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NADH OXIDATION AT MELDOLA BLUE MODIFIED GLASSY CARBON ELECTRODES. A COMPARATIVE STUDY

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ABSTRACT. Three types of modified electrodes based on glassy carbon (powder and rod) and Meldola Blue (MB) were used for NADH electrocatalytic oxidation. MB was immobilized by simple adsorption on two different phosphates materials, crystalline zirconium phosphate (α-ZP) and crystalline titanium phosphate (α-TP), as well as on carbon aerogel (CA). The phosphate materials were incorporated in carbon paste, while CA was immobilized on a glassy carbon rod using a chitosan matrix. The basic electrochemistry of adsorbed MB and its ability to catalyze NADH electrooxidation have been investigated by cyclic voltammetry, performed in different experimental conditions (pH, potential scan rate, NADH concentration). The electroanalytical parameters of the investigated modified electrodes showed that: (i) irrespective the material, the formal standard potential of the immobilized MB was found pH dependent; (ii) the best electrocatalytic response was obtained for MB-αTP modified electrode.

***Keywords**: electrocatalysis, NADH electrooxidation, glassy carbon electrode, redox dyes*

INTRODUCTION

Dehydrogenases represent the majority of redox enzymes. They require for the operation the presence $NAD(P)^+$ / NAD(P)H coenzyme. Consequently, the electrochemical regeneration of this coenzyme - in fact the oxidation of the reduced form, NAD(P)H - is an important issue. Indeed, for many years the electrochemical oxidation of NADH was studied, and it was concluded that in order to achieve this process at different conventional electrode materials relatively high overpotentials are required [1, 2] or is necessary the presence of a suitable redox mediator [3]. It is worth to add, that the direct electrochemical oxidation of NADH is accompanied by the electrode fouling, which is due to the NAD^+ adsorption on the electrode surface or to the side reactions involving the radicals generated during the coenzyme oxidation.

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One of the best mediators for NADH catalytic oxidation is Meldola Blue (MB), a phenoxazine compound involving two electrons and two protons [4, 5].

Excepting the water solubility of the MB, another reason for its immobilization onto different type of support materials is the shift of its formal standard potential (E°') towards more positive potentials, at a value allowing the operation of the modified electrode within the potential window essentially free of interfering reactions [6]. Previous studies showed that, when MB was directly adsorbed onto pyrolytic graphite, its E°' value was –175 mV *vs.* Ag|AgCl (at pH 7) [4], which is outside of the optimum window for amperometric detection [7]. Additionally, an increase of this E°' value will indirectly induce an increase of the rate constant for NADH electrocatalytic oxidation, which, actually, is large enough to be limited by the mass transport process [8, 9].

Due to their peculiar properties crystalline phosphates were used in many research areas as catalysts, ion exchangers, molecular sieves, and electrode materials [10]. Thus, because of their negatively charged phosphate groups, crystalline phosphates are good ion conductors and good ion exchangers (strong acidic materials). Additionally, they are layered materials, which present two important features: (i) the interlayer spaces can be adjusted to accommodate guests of different size; (ii) they have the control over the orientation, aggregation and distribution of the adsorbed guests [11].

Recently, carbon aerogels (CAs) were used as support material for the immobilization of different chemical species with catalytic properties [12]. CAs have interesting properties, such as high specific surface area, porosity, and electrical conductivity, associated with a good chemical stability. Therefore, CAs are attractive materials for different applications such as electrode materials in supercapacitors and rechargeable batteries, catalyst supports, adsorbent materials and thermal insulators [13].

In the present work carbon aerogel (CA), a high mesoporous material, was compared with crystalline zirconium phosphate (α-ZP) and crystalline titanium phosphate (α-TP) when all were used as support material for MB immobilization. The resulting modified materials, MB-αTP, MB-αZP and MB-CA, were deposited on two types of glassy carbon, Sigradur K powder (SK) or glassy carbon rod (GC) in order to obtain two MB modified carbon paste electrodes, (MB-αTP-SK-CPE) and MB-αZP-SK-CPE, and MB modified glassy carbon electrodes (MB-CA/GCEs). Aiming to estimate and compare their electroanalytical parameters, the electrochemical behavior of the obtained modified electrodes and their ability to catalyze the electrooxidation of NADH have been investigated by cyclic voltammetry, performed in different experimental conditions (pH, potential scan rate, NADH concentration).

