

Carmen Ioana Ladiu · Ionel Catalin Popescu · Lo Gorton

Electrocatalytic oxidation of NADH at carbon paste electrodes modified with meldola blue adsorbed on zirconium phosphate: effect of Ca^{2+} and polyethyleneimine

Received: 30 August 2004 / Revised: 7 October 2004 / Accepted: 21 October 2004 / Published online: 18 March 2005
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Abstract The basic electrochemistry of carbon paste electrodes modified with Meldola Blue adsorbed on zirconium phosphate (MB-ZP-CPEs) and their ability to oxidize NADH have been investigated. Three types of carbon powder (graphite and glassy carbon-type Sigradur K and G) were used to obtain MB-ZP-CPEs. On comparing cyclic voltammograms recorded at MB-ZP-CPEs, similarly prepared from the three different carbon powders, those made with Sigradur K exhibited the lowest background current, and the best MB electrochemistry, seen as the highest peak intensities and smallest peak separation. Using MB-ZP-CPEs based on Sigradur K a study on NADH oxidation was done focusing on the effect of the Ca^{2+} concentration in the contacting solution and on the addition of polyethyleneimine (PEI) into the paste. It can be stated that MB-ZP-CPEs based on Sigradur K and containing 1.23% (w/w) PEI exhibited the best behavior for NADH oxidation, measured by the highest electrocatalytic rate constant ($8.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$).

Keywords NADH oxidation · Modified electrodes · Electrocatalysis · Zirconium phosphate · Redox dyes

This work is dedicated to Professor Horanyi on the occasion of his 70th birthday in recognition of his outstanding contribution to electrochemistry

C. I. Ladiu · I. C. Popescu
Department of Physical Chemistry,
Babes-Bolyai University,
400028, Cluj-Napoca,
Romania

L. Gorton (✉)
Department of Analytical Chemistry,
Lund University,
P.O. Box 124,
22100 Lund,
Sweden
E-mail: Lo.Gorton@analykem.lu.se

Introduction

For practical applications of mediator-modified electrodes for NADH oxidation in e.g., biosensors and in biofuel cells, one would need as high a second-order rate constant (k_{obs}) between NADH and the immobilized mediator as possible, preferably so high that the reaction is controlled rather by mass transfer than by kinetics [1]. There seems to be a linear relationship between the $\log -k_{\text{obs}}$ versus the formal standard potential of the mediator ($E^{\circ'}$) and to reach high values of k_{obs} of about 10^6 – $10^7 \text{ M}^{-1} \text{ s}^{-1}$ one would need the mediator to have an $E^{\circ'}$ value of at least around +200/+300 mV versus Ag|AgCl (pH 7) [2]. Such high values of the $E^{\circ'}$ would compromise the selectivity of a biosensor based on an $\text{NAD(P)}^+/\text{NAD(P)H}$ -dependent dehydrogenase as the necessary applied potential (E_{appl}) would need to be more positive than the $E^{\circ'}$ of the mediator and would therefore open up the sensing system to interfering reactions caused by direct electrochemical oxidation. For biofuel cells, such high potentials would lead to loss of energy.

In this work, we have used one of the classical mediators for catalytic NADH oxidation, Meldola Blue (two-electron–proton type) [3, 4], however, not directly immobilized onto the electronically conducting electrode but rather onto zirconium phosphate (ZP) and the Meldola Blue–ZP complex was mixed into carbon paste electrodes. ZP is a good ion conductor due to its phosphate groups (negatively charged), a good ion exchanger (strongly acidic material), and a layered material, which presents one important feature: the interlayer spaces can be adjusted to accommodate guests of any size and due to its charge field ZP has the control over the orientation, aggregation and distribution of the adsorbed guests [5]. Moreover, the reason Meldola Blue is adsorbed onto ZP is because when adsorbed directly onto carbon, the $E^{\circ'}$ of Meldola Blue is -175 mV versus Ag|AgCl (at pH 7), but when adsorbed onto ZP the $E^{\circ'}$ value is shifted in the positive direction with more than 200 mV [6, 7], to a value that would allow the modified electrode to operate within

a potential window essentially free of interfering reactions [8]. Additionally, when adsorbed onto ZP, the E° of Meldola Blue does not vary with pH [6, 7], in contrast to when directly immobilized to conventional electrode materials [3]. The increase in E° would also increase the k_{obs} to higher values, unfortunately not enough to reach a value where the electrocatalytic current for NADH oxidation would be limited by mass transfer [1, 2].

In a series of previous publications on electrocatalytic oxidation of NADH at mediator-modified electrodes, it has been shown that addition of Ca^{2+} to the contacting solution largely increases k_{obs} up to at about one order of magnitude [9]. The true reason for this effect is maybe not fully elucidated yet even though a fully plausible explanation to the effect has been given [10]. The effect of addition of Ca^{2+} to the contacting solution was therefore investigated to see whether k_{obs} could be further increased to reach the desired level.

Another approach was also investigated. Instead of adding Ca^{2+} to the contacting solution, polyethyleneimine (PEI), a highly positively charged polyelectrolyte was added to the carbon paste mixture, thus minimizing additions of reactants to the conducting buffer to perform experiments. Previously it was noticed that adding PEI to enzyme-modified carbon paste resulted in a remarkable increase in the sensitivity of the biosensor [11, 12].

Recently, it was shown that the electrochemical behavior of carbon paste electrodes could be improved when the ordinary highly porous graphite powder is exchanged for non-porous glassy carbon powder [13–16]. In this context, two types of glassy carbon powder, with low adsorption capability [13], were used for preparation of Meldola Blue-modified carbon paste electrodes. The electrochemical behavior of the redox dye was recorded for these electrodes, in different experimental conditions, and compared with that observed for a carbon paste electrode made from ordinary graphite powder.

Materials and methods

Synthesis of zirconium phosphate

Zirconium phosphate (ZP) was obtained according to the method reported by Larsen et al. [17]. Five-hundred milliliter of 0.2 M phosphoric acid (Merck, Darmstadt, Germany) were added to 500 ml of 0.1 M zirconium chloride (Fluka, Buchs, Switzerland) prepared in 0.1 M HCl (Merck), until the phosphoric acid was present in slight excess. A solution of 1 M NaOH (Merck) was added afterwards, until the pH of the solution became higher than 3 [7]. The gelatinous precipitate obtained was washed with de-ionized water (Milli-Q System, Millipore, Milford, MA, USA) ten times. A thermostated oven was used for 4 h at 80 °C to dry this precipitate, and after drying it was ground until the particle

size diameter became smaller than 0.05 mm and was drizzled in a sieve.

Immobilization of organic dye (Meldola Blue)

The immobilization process was carried out using an aqueous solution of the organic dye (Meldola Blue, MB; Sigma, St. Louis, MO, USA) in a concentration of 0.001% (w/v). The procedure of immobilization was as follows: 50 mg of ZP was added to 50 ml of dye solution and the mixture was shaken for 1 h. The colored precipitate was filtered, washed with de-ionized water and dried at room temperature.

Preparation of modified carbon paste electrode

The carbon paste electrodes were prepared by thoroughly mixing different quantities of carbon powder and MB-ZP with paraffin oil (Fluka, Buchs, Switzerland) in an agate mortar. For 20 mg of carbon powder and MB-ZP mixture, with a ratio of carbon powder to MB-ZP of 1:1 (w/w) or 2.3:1 (w/w), 5 μL of paraffin oil were added. The carbon powders used were graphite powder (Fluka, Buchs, Switzerland), and two types of glassy carbon powder: Sigradur G and Sigradur K (HTW, Hochtemperatur-Werkstoffe GmbH, Bonn, Germany). The size of the spherical glassy carbon particles was 0.4–1.2 μm .

Polyethyleneimine (Sigma; 50% w/v aqueous solution; PEI) was added to carbon pastes as 100 mg in 2 ml of 0.1 M Tris buffer (pH 7) and after addition the mixture was dried at room temperature.

Electrochemical measurements

The pastes obtained were put into the cavity of a Teflon holder, in the bottom of which a piece of pyrolytic graphite was used for electric contact. The Teflon holder then was screwed onto a rotating disc electrode device (EG-G PAR, model 616, Princeton, USA) and was used as working electrode for cyclic voltammetry and rotating disc electrode (RDE) measurements. The geometrical area was 0.049 cm^2 . The counter electrode was a platinum ring and the reference electrode was Ag|AgCl (KCl_{sat}). The measurements were performed using a BAS 100 W Electrochemical Analyzer (Bioanalytical Systems, West Lafayette, IN, USA), which was connected to a PC microcomputer for potential control and data acquisition.

The supporting electrolyte used in the electrochemical cell was a 0.1 M solution of Tris (Sigma). The pH of the buffer solutions was adjusted to the desired values by adding HCl or KOH (Merck). All experiments were performed in deoxygenated electrolytes by bubbling nitrogen for 20 min before measurements. A cyclic voltammogram was recorded, for each modified electrode, for surface coverage (Γ , mol/cm^2) estimation.

For flow injection measurements the holder was replaced with a plastic syringe holder (ONCE, ASIK, Denmark), which was packed with unmodified carbon paste in the bottom and modified carbon paste on the top. Electrical contact was realized by inserting a silver wire into the unmodified carbon paste. The electrode was than fitted into a Teflon holder and inserted into a flow-through "wall-jet" amperometric cell [18]. The auxiliary electrode was a platinum wire and the reference electrode was Ag|AgCl (0.1 M KCl). The electrodes were connected to a three-electrode low noise potentiostat (Zäta Elektronik, Lund, Sweden), controlling the applied potential of the working electrode. The current output was monitored on a chart strip recorder (Kipp and Zonen, Delft, The Netherlands). In this type of cell it is possible to vary the distance between the inlet and the electrode surface to give the maximum sensitivity [18], and in all the following experiments the same distance has been used: 2 mm. The flow injection experiments were carried out using a one-channel flow system connected to the flow cell. The carrier was a 0.1 M Tris buffer at pH 7, which was carefully degassed before use. The carrier flow of 0.5 ml/min was maintained with a peristaltic pump (ALITEA, Stockholm, Sweden). The injection of the sample into the carrier flow was realized using an electrically controlled six-port valve (LabPRO, Rheodyne, Cotati, CA, USA) with an injection loop volume of 50 μ l. A dispersion coefficient of 4.2 was estimated for the whole flow injection system, using an unmodified CPE and 0.5 mM $K_3[Fe(CN)_6]$ (Merck) solution.

NADH electro-oxidation study

The electrocatalytic oxidation of NADH at the modified carbon paste electrodes with MB adsorbed onto ZP was investigated through addition of a freshly prepared NADH (Sigma) solution to the electrolyte solution. Investigations on the electrocatalytic oxidation of NADH were performed using cyclic voltammetry, rotating disc electrode and flow injection techniques. For cyclic voltammetry the initial potential was -250 mV versus Ag|AgCl, KCl_{sat} , and the experiments were performed in the potential range: -250 to 250 mV versus Ag|AgCl, KCl_{sat} . The RDE experiments were carried out at an applied potential of $+170$ mV versus Ag|AgCl, KCl_{sat} within the rotating speeds range from 100 rpm to 1,000 rpm. All measurements in FI were performed at an applied potential of $+200$ mV versus Ag|AgCl, KCl_{sat} .

The influence of Ca^{2+} on the electrocatalytic oxidation of NADH was studied through addition of different concentrations of Ca^{2+} into the contacting buffer. Different amounts of PEI incorporated into the paste electrode were additionally investigated as a means to increase the efficiency of the electrocatalytic oxidation of NADH at the Meldola Blue-modified carbon paste electrodes.

Results and discussion

Electrochemical behavior of MB-ZP-CPEs

Cyclic voltammetry was used to compare the electrochemical behavior of the MB-ZP-CPEs obtained from graphite powder and two different glassy carbon powders: Sigradur K and Sigradur G. Figure 1 shows typical cyclic voltammograms obtained for a MB-ZP-CPE

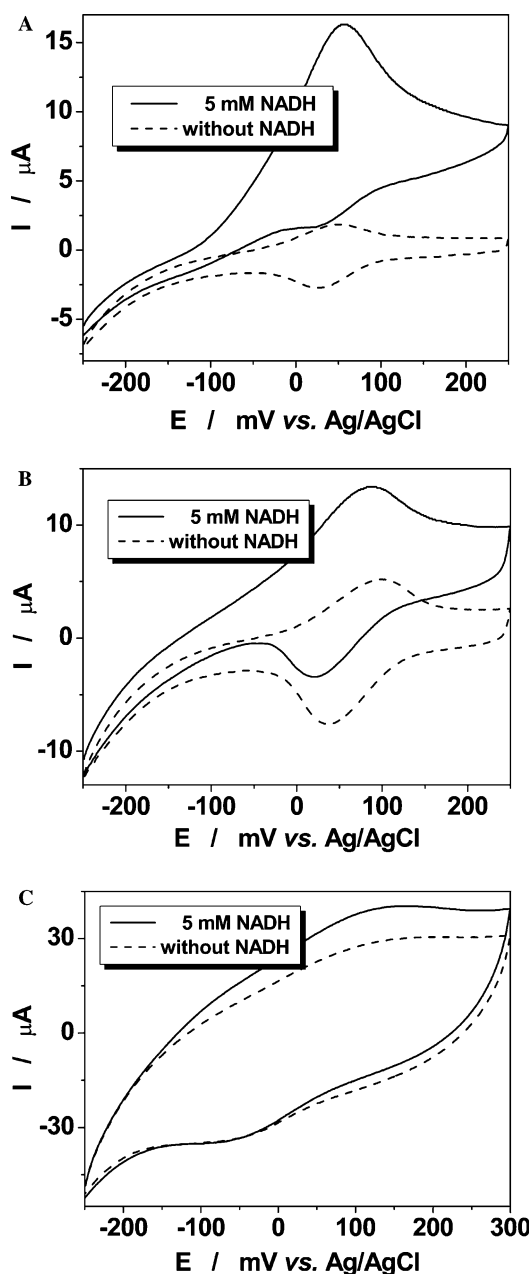


Fig. 1 Cyclic voltammograms recorded at MB-ZP-CPEs made from Sigradur K (2.3:1; w/w) (A), graphite powder (1:1; w/w) (B), and Sigradur G (2.3:1; w/w) (C) in the presence of 5 mM NADH and in its absence. Experimental conditions: potential scan rate, 20 mV/s; supporting electrolyte, 0.1 M Tris buffer (pH 7)

based on Sigradur K, a MB-ZP-CPE based on graphite powder, and a MB-ZP-CPE based on Sigradur G, recorded in 1.0 M Tris buffer at pH 7. For electrodes based on Sigradur K (Fig. 1A) and graphite powder (Fig. 1B) clear voltammetric waves for Meldola Blue are seen, whereas those recorded on Sigradur G (Fig. 1C) are less obvious. Additionally, for the MB-ZP-CPEs based on Sigradur K the background current is much lower than its equivalent counterpart based on graphite powder, whereas for the same material the electrochemical response of the adsorbed MB was much higher than that recorded for Sigradur G.

The formal potential ($E^{\circ'}$) of adsorbed Meldola Blue (Table 1), evaluated as the average of the oxidation and the reduction peaks of the voltammograms [19], is around +40 mV versus Ag|AgCl (KCl_{sat}), for the electrode based on Sigradur K, +30 mV versus Ag|AgCl (KCl_{sat}), for the electrode based on Sigradur G, and around +50 mV versus Ag|AgCl (KCl_{sat}), for the electrode based on graphite powder, i.e., a large shift in the positive direction was observed [20, 21], compared with an $E^{\circ'}$ of -175 mV versus SCE for Meldola Blue adsorbed directly onto graphite.

The reason for these different electrochemical behaviors is obviously due to the different structures of the carbon powders used. It was reported that the particle size and the orientation of the crystallites influence the texture and electrochemical properties of carbon paste electrodes [22]. Graphite powders are porous, have a particle size between 5 μm and 20 μm , and have a rough texture and unfavorable physical and electrochemical properties causing a higher background current [23]. In contrast, glassy carbon powders (Sigradur K and Sigradur G) are nonporous, have a globular particle size between 0.4 μm and 12 μm , and exhibit electrochemical properties similar to those of solid glassy carbon, having the widest potential range of all carbon electrodes [14, 15, 24, 25]. However, there seems to be a great difference in the electrochemical behavior of the two different kinds of glassy carbon materials, probably due to the different adsorption capabilities [13]. Sigradur K and Sigradur G are also available as solid electrode materials and when electrodepositing Prussian Blue onto the surface of these two glassy carbon materials, only

Table 1 Electrochemical and kinetic parameters for NADH electrooxidation at MB-ZP-CPEs made from different carbon powders (0.1 M Tris buffer pH 7)

Parameters	Graphite powder [1:1(w/w)]	Sigradur G [1:1(w/w)]	Sigradur K [2.3:1(w/w)]
$E^{\circ'}/\text{mV}$ versus Ag AgCl	50	30	40
$\Gamma/\text{mol cm}^{-2}$	6.2×10^{-10}	1.3×10^{-9}	5.6×10^{-11}
K_M/mM	2	2.5	2.5
$k_{\text{obs}[\text{NADH}]_0}/\text{M}^{-1} \text{s}^{-1}$	8.5×10^2	2.9×10^2	7.2×10^3
k_{+2}/s^{-1}	1.7	0.73	18
No. of transferred electrons	1.94	1.96	1.60

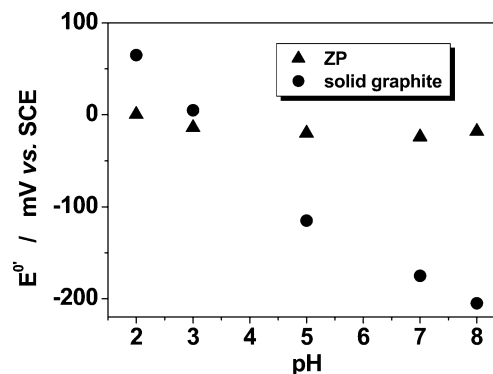


Fig. 2 pH influence on the formal standard potential ($E^{\circ'}$) of Meldola Blue adsorbed on ZP, entrapped in MB-ZP-CPEs made from Sigradur K (2.3:1; w/w) (filled triangle), and adsorbed on solid graphite (filled circle) [3]

Sigradur K revealed close to reversible and stable electrochemistry, whereas both the electrochemistry and the stability of the Prussian Blue film on Sigradur G were much poorer [26]. Obviously, Sigradur K is the better choice for our purposes and therefore further experiments were mainly performed with this carbon material.

Figure 2 shows the variation of the $E^{\circ'}$ with the pH value of the contacting solution for MB-ZP-CPEs based on Sigradur K. It is clear that the $E^{\circ'}$ remains constant in contrast to when dissolved in aqueous buffer or when adsorbed on graphite [3]. Previously, it was shown for a whole range of different aromatic quinoid redox compounds (phenazines, phenothiazines, phenoxazines, flavins, indophenols, naphthoquinones), immobilized on ZP and mixed into carbon paste electrodes prepared from graphite powder, that the $E^{\circ'}$ of the immobilized redox compound did not vary when changing the pH of the contacting electrolyte between pH 1 and 9 [6, 27–30]. In a number of investigations drastic spectral changes have been noticed for these kinds of compounds when intercalated into layered phosphates [5, 16, 31–40]. It is thus obvious that there are strong interacting forces between the host material (ZP) and the guest molecules (such as Meldola Blue). However, so far there has been only some attempts to correlate the observed independence of the $E^{\circ'}$ on the pH with the spectral changes [41, 42] and thus more work is needed to fully explain this effect.

Electrocatalytic NADH oxidation

Equivalently prepared MB-ZP-CPEs, based on the two glassy carbon powders and on graphite powder were screened for their electrocatalytic effect for NADH oxidation with cyclic voltammetry. The efficiency of the catalytic oxidation of NADH varies with the carbon powder used (Fig. 1). Even though the noticeable surface coverage of immobilized Meldola Blue was the lowest for the MB-ZP-CPE based on Sigradur K (Table 1), the catalytic current for NADH oxidation was

higher for Sigradur K than for graphite powder and the lowest for Sigradur G (Fig. 1).

To investigate the kinetics and mechanism of the electrocatalytic oxidation of NADH with this type of bound mediators, amperometric experiments were performed using a rotating disc electrode at different concentrations of NADH. The applied potential was set approximately 100 mV more positive than the $E^{\circ'}$ of Meldola Blue adsorbed onto ZP, in order to avoid the reoxidation of the mediator as rate limiting step [3, 6]. For evaluation of the rate constants it is essential to perform the experiments with a surface coverage less than what is expected to be the equivalent to a monolayer. For carbon pastes the real surface area remains unknown, but in recent investigations it was shown that for equivalently prepared carbon paste electrodes, the catalytic current for a constant NADH concentration varies linearly with mediator coverage up to about 10^{-10} mol cm $^{-2}$ [29, 30] and for a higher coverage the catalytic current levels off to a constant value. This can be expected to be a reason both because the reaction between NADH and the mediator occurs according to a reaction sequence similar to Michaelis-Menten enzyme kinetics and because for mediator coverage higher than a monolayer, NADH does not have direct and easy access to all electrochemically active mediator molecules. For evaluation of the rate constants and to be able to compare with previously published data, these investigations were performed with a surface coverage below 10^{-10} mol cm $^{-2}$.

Table 1 summarizes the values of the kinetic parameters for the heterogeneous catalytic reaction [the second-order rate constant ($k_{\text{obs[NADH]}=0}$), the Michaelis-Menten constant (K_M), and the turnover number (k_{+2})] for all types of investigated carbon powder: graphite, Sigradur G and Sigradur K. To calculate these parameters the Koutecký-Levich approach was used [3].

As expected, the values of k_{obs} were strongly dependent on NADH concentration [3]. Using the relation describing the dependence between k_{obs} and NADH concentration:

$$k_{\text{obs}} = \frac{k_{+2}}{K_M + [\text{NADH}]}$$

the extrapolated value of $k_{\text{obs[NADH]}=0}$ was calculated from the intercept of the linear regression $1/k_{\text{obs}}$ versus $[\text{NADH}]$.

Comparing the values for all three electrode-systems, it can be concluded that the Sigradur K-modified paste electrodes have the best response for NADH electrooxidation: for the lowest coverage it presents the highest heterogeneous second-order reaction rate constant. For this reason, we have continued our experiments focused on Sigradur K-modified paste electrodes. The variation of the pH of the buffer solution will not affect the $E^{\circ'}$ of the immobilized mediator, only that of the NAD^+/NADH redox couple will influence the thermodynamic driving force for the catalytic reaction. It is worth

mentioning that the $E^{\circ'}$ of the NAD^+/NADH redox couple increases by 30 mV for each tenfold increase in $[\text{H}^+]$ [43].

The effect of Ca^{2+} on electrocatalytic NADH oxidation

Previous studies of the reaction rate between NADH and different mediators both in solution [44, 45] and when adsorbed on electrodes [46] have shown that positively charged mediators are more efficient than corresponding neutral ones having $E^{\circ'}$ values in the same potential range. Moreover, some of these mediators can form strong complexes with metal cations and transform the mediator into a molecule with a net positive charge [2]. The addition of divalent cations such as Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} [9, 10, 47, 48] into the buffer solution increases the electrocatalytic response towards NADH at various modified electrodes: gold-modified electrodes [9], glassy carbon electrodes [47, 49], modified carbon paste electrodes, and a fully plausible explanation to this effect was recently given [10].

Based on these previous results, a study on the effect of NADH electrooxidation by adding Ca^{2+} was performed. The catalytic current observed after a 10 mM addition of Ca^{2+} was 175% higher than that recorded for the same NADH concentration (5 mM) but in the absence of Ca^{2+} (Fig. 3). Mano et al. [48, 49] have shown that this enhancement is not due to the ionic strength variation of the supporting electrolyte and, under certain conditions, is a result of complexation of Ca^{+2} with the phosphate groups of NADH (an increased hydrophobic character of NADH), or with the mediator molecule, which has carboxyl groups (increase of the hydrophilic character of the mediator). Moreover, Ca^{2+} ions could act like a bridge between NADH and the mediator.

Cyclic voltammetry was used to investigate the effect of Ca^{2+} ions on the catalytic current. The influence of

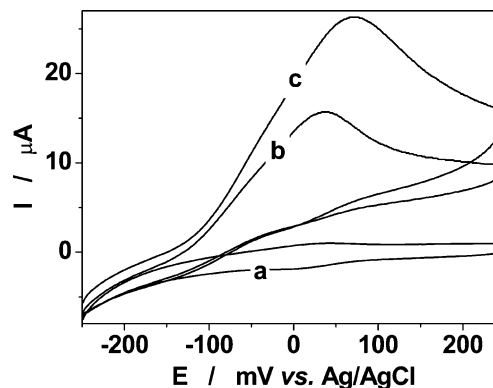


Fig. 3 NADH electrocatalytic oxidation at MB-ZP-CPEs made from Sigradur K (2.3:1; w/w): no NADH (a); 5 mM NADH (b); 5 mM NADH containing 10 mM Ca^{2+} (c). Experimental conditions: potential scan rate, 10 mV/s; supporting electrolyte, 0.1 M Tris buffer (pH 7)

the concentration of Ca^{2+} on the electrocatalytic oxidation of NADH at MB-ZP-CPEs based on Sigradur K can be observed in Fig. 4. In the presence of a low concentration of Ca^{2+} the catalytic current increases, but the optimal concentration of Ca^{2+} for the electrooxidation of NADH is 0.25 mM. In these conditions, the catalytic current obtained is more than twice higher than in the absence of Ca^{2+} . For high concentrations of Ca^{2+} the catalytic current starts to decrease, but it is still higher than the value of the catalytic current obtained in the absence of Ca^{2+} . For graphite powder paste-based modified electrodes the optimal concentration of Ca^{2+} was found to be 0.2 M, and at 0.45 M Ca^{2+} and higher the NADH oxidation reaction becomes inhibited. What is also clear from the results shown in Fig. 4 is that the catalytic current is very much dependent on the concentration of Ca^{2+} and any small variation in its concentration would very much affect the response. Therefore, the analytical use of addition of Ca^{2+} to the contacting solution to increase the response for NADH oxidation would be of little benefit for this system.

PEI-influence on NADH electrooxidation

The addition of supplementary reagents (for example Ca^{2+}) to the conducting buffer in order to enhance the rate of the electrocatalytic process for NADH oxidation, was avoided by incorporating PEI, a highly positively charged polyelectrolyte, into the carbon paste mixture. Previously, it was noticed that the addition of this polycation to enzyme-modified carbon pastes resulted in a remarkable increase in the sensitivity of the biosensor [11, 12]. As expected, the addition of PEI to the modified carbon paste electrodes induced a large increase in the catalytic current. Figure 5 presents a comparison between the voltammetric responses of two MB-ZP-CPEs based on Sigradur K, prepared with different amounts of

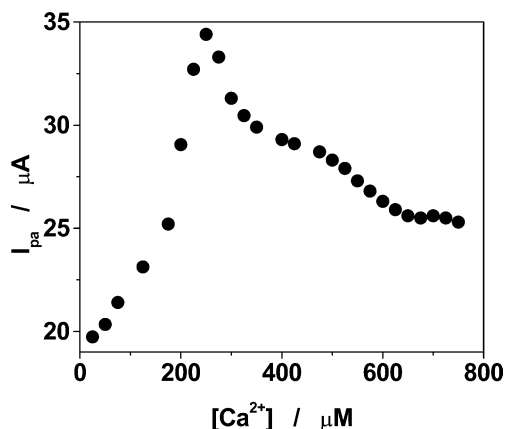


Fig. 4 Variation with Ca^{2+} concentration of the peak currents for NADH oxidation, recorded at MB-ZP-CPEs made from Sigradur K (1:1; w/w). Experimental conditions: potential scan rate, 20 mV/s; supporting electrolyte, 0.1 M Tris buffer (pH 7) containing 5 mM NADH

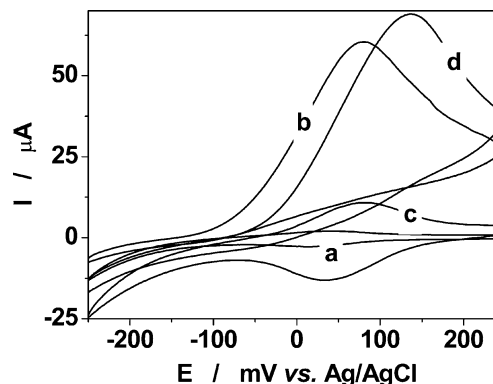


Fig. 5 Influence of PEI addition on the NADH electrocatalytic oxidation at MB-ZP-CPEs made from Sigradur K (1:1; w/w): 1.23% PEI (a, b), 2.44% PEI (c, d). Experimental conditions: potential scan rate, 20 mV/s; supporting electrolyte, 0.1 M Tris buffer (pH 7) (a, c), and containing 5 mM NADH (b, d)

PEI. One can conclude that a high amount of PEI (2.44% PEI) immobilized in the carbon paste provides the best response for NADH electrooxidation. Furthermore, comparing the response of MB-ZP-CPEs based on Sigradur K, with the same amount of PEI (1.23% PEI) but having different Sigradur K|MB-ZP ratios (2.3:1 and 1:1), it was noticed that a high amount of the MB-ZP induced a significant increase in the electrocatalytic current (results not shown).

In order to obtain more information on the behavior of MB-ZP-CPEs based on Sigradur K, calibration curves for NADH were performed using the “wall-jet” FI system for electrodes with different contents of PEI (Fig. 6). As expected, the catalytic current increases with the content of PEI, being almost twice higher for a paste containing 2.44% PEI when compared with a paste without PEI.

Table 2 summarizes the analytical and the kinetic parameters, estimated from FI and RDE measurements, respectively, for MB-ZP-CPEs based on Sigradur K with different contents of PEI and different Sigradur K|MB-

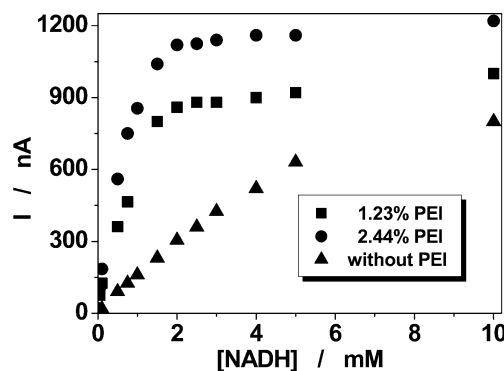


Fig. 6 Flow injection calibration curves to NADH for MB-ZP-CPEs made from Sigradur K (2.3:1; w/w). Experimental conditions: applied potential, +200 mV versus Ag/AgCl; flow rate, 0.5 ml/min; supporting electrolyte, 0.1 M Tris buffer (pH 7)

Table 2 Influence of PEI addition on the analytical and kinetic parameters for NADH electrooxidation at MB-ZP-CPEs made from Sigradur K

Parameters	Sigradur K/MB-ZP					
	2.3:1 (w/w)			1:1 (w/w)		
	PEI/% (w/w)					
	–	1.23	2.44	–	1.23	2.44
$I_{\max}^a/\mu\text{A}$	0.8	1.0	1.2	2.8	5.5	6.5
K_M^a/mM	2.5 ^c	1.0	0.8	2.2	1.0	(0.5) ^c
$S^b/\mu\text{A M}^{-1}$	169	590	958	700	1,929	1,933
$\Gamma/\text{mol cm}^{-2}$	5.6×10^{-11}	–	–	–	4.4×10^{-11}	–
$k_{\text{obs}}[\text{NADH}]_0/\text{M}^{-1} \text{ s}^{-1}$	7.2×10^{3c}	–	–	–	8.2×10^{3c}	–
k_{+2}/s^{-1}	18 ^c	–	–	–	8.1 ^c	–

^aEstimated from the Lineweaver–Burk linearization (FI amperometric measurements)

^bEstimated as the slope of the linear range (FI amperometric measurements)

^cEstimated from K-L interpretation of RDE measurements

ZP ratios. The highest sensitivity was found for electrodes with a Sigradur K|MB-ZP ratio of 1:1. Moreover, irrespective of the content of PEI, these electrodes showed the highest catalytic currents for NADH oxidation and the lowest Michaelis–Menten constants. Thus, in spite of a low K_M , involving a narrower linear range, these electrodes are recommended as NADH sensors, or transducers for amperometric biosensors based on NADH-dependent dehydrogenases.

Due to the hydrophilic nature of PEI, the mechanical stability of the modified electrodes is strongly affected by the presence of PEI in the carbon paste. For this reason, the short-term operational stability was taken as a key parameter for establishing the best composition of the carbon paste, used to obtain MB-ZP-CPEs based on Sigradur K. A PEI content of 1.23% (w/w) was selected as optimum, because these electrodes showed a much higher short-term operational stability than those containing 2.44% (w/w) PEI.

Conclusions

When used for preparing carbon paste electrodes modified with Meldola Blue adsorbed on zirconium phosphate, the glassy carbon powder-type Sigradur K exhibits the best behavior for NADH electrooxidation, seen as: low background current, high catalytic current, and high catalytic rate constant. The presence of Ca^{2+} in the contacting solution and the incorporation of PEI in the carbon paste significantly both improve the electrocatalytic activity for MB-ZP-CPEs.

As a general conclusion, it can be stated that MB-ZP-CPEs based on Sigradur K-type glassy carbon and containing 1.23% (w/w) PEI showed the best behavior for electrocatalytic oxidation of NADH, expressed by a

high electrocatalytic rate constant ($8.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$). The kinetic parameters obtained for this electrode open up promising possibilities in various fields like biosensors, biofuel cells and enzyme-driven organic synthesis.

Acknowledgements The authors thank the following organizations for financial support: The Swedish Research Council (VR); The Swedish Institute (SI) for financially supporting the research stay for Ioana Ladiu at the Department of Analytical Chemistry, Lund University (Sweden); The Romanian Academy (GAR 40/2004).

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