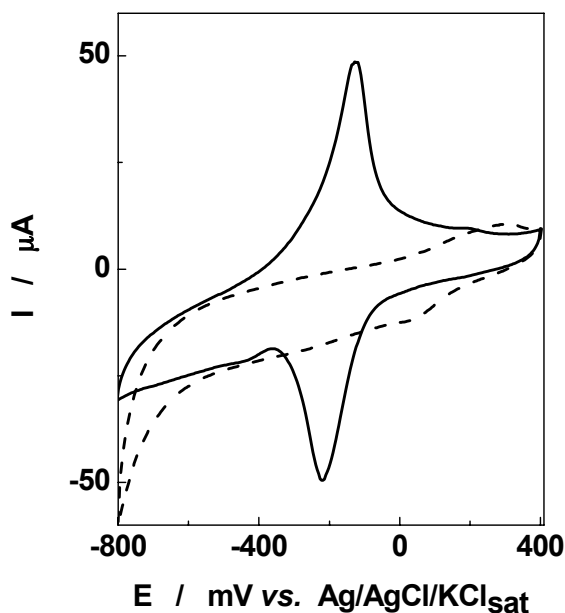
Fig. 2 – Cyclic voltammograms for **Z-CPEs** (---) and **MB-Z-CPEs** (—). Experimental conditions: starting potential, -800 mV *vs.* Ag|AgCl/KCl$_{sat}$; potential scan rate, 10 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer.
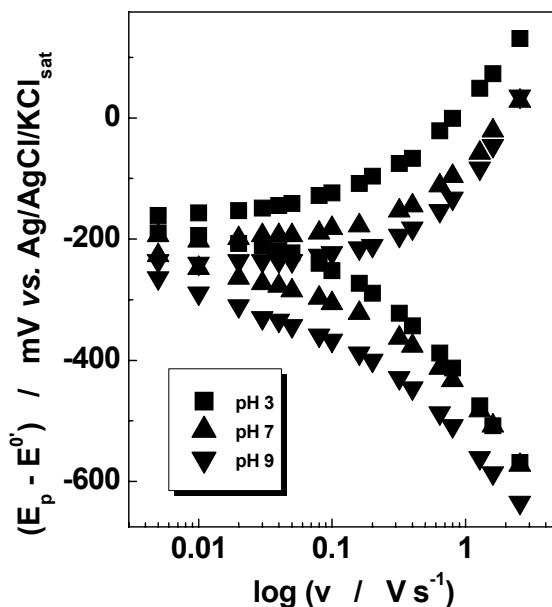
Fig. 3 – Experimental dependence of $(E_p - E^{0'})$ *vs.* logarithm of the scan rate for **MB-Z-CPEs**. Experimental conditions: starting potential, -800 mV *vs.* Ag|AgCl/KCl$_{sat}$; supporting electrolyte, 0.1 M phosphate buffer.
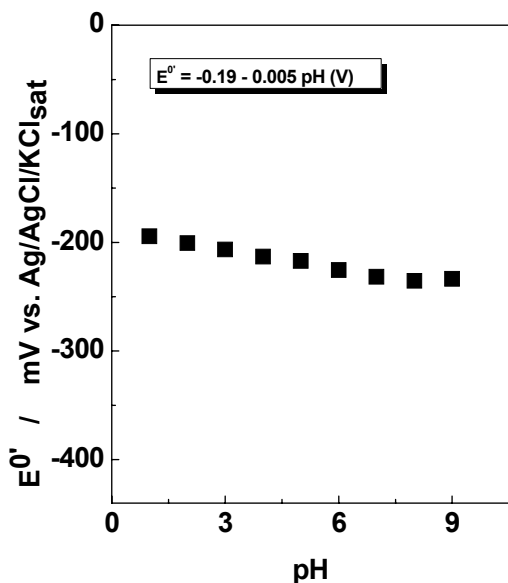
Fig. 4 – Variation of $E^{0'}$ with pH for **MB-Z-CPEs**. Experimental conditions: starting potential, -800 mV *vs.* Ag|AgCl/KCl$_{sat}$; potential scan rate, 50 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer.

Electrocatalytic reduction of H$_2$O$_2$

Hydrogen peroxide electroreduction studies were performed by cyclic voltammetry on **MB-Z-CPEs** immersed in H$_2$O$_2$ solutions of different concentrations (phosphate buffer, pH 7) (Fig. 5). In the presence of H$_2$O$_2$ an enhancement of the cathodic currents and a small peak potential shift towards negative direction with the increase of H$_2$O$_2$ concentration were observed.

The amperometric response of the **MB-Z-CPEs**, at an applied potential of -400 mV *vs.* Ag|AgCl/KCl$_{sat}$, under magnetic stirring conditions (200 rpm), in phosphate buffer solution (pH 7) with successive injections of H$_2$O$_2$ is presented in inset of Fig. 6. The dependence of the catalytic current intensities on H$_2$O$_2$ concentrations was shown to be linear (Fig. 6). The detection limits, (DL) considering the formula:

$$DL = \frac{3*SD}{B} \qquad (1)$$

(where SD is the standard deviation and B is the sensitivity[37]) were $7.9*10^{-4}$ M (Ph = 7) and $1.3*10^{-4}$ M (Ph = 6). The linear response range obtained for **MB-Z-CPEs** was between 10^{-3} and 10^{-1} M (Ph = 7) and between $8*10^{-5}$ and 10^{-3} M (Ph = 6) and the curve was fitted by the equation I = $1.35 \cdot 10^{-3}$ $(\pm 0.38 \cdot 10^{-3})[H_2O_2]$ / $[H_2O_2]+0.81(\pm 0.25)$, with a

correlation coefficient R^2 of 0.997 for n = 59, where I is the current in A and $[H_2O_2]$ is the H_2O_2 concentration in mol l^{-1}. The reproducibility of the electrocatalytic effect of **MB-Z-CPEs** was confirmed by repetitive amperometric measurements ($\sim$ 3 measurements).

The good electrocatalytic effect toward H_2O_2 reduction proves the possibility of using this kind of modified electrodes as transducers for amperometric biosensors based on H_2O_2 detection.

Fig. 5 – Cyclic voltammograms obtained at **MB-Z-CPEs**, in the absence and in the presence of H_2O_2. 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 6.0.
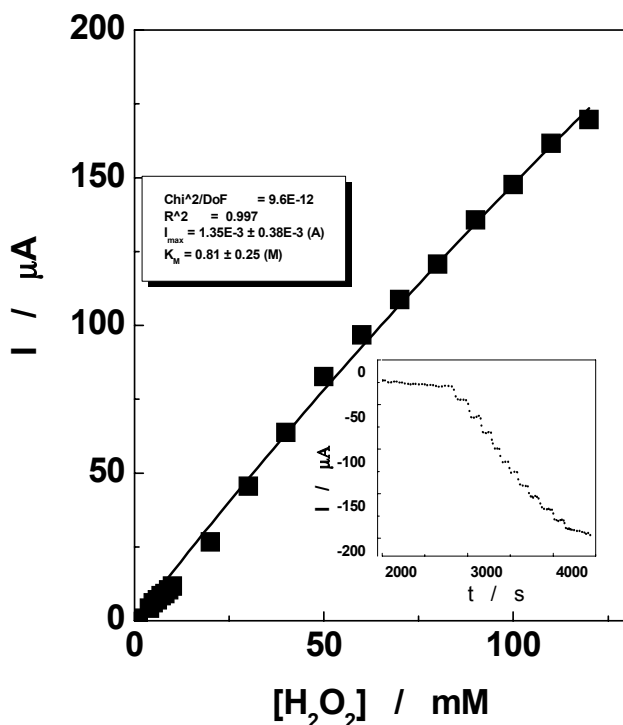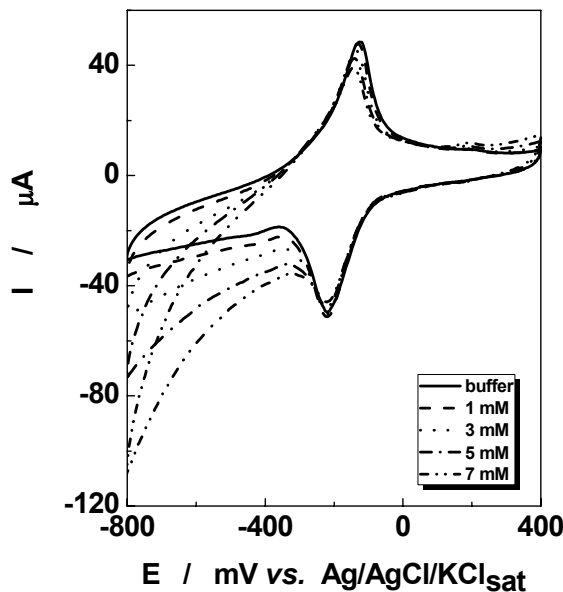

Fig. 6 – Amperometric response to successive increments of 10 mM H_2O_2 (inset) and H_2O_2 calibration at **MB-Z-CPEs**. Experimental conditions: applied potential, -400 mV *vs.* Ag|AgCl/KCl$_{sat}$; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0.

CONCLUSIONS

The 13X type zeolite can be successfully used as support for immobilization of Methylene Blue in order to develop new sensors for hydrogen peroxide. The strong adherence of the dye in the matrix, associated with the constant $E^{0'}$ value of **MB-Z-CPEs** at different pH values of supporting electrolyte, account for our high interest in studying the preparation of the present material and its use for analytical purposes. The $E^{0'}$ independence towards the pH of the solution should be investigated in more details, using other types of zeolite and organic dyes, in order to understand the mechanism of the redox process of the dyes in the host matrix.

The simple method of electrode preparation in combination with its relatively low detection limit and high selectivity recommend **MB-Z-CPEs** as amperometric sensors for H_2O_2.

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