RESULTS AND DISCUSSIONS

Electrochemical behavior of the MB modified GC electrodes

A comparison between the cyclic voltammograms (CVs) recorded at MB modified electrodes with similar measurements performed at electrodes without immobilized MB lead to observe that, as expected, no redox process occurred at electrodes in the absence of MB, while MB-αTP-SK-CPE (Sigradur K : MB-αTP = 19:1; w/w), MB-αZP-SK-CPE (Sigradur K : MB-αZP = 19:1; w/w), and MB-CA/GCE present one voltammetric peaks pair, which is due to the oxido-reduction process involving the mediator redox couple (Figure 1).

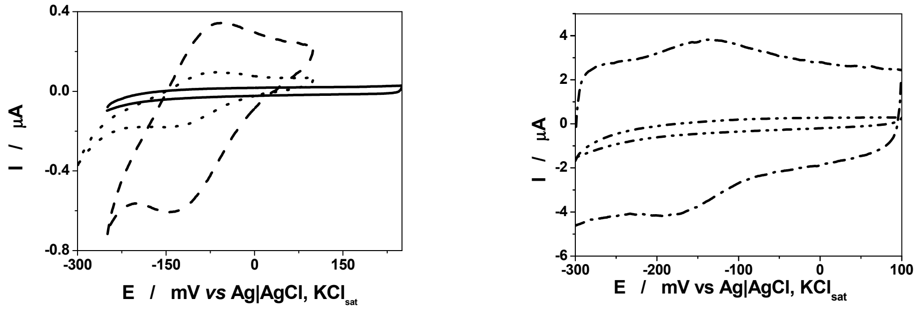

Figure 1. Cyclic voltammogram recorded at MB-αTP-SK-CPE (····), MB-αZP-SK-CPE (----), SK-CPE (—), MB-CA/GCE (-·-·-) and GCE (-··-). Experimental conditions: scan rate, 20 mV/s, supporting electrolyte Tris buffer, 0.1 M, pH 7.

The background current observed on the recorded CVs was higher for all MB modified electrodes than that evidenced for the corresponding unmodified electrodes (SK-CPE or GCE) (Figure 1). A plausible explanation of this fact should take into consideration that all support material used for mediator immobilization (α-TP, α-ZP, and CA), have high specific surface area, conferring a high electrochemical active surface to the modified electrodes. Indeed, the highest background current among the compared modified electrodes was observed in the case of the electrode modified with CA, which is a high mesoporous material (Figure 1) [14].

The electrochemical parameters of the MB modified GCEs are summarized in Table 1. In all cases, the peak separation values (ΔE_p), the width at half maximum current intensity ($W_{1/2}$) and the ratio between the intensity of the cathodic and anodic peaks (I_{pa}/I_{pc}) point to a quasi-reversible redox process [15].

The values of the formal standard potential (E°’) for all MB modified GCEs (Table 1) are in the optimal potential range for electroanalytical applications [7], and assure an efficient production of enzyme active NAD^+ [6, 8]. Among the prepared modified electrodes, the MB-αZP-SK-CPE and MB-αTP-SK-CPE exhibit identical values of (E°’) for MB redox couple, which are slight lower than that corresponding to MB-CA/GCE.

Table 1. Electrochemical parameters of the investigated GC modified electrodes.

Electrode	$E^{o'}$ (mV)	ΔE_p (mV)	$W_{1/2}$ (mV)		I_{pa}/I_{pc}
			anodic	catodic	
MB-CA/GCE	-140	40	110	100	1.1
MB-αZP-SK-CPE	-100	65	120	100	1.07
MB-αTP-SK-CPE	-100	60	100	90	0.87

$\Delta E_p = E_{pa} - E_{pc}$, where E_{pa} and E_{pc} are the cathodic and anodic peak potentials.

The short-term stability of the modified electrodes was estimated by continuous voltammetric cycling within the potential window of practical interest. The time evolution of the recorded CVs revealed that the peak current and the peak potential corresponding to the MB redox wave show a good stability. Thus, by using the relation $\frac{(I_{pa})_{100^{th}\ cycles}}{(I_{pa})_{1^{st}\ cycle}} \times 100\%$, the current stability was found as follows: ~80% for MB-αTP-SK-CPE; ~101% for MB-αZP-SK-CPE was; and ~105% for MB-CA/GCE. These results show a low decrease or even an increase of the electrode activity due to its activation induced by the continuous potential cycling. Consequently, the simple adsorption of MB on the α-TP, α-ZP or CA support materials, followed by the incorporation MB / support material in the electrodes composition, lead to stable and functional modified electrodes.

For all prepared modified electrodes based on MB-αTP, MB-αZP and MB-CA the $E^{o'}$ value depends on the pH in the same manner as that reported for MB dissolved in aqueous solution [16], MB adsorbed on spectrographic graphite [4] or on α-ZP [17]. In good agreement with previously published results [4], the $E^{o'}$ *vs.* pH dependence shows two linear regions: one with the slope of ~60 mV/pH, and the second having the slope of ~30 mV/pH unit (Figure 2). This behavior indicates that, irrespective of the support material, the pK_a value for the adsorbed MB is around 4 [4]. Indeed, all experimental data were well fitted to the following nonlinear regression equation: $E = E^{0'} - 0.059 * pH + 0.029 * \log(1 + 10^{pH - pK_a})$. The calculated pK_a values for MB-αTP-SK-CPE, MB-αZP-SK-CPE and MB-CA/GCE are presented in Table 2. These pK_a values are lower than that reported for MB adsorbed on spectrographic graphite (pK_a = 5) [4]. This difference suggests that, due to the interactions with the support materials the MB immobilized onto α-ZP, α-TP or CA becomes more acidic than when adsorbed onto graphite.

The cyclic voltammograms recorded at pH 7, in a wide range of potential scan rates (0.001 – 18 Vs^{-1}), showed a linear dependence of the peak currents (I_{pa}) on the scan rate (*v*). Thus, the slopes of log(I_{pa}) *vs.* log(v) dependencies (Figure 3) were close to the theoretical value (1), confirming the immobilization of the redox mediator MB on the support material (α-ZP, α-TP or CA) (Table 3).

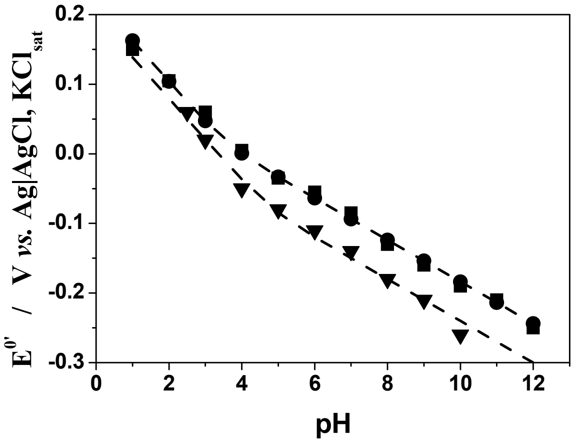

Figure 2. pH influence on the E°' value of MB adsorbed on: α-ZP [SK : MB-αZP = 19:1 (w/w)] (●) [18]; α-TP [SK : MB-αTP = 19:1 (w/w)] (■) and CA (▼).

Table 2. pK_a calculated values for MB-αTP-SK-CPE, MB-αZP-SK-CPE, and MB-CA/GCE.

Electrode	pK_a	chi^2	R^2	N
MB-αZP-SK-CPE	4.3 ± 0.4	0.0002	0.9866	11
MB-αTP-SK-CPE	3.6 ± 0.2	0.00007	0.9963	12
MB-CA/GCE	4.7 ± 0.3	0.0001	0.9899	9

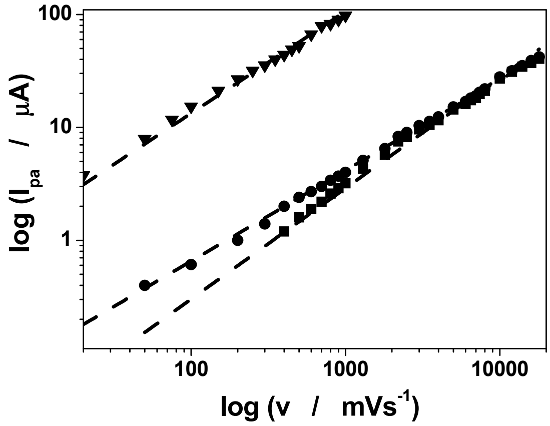

Figure 3. Influence of the potential scan rate on the anodic peak current recorded at MB-αTP-SK-CPE (■), MB-αZP-SK-CPE (●), and MB-CA/GCE (▼). Experimental conditions: supporting electrolyte, 0.1 M Tris buffer (pH 7).

Table 3. The slopes of the log(I_{pa}) *vs.* log(v) for MB-αZP-SK-CPE, MB-αZP-SK-CPE and MB-CA/GCE modified electrodes.

Electrode	Slope	R	N
MB-CA/GCE	0.90 ± 0.02	0.994	19
MB-αZP-SK-CPE	0.80 ± 0.01	0.997	32
MB-αTP-SK-CPE	0.98 ± 0.01	0.994	26

Electrocatalytic NADH oxidation at MB modified GC electrodes

MB-αZP-SK-CPE, MB-αTP-SK-CPE and MB-CA/GCE showed clear electrocatalytic activity for NADH oxidation. Thus, the cyclic voltammograms recorded at these electrodes in the presence of NADH (Figure 4, solid lines) present all the features of an electrocatalytic process: the oxidation peak is drastically increased, simultaneously with the disappearance of the reduction peak.

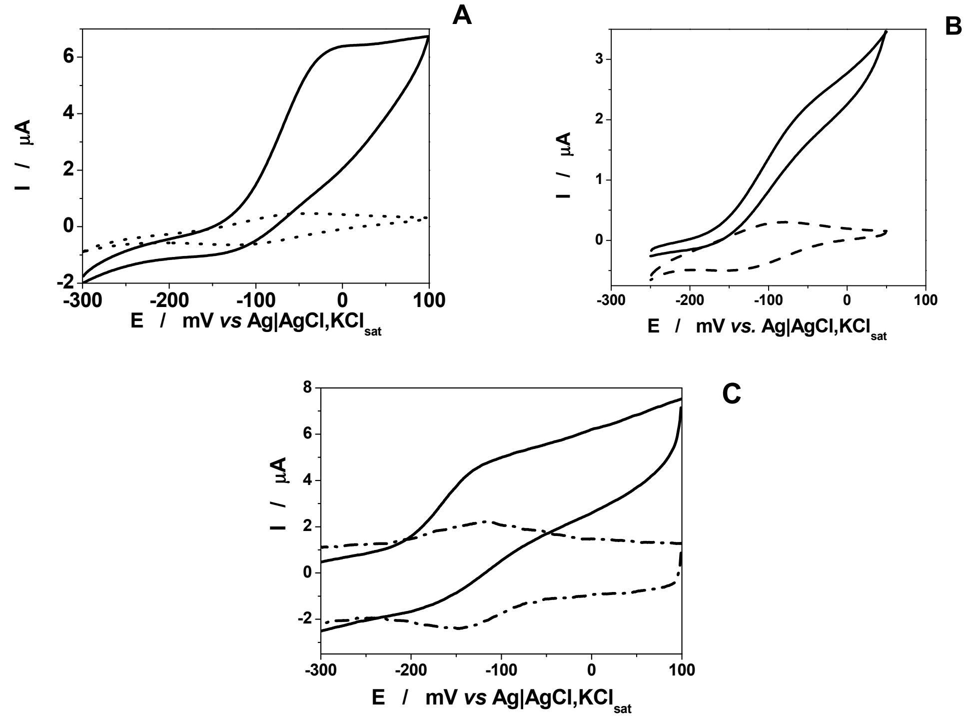

Figure 4. Electrocatalytic oxidation of NADH at MB-αTP-SK-CPE (A), MB-αZP-SK-CPE (B) and MB-CA/GCE (C) in the absence and in the presence of 5 mM NADH. Experimental conditions: scan rate, 20 mV/sec; supporting electrolyte, 0.1 M Tris buffer, (pH 7).

The efficiency (*Eff*) of the electrocatalytic oxidation of NADH at the investigated modified electrodes (MB-αTP-SK-CPE, MB-αZP-SK-CPE and MB-CA/GCE) was calculated according to the following relation: $\mathbf{Eff = (I_{cat} - I_0)/I_0}$, where I_{cat} is the oxidation current intensity in presence of 5 mM NADH and I_0 is the oxidation current intensity observed in its absence. Among the calculated electrocatalytic efficiencies, the highest value was obtained for MB-αTP-SK-CPE, and decrease in the following sequence: $Eff_{MB\text{-}\alpha TP\text{-}SK\text{-}CPE} > Eff_{MB\text{-}\alpha ZP\text{-}SK\text{-}CPE} > Eff_{MB\text{-}CA/GCE}$ (Table 4).

The surface coverage (Γ) was estimated by using the equation $\mathbf{\Gamma = Q/nFA}$, *where* Q is the electric charge obtained by integrating the anodic peak, corrected for the background current; *n*, *F* and *A* are the number of electrons transferred in redox reaction, Faraday’s constant and the electrode geometric area, respectively.

Table 4. Electrocatalytic efficiencies for NADH oxidation at modified GC electrodes.

Electrode	**Eff**	**Γ** **10^{10} mol cm^{-2}**
MB-CA/GCE	2.2	1.85
MB-αZP-SK-CPE	7.5	0.32
MB-αTP-SK-CPE	19.4	0.08

Surprisingly, the MB coverage value for MB-αTP-SK-CPE was the lowest, comparing with those obtained for MB-αZP-SK-CPE or MB-CA/GCE, but is associated with the highest electrocatalytic efficiency. A plausible explanation for this behavior could due to the differences existing between the structure of the support material used for mediator immobilization. Therefore, when CA, a high mesoporous material, was used as support material the free access of NADH to the immobilized MB was more difficult due to some steric hindrances.

CONCLUSIONS

Two different types of glassy carbon (powder and rod) and three types of support materials have been successfully used for the preparation of MB-αZP-SK-CPE, MB-αTP-SK-CPE and MB-CA/GCE modified electrodes. The electrochemical parameters of the modified electrodes point out the presence of the immobilized MB species on the electrode surface. All E°’ values are placed in the optimal potential range for the amperometric detection. The pH dependence of E°', for all investigated modified electrodes, was fitted using a nonlinear model, allowing the estimation of the pK_a value for the immobilized MB. The electrochemical and electroanalytical parameters of the investigated electrodes recommend them as stable and reproducible sensors for NADH. Among them MB-αTP-SK-CPE presents the best electrocatalytic response for NADH electrooxidation.

EXPERIMENTAL SECTION

Synthesis of CA. CA was prepared using a mixture of resorcinol (98% purity, Aldrich), formaldehyde (37% solution, Aldrich), Na_2CO_3 (99.9% purity, Aldrich), and deionized water, according to a previously reported sol-gel method [14].

Synthesis of acidic alpha- zirconium phosphate (α-ZP) and alpha-titanium phosphate (α-TP). α-ZP and α-TP were prepared according to a previously reported method [17], and [19], respectively.

Immobilisation of the organic dye (Meldola Blue) on the support material surfaces. 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 support material (α-ZP, α-TP and CA) was added to 50 ml of dye solution and the mixture was shaken for 1 h. The precipitate (MB-αZP, MB-αTP and MB-CA) was filtered, washed with de-ionized water and dried at room temperature.

Preparation of the modified glassy carbon electrode. The carbon paste electrodes were prepared by thoroughly mixing glassy carbon powder (Sigradur K, HTW, Hochtemperatur-Werkstoffe GmbH, Bonn, Germany) and MB-αZP or MB-αTP with paraffin oil (Fluka, Buchs, Switzerland) in an agate mortar. For 20 mg of carbon powder and MB-αZP or MB-αTP mixture, with a ratio of glassy carbon powder to MB-αZP or MB-αTP of 19:1 (w/w), 5 μL of paraffin oil were added. The size of the spherical glassy carbon particles was 0.4 – 1.2 μm. The obtained pastes 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.

The home made glassy carbon rod electrodes were prepared by introducing glassy carbon rod into a Teflon holder, then was screwed onto a rotating disc electrode device (Radiometer Analytical, France) and was used as working electrode for cyclic voltammetry. The glassy carbon rod was thoroughly polished on alumina (1 μm Stuers, Copenhagen, Denmark), then rinsed with Milli-Q water. Chitosan solution was prepared by adding 10 mg chitosan (Sigma-Aldrich) to 10 mL of acetic acid (glacial acetic acid, Sigma) 0.1 M. Then a suspension of 1 g/L MB-CA in this solution was prepared. 5 μL from this suspension were placed onto clean GC electrode surface, and let to dry at room temperature.

The geometrical area of the prepared modified electrodes was 0.049 cm^2.

Electrochemical measurements. 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 modified GCEs were used as working electrode, a platinum ring as the counter electrode, and Ag|AgCl,KCl$_{sat}$ as reference electrode. Cyclic voltammetry was carried out in 0.1 M solution of Tris (Sigma). The pH of the electrolyte solutions was adjusted to the desired values by adding HCