Electrochemical property of methylene blue redox dye immobilized on porous silica–zirconia–antimonia mixed oxide

Galina Zaitseva [b], Yoshitaka Gushikem [a],*, Emerson S. Ribeiro [a], Simone S. Rosatto [a]

[a] *Instituto de Química, Unicamp, CP 6154, 13083-970 Campinas, SP, Brazil*
[b] *Institute of Sorption and Endoecology Problems, Kiev 252680, Ukraine*

Abstract

The mixed oxide SiO_2/ZrO_2 obtained by the sol–gel processing method adsorbs Sb(V) from acid solution, forming a new phase on the matrix surface designated as $SiO_2/ZrO_2/Sb_2O_5$. The amount of Zr(IV) in the matrix was 8.1 wt.%, corresponding to 0.89 mmol g^{-1}, and the amount of Sb(V) incorporated in the matrix was 6.3 wt.% (0.52 mmol g^{-1}). This material presented a specific surface area, $S_{BET} = 590$ m^2 g^{-1}. The immobilized Sb_2O_5 is a good cation exchanger and adsorbs methylene blue (MB) cationic dye with an average surface density $\delta = 1.1 \times 10^{-11}$ mol cm^{-2} (about 0.07 molecule nm^{-2}). The dye is strongly entrapped in the pores of the matrix and it is not leached off in KCl solutions as concentrated as 1.0 M. A carbon paste of the $SiO_2/ZrO_2/Sb_2O_5/$MB material showed a reversible redox pair with midpoint potential $E_m = -0.1$ V versus SCE. This midpoint potential is not affected by solution pH change between 2 and 7. The immobilized dye mediated NADH catalytic oxidation at ca. 50 mV. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Silica–zirconia–antimonia; Sol–gel processing; Methylene blue; Modified carbon paste electrode; NADH electrocatalytic oxidation

1. Introduction

Antimony oxide, Sb_2O_5, amorphous or as a crystalline phase [1,2] is normally obtained as a fine powder presenting low mechanical and thermal resistance, a limiting factor for its wider use. The oxide presents good Brønsted acid properties. Thus, in order to improve its mechanical properties, Sb_2O_5 has been obtained in a highly dispersed form by the grafting reaction between $SbCl_5$ and SiO_2 from a non aqueous solvent [3]. However, the use of the material obtained according to this method has been limited to neutral aqueous solutions because the dispersed oxide is leached from the surface due to the ease of Si–O–Sb hydrolysis in an acid solution.

An alternative way to obtain chemically stable Sb_2O_5 has been to adsorb Sb(V) on a binary mixed oxide matrix obtained by the sol–gel processing method [4]. This procedure has been used in order to obtain Sb_2O_5

dispersed on SiO_2/TiO_2 matrix, with the antimony oxide becoming practically insoluble in acid solutions due to the formation of the titanium(IV) antimonate phase.

In this work a procedure is described to adsorb antimony(V) oxide on SiO_2/ZrO_2, previously obtained by the sol–gel processing method, aiming to obtain Sb_2O_5 homogeneously dispersed on the matrix and thus less soluble in an acid solution. The matrix obtained is a good ion exchanger [5] and was used to adsorb, by an ion exchange reaction, methylene blue (MB), a cationic dye whose electrochemical properties are very well known in the solution phase. It has been used as a redox indicator since its formal potential, E^0, is between 0.08 and -0.25 V (vs. saturated calomel electrode (SCE)) in solution with pH between 2 and 8 [6,7]. This redox potential is close to that of most biomolecule redox potentials and a modified carbon electrode based on this dye as an electron mediator system may be of great interest. Methylene blue has been immobilized on a Nafion-modified polymeric matrix [8,9] or on zirconium phosphate by an ion exchange

---

* Corresponding author. Fax: + 55-197-883023.
E-mail address: gushikem@iqm.unicamp.br (Y. Gushikem).

process [10], to study the redox process and the effects of the matrices. In the present work, procedures for preparation of $SiO_2/ZrO_2/Sb_2O_5$ and further immobilization of MB are described. The material was used to prepare a carbon paste electrode which was tested for mediation of oxidation of NADH. This dye is known to have efficient electrocatalytic property for NADH oxidation [11]. This characteristic is very important for the development of dehydrogenase electrodes that usually rely on the anodic detection of the liberated dihydronicotinamide adenine dinucleotide (β-NADH).

2. Experimental

2.1. Preparation of SiO_2/ZrO_2

The silica–zirconium mixed oxide was prepared by the sol–gel method, as previously described [12]. In a reaction flask containing 250 cm^3 of tetraethylorthosilicate (TEOS, from Aldrich) about 14 cm^3 of 3 M aq. HCl solution was added dropwise and the resulting solution was stirred for 3 h at 333 K. A solution containing a mixture of 28 cm^3 of zirconium tetrabutoxide (ZrBut; Aldrich; 0.061 mol) and 80 cm^3 of dry EtOH was added, under an Ar atmosphere, and the mixture was stirred for 1.5 h. About 7 cm^3 of 3 M HCl solution was added dropwise and the resulting mixture was allowed to rest for 16 h. The solvent was evaporated by gently heating the mixture at 353 K. The xerogel formed was ground and sieved to between 200 and 250 mesh, washed with EtOH in a soxhlet apparatus and then immersed in 0.1 M HCl solution for 24 h, filtered and dried under vacuum at 333 K.

2.2. Reaction of SiO_2/ZrO_2 with Sb(V)

About 20 g of SiO_2/ZrO_2 xerogel was immersed in 50 cm^3 of 0.45 M Sb(V) solution containing 2.5 M HCl. The volume was completed to 800 cm^3 with pure water and the mixture was stirred at 333 K for 7 h. The solid was filtered, washed with water until the filtrate solution was neutral, and dried at 333 K in an oven.

2.3. Chemical analyses and specific surface area

About 0.1 g of $SiO_2/ZrO_2/Sb_2O_5$ was treated with few drops of 40% (v/v) HF solution until complete dissolution of the solid. HF was eliminated from the solution with a HNO_3–HCl mixture (1:15 v/v) and the content of Sb and Zr analyzed by using atomic absorption spectroscopy on a Perkin–Elmer Optima 3000 DV apparatus.

Specific surface area (S_{BET}) was determined by the BET method by using an ASAP 2010 apparatus from Micromeritics.

2.4. Immobilization of MB on $SiO_2/ZrO_2/Sb_2O_5$

About 0.2 g of the $SiO_2/ZrO_2/Sb_2O_5$ was immersed in 20 cm^3 of a 10^{-3} M MB aq. solution and shaken for 24 h. The resulting solid was filtered, washed with 10^{-2} M HCl solution to remove the excess of MB, with demineralized water and then dried at room temperature (r.t.). The amount of adsorbed MB was determined by using a spectrophotometry technique.

2.5. Electrochemistry studies

A modified carbon paste electrode was prepared by mixing 30 mg of $SiO_2/ZrO_2/Sb_2O_5/MB$ with 30 mg of graphite and a drop of mineral oil. This paste was deposited into a cavity in contact with a platinum disk fused at the end of a glass tube with 3 mm internal diameter.

The measurements were carried out under pure Ar atmosphere using the $SiO_2/ZrO_2/Sb_2O_5/MB$ modified carbon paste electrode as the working electrode, a SCE as the reference electrode and a Pt wire as the auxiliary electrode. The electrochemical properties of the adsorbed MB on the surface were studied by means of the cyclic voltammetry technique with a PGSTAT-20 Autolab potentiostat. The experiments were carried out in 0.5 M KCl electrolyte solution and the pH was adjusted by adding HCl or NaOH solutions.

2.6. Application for electrocatalytic oxidation of NADH

The study of the electrocatalytic oxidation of NADH was carried out in 0.2 M KCl + 0.06 M phosphate buffer solution at pH 6.8. In the electrochemical cell containing 5 cm^3 of this solution, 25 mm^3 of 0.02 M NADH was successively added for a fixed applied potential of 50 mV versus SCE. The NADH solution was immediately used after preparation and all experiments were made at r.t. under an Ar atmosphere.

3. Results and discussions

### 3.1. Characteristics of the material

The amount of Zr(IV) loading in the matrix was 8.1 wt.%, corresponding to 0.89 mmol g^{-1} and the amount of incorporated Sb(V) in the matrix was 6.3 wt.% (0.52 mmol g^{-1}). Incorporation of Sb(V) into the SiO_2/ZrO_2 matrix can be described by the reaction [12]:

$$n\equiv ZrOH + Sb(OH)_5 \rightarrow (\equiv ZrO)_n Sb(OH)_{5-n} + nH_2O$$

(1)
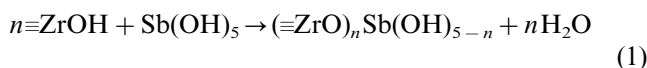
where $\equiv ZrOH$ stands for hydrated zirconium oxide bonded to the SiO_2 surface.

The specific surface area of this matrix was $S_{BET} = 590$ m^2 g^{-1}, and the average pore diameter was 0.73 nm.

The cationic form of the dye, MB$^+$Cl$^-$, whose structure is shown in Fig. 1, was adsorbed on the matrix surface by an ion exchange reaction:

$$SbOH + MB^+ \rightleftarrows SbOMB + H^+ \qquad (2)$$
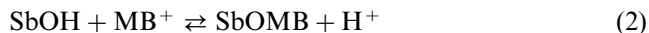
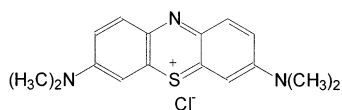where SbOH stands for hydrated antimony(V) dispersed on SiO$_2$/ZrO$_2$ surface. The adsorbed amount of
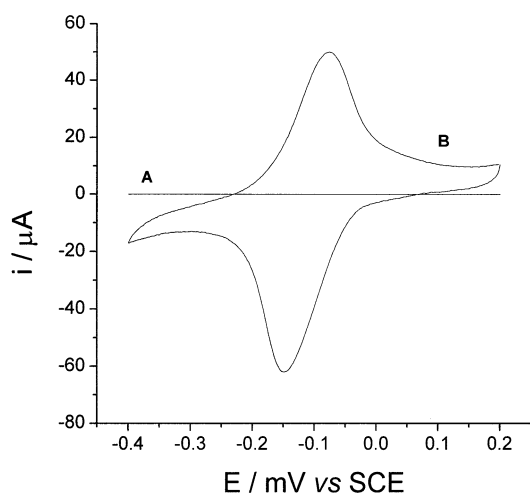

Fig. 1. Structure of methylene blue.

Fig. 2. Cyclic voltammograms of a carbon paste electrode with SiO$_2$/ZrO$_2$/Sb$_2$O$_5$ (A) and SiO$_2$/ZrO$_2$/Sb$_2$O$_5$/MB (B), obtained in 0.5 M KCl solution with a scan rate of 20 mV s^{-1}.
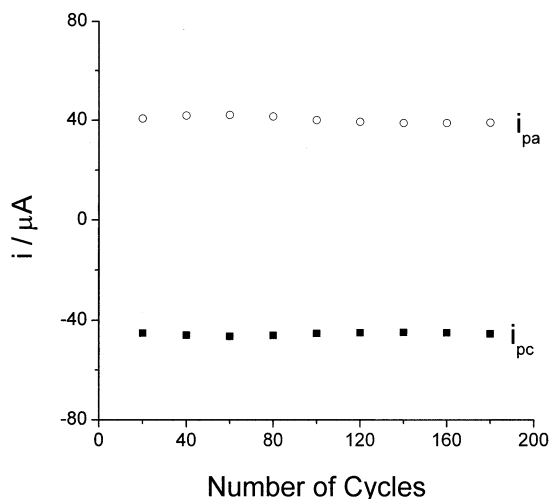
Fig. 3. The peak current dependence on the number of cycles for a SiO$_2$/ZrO$_2$/Sb$_2$O$_5$/MB modified carbon paste electrode, obtained in 1 M KCl solution with a scan rate of 20 mV s^{-1}.

the dye is 68 μmol g^{-1} and it is strongly retained on the surface.

The high degree of dispersion of the dye on the matrix is another important characteristic of the matrix that must be taken into consideration, as the objective is to use as base material for preparing electrochemical sensor. In the present case it can be estimated by assuming that the dye molecule is uniformly dispersed on the matrix surface. Under such conditions, the average surface density δ (where $\delta = n/S_{BET}$, n = amount of immobilized dye) is 1.1×10^{-11} mol cm^{-2} (about 0.07 molecule nm^{-2}). Such a condition is desirable in order to minimize molecular association as dimmer or other higher order aggregates formation in the matrix surface, as occurs in the solution phase [13,14].

3.2. Electrochemical studies

A cyclic voltammetry experiment using the carbon paste electrode modified with the material was carried out. For electrodes modified with SiO$_2$/ZrO$_2$/Sb$_2$O$_5$, Fig. 2A, no cathodic or anodic peaks are observed. Fig. 2B shows the cyclic voltammetry curve obtained for a carbon paste electrode modified with SiO$_2$/ZrO$_2$/Sb$_2$O$_5$/MB. In this case, a redox couple of the mediator peak with midpoint potential $E_m = -0.1$ V ($E_m = (E_{pa} + E_{pc})/2$ where E_{pa} and E_{pc} are the anodic and cathodic peak potential, respectively) is observed. Comparing this potential with that for the free dye in solution phase, for the same pH solution, it is about 120 mV more positive. Similar shift of the midpoint potential toward the positive direction in pH neutral solution was also observed for MB adsorbed onto zirconium phosphate or titanium phosphate and has been partly assigned to the acidity of the host matrix [6,15,16]. This low midpoint potential and the anodic peak potential near zero are very important for sensor/biosensor development.

The immobilized dye is strongly adhered on the matrix surface and evidence of this is observed by cycling the potential and recording the anodic peak, i_{pa}, and the catodic peak, i_{pc}, currents for the electrode immersed in 1.0 M KCl solution (Fig. 3). The current intensities remain practically constant, otherwise it should decrease due to dye being released to the solution, indicating that the dye strongly adheres to the surface, as it is not leached off in up to 180 redox cycles.

Another interesting behavior of the immobilized dye is that the midpoint potential, E_m, practically does not change for solution pH between 2 and 7. It is very well known that MB midpoint potential in the solution phase changes between 0.08 and about -0.25 V as the pH varies between 2 and 8 [6,7]. The dye is certainly strongly entrapped in the SiO$_2$/ZrO$_2$/Sb$_2$O$_5$ pores, as is demonstrated by the results shown in Fig. 4, the reason
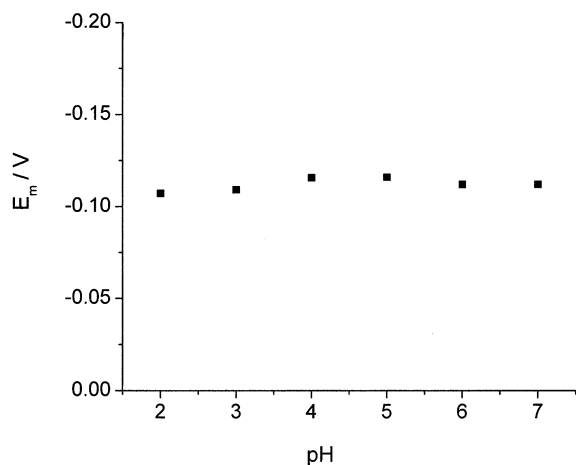
Fig. 4. The influence of solution pH on the midpoint potential, E_m, for methylene blue immobilized on $SiO_2/ZrO_2/Sb_2O_5$. 0.5 M KCl solution and scan rate of 20 mV s^{-1}.
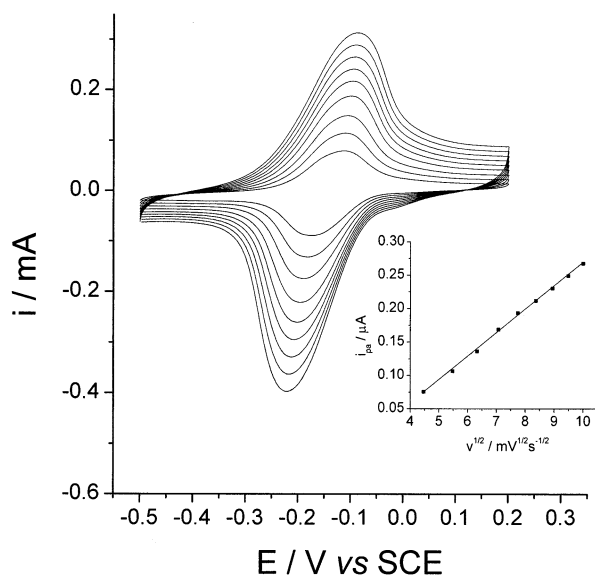
Fig. 5. Cyclic voltammetry curves for various scan rates (from the bottom to top: 20–100 mV s^{-1}) using the $SiO_2/ZrO_2/Sb_2O_5/MB$ modified electrode in 0.5 M KCl. Inserted figure shows the peak current dependence on the square root of the scan rate, $v^{1/2}$.

why the potential is insensitive to the pH change. This result is similar to that obtained for MB immobilized on a zirconium phosphate [6] or titanium phosphate matrix [16] and has been attributed to the protection effect of the matrix since the nitrogen atom is interacting in a strong way with the acidic site, avoiding the proton interaction. Various phenoxazine and phenothiazine-type mediators have had their E_m almost constant with a change in pH when immobilized onto zirconium phosphate [6,15,17–21] or titanium phosphate [16].

Cyclic voltammograms obtained at different scan rates indicate that ΔE_p ($\Delta E_p = E_{pa} - E_{pc}$) increased at higher scan rates (Fig. 5). This is an indication that the kinetics of electron transfer on the electrode surface is

not sufficiently fast as a consequence of the matrix whose resistance is considerable. The correlation of the peak current, i_{pa}, against $v^{1/2}$ (v is the scan rate) is linear (Fig. 5 insert) which is very similar to a diffusion controlled process. Since the electroactive species strongly adheres to the matrix, as was shown earlier, the mechanism may be explained by the transport of the ion of the supporting electrolyte to and from the electrode surface for charge compensation [22].

The nature of the supporting electrolyte did not produce a significant effect on the voltammetric response, as can be seen in Table 1, except in the case of NaOAc. For this electrolyte, the midpoint potential shifted to more negative values and the ΔE value for a given concentration, in comparison with other electrolytes, increased. This fact can be related to an interaction of acetate ion with the mediator, increasing the electron density and stabilizing the oxidized form of MB.

The E_m values varied between -0.10 and -0.14 V for KCl, NaCl, NH$_4$Cl and NaNO$_3$ and between -0.15 and -0.17 V for NaOAc, for concentrations increasing from 0.2 up to 1.0 M. These results indicate that the electrolytes are not interacting with the electron mediator and, an important aspect, they are not displacing MB and releasing this to the solution phase by an ion exchange reaction.

The observed values for ΔE, the cathodic and anodic peak separation, decrease for higher electrolyte concentrations, since the charge transport is made by the electrolytes. The conductivity of the medium is very important to give a good electrochemical response, i.e. the electrical resistance for electron transfer decreased for higher concentration of the supporting electrolyte.

Table 1
Electrochemical response of $SiO_2/ZrO_2/Sb_2O_5/MB$ modified carbon paste electrode on different supporting electrolyte solutions

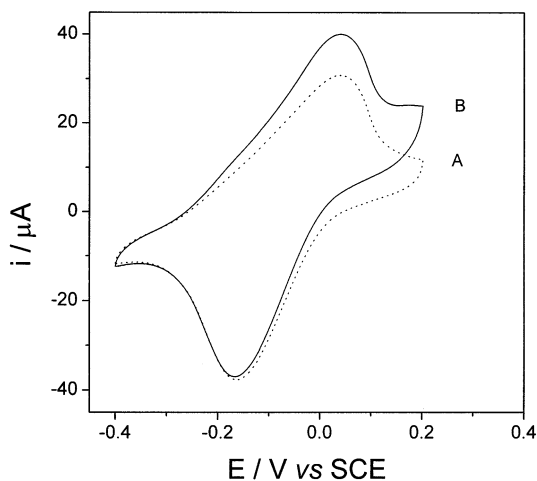| Electrolytes	[Electrolyte] (mol l^{-1})	E_m (mV)	ΔE (mV)
KCl	0.2	−104	113
	0.5	−107	78
	1.0	−103	61
NaCl	0.2	−117	105
	0.5	−120	81
	1.0	−121	63
NH$_4$Cl	0.2	−126	59
	0.5	−122	61
	1.0	−116	49
NaNO$_3$	0.2	−131	97
	0.5	−139	78
	1.0	−141	58
NaOAc	0.2	−154	125
	0.5	−158	107
	1.0	−167	86

Measurements in pH 6 and scan rate of 20 mV s^{-1}.

Fig. 6. Cyclic voltammograms of a $SiO_2/ZrO_2/Sb_2O_5/MB$ modified carbon paste electrode obtained in 0.2 M KCl + 0.06 M phosphate buffer solution at pH 6.8, with a scan rate of 20 mV s^{-1}, without NADH (A) and with 10 mM NADH present (B).

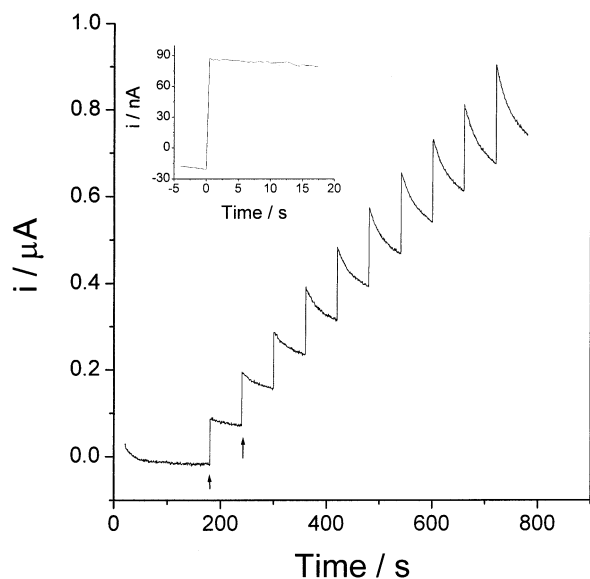Fig. 7. Typical amperometric curve obtained with a $SiO_2/ZrO_2/Sb_2O_5/MB$ modified carbon paste electrode in 5 cm^3 of 0.2 M KCl + 0.06 M phosphate buffer solution at pH 6.8 and an applied potential of 50 mV versus SCE. Successive additions of 25 mm^3 of a 0.02 M NADH solutions.

3.3. Electrocatalytic oxidation of NADH

Fig. 6 shows the cyclic voltammetric curves obtained using the carbon paste electrode of $SiO_2/ZrO_2/Sb_2O_5/$ MB. Fig. 6A was obtained by cycling the potential in a solution in the absence of NADH and Fig. 6B in a solution containing 10 mM of NADH. The increase of the anodic peak current intensity results from the electrocatalytic activity of the immobilized mediator near 50 mV. This potential is about 500 mV lower when compared with NADH oxidation on a graphite surface

[23]. The shift to lower potential of such a magnitude is important since many other chemical species which could be oxidized on the rough electrode surface can be avoided at this lower potential [24].

Fig. 7 shows the chronoamperometric curve obtained by adding, successively, 25 mm^3 of 0.02 M NADH at a 50 mV applied potential. The response time in this case is very fast, about 0.5 s for the current to achieve the maximum value (see the figure inserted in Fig. 7). The slower decrease of the current intensity, as observed after the current achieved the maximum value, is due to the slow diffusion of NADH at the solid–solution interface. So, 1 min of elapsed time, between one addition to other of the NADH solution, was maintained. By plotting the current i against NADH concentration, a linear relationship described by the equation, $i = -5.93 \times 10^{-3} + 7.45 \times 10^{-4}$[NADH], with linear correlation $r = 0.999$ and $n = 10$, was obtained. This linear equation was obtained for i expressed in μA and NADH concentration in μM. The detection limit, considering a signal/noise ratio equal to three, was 36 μM. The proposed electrode present a lower sensitivity and higher detection limit to NADH in comparison with MB adsorbed on similar inorganic materials reported earlier [6,15,16], but the applied potential is relatively lower which is interesting for NADH detection. This behaviour suggests that the thermodynamic driving force is very important in the reaction between MB and NADH.

4. Conclusions

SiO_2/ZrO_2 mixed oxide obtained by a sol–gel processing method adsorbs Sb(V) from acid solution forming a new phase on the matrix surface. The material obtained, $SiO_2/ZrO_2/Sb_2O_5$, has a surface area $S_{BET} = 590$ m^2 g^{-1}.

The midpoint potential of MB dye is not influenced by the solution phase pH because it is strongly entrapped in the pores of the $SiO_2/ZrO_2/Sb_2O_5$ matrix.

The electrode response to NADH in chronoamperometry experiments showed that, despite the slow diffusion process of NADH at the solid–solution interface the electrode had, a good activity. Electrooxidation of the NADH coenzyme occurs in the present case at a considerably lower potential, as compared to that needed on a rough electrode surface, for instance platinum or carbon.

References

[1] M. Abe, T. Ito, Bull. Chem. Soc. Jpn. 41 (1968) 2366.

[2] M. Abe, R. Chitrakar, M. Tsuji, K. Fukumoto, Solv. Extr. Ion Exch. 3 (1985) 149.

[3] E.V. Benvenutti, Y. Gushikem, C.U. Davanzo, S.C. de Castro, I.L. Torriani, J. Chem. Soc. Faraday Trans. 88 (1992) 3193.

[4] J.E. Gonçalves, Y. Gushikem, S.C. de Castro, J. Non-Cryst. Solids 260 (1999) 125.

[5] G. Zaitseva, Y. Gushikem, J. Braz. Chem. Soc., submitted for publication.

[6] C.A. Pessoa, Y. Gushikem, L.T. Kubota, L. Gorton, J. Electroanal. Chem. 431 (1997) 23.

[7] J.M. Ottaway, in: E. Bishop (Ed.), Indicators, Pergamon, Oxford, 1972.

[8] Z. Lu, S. Dong, J. Chem. Soc. Faraday Trans. I 84 (1988) 2979.

[9] A.R. Guadalupe, K.E. Liu, H.D. Abruña, Electrochim. Acta 36 (1991) 881.

[10] C.A. Pessoa, Y. Gushikem, L.T. Kubota, Electroanalysis 9 (1997) 800.

[11] B. Person, L. Gorton, J. Electroanal. Chem. 292 (1990) 115.

[12] A.A.S. Alfaya, Y. Gushikem, S.C. de Castro, Micropor. Mesopor. Mater. 39 (2000) 57.

[13] L. Antonov, G. Gergov, V. Petrov, M. Kubista, J. Nygren, Talanta 49 (1999) 99.

[14] C.U. Ferreira, Y. Gushikem, L.T. Kubota, J. Solid State Electrochem. 4 (2000) 298.

[15] L.T. Kubota, L. Gorton, Electroanalysis 11 (1999) 719.

[16] L.T. Kubota, F. Munteanu, A. Roddick-Lanzilotta, A.J. McQuillan, L. Gorton, Quím. Anal. 19 (2000) 15.

[17] L.T. Kubota, L. Gorton, J. Solid State Electrochem. 3 (1999) 370.

[18] A. Malinauskas, T. Ruzgas, L. Gorton, Bioelectrochem. Bioenerg. 49 (1999) 21.

[19] A. Malinauskas, T. Ruzgas, L. Gorton, L.T. Kubota, Electroanalysis 12 (2000) 194.

[20] A. Malinauskas, T. Ruzgas, L. Gorton, J. Colloid Interface Sci. 224 (2000) 325.

[21] F.D. Munteanu, L.T. Kubota, L. Gorton, J. Electroanal. Chem. 509 (2001) 2.

[22] L.T. Kubota, Y. Gushikem, J. Perez, A. Tanaka, Langmuir 11 (1995) 1009.

[23] A. Tortensson, L. Gorton, J. Electroanal. Chem. 130 (1981) 199.

[24] J. Moiroux, P.J. Elving, Anal. Chem. 50 (1978) 1056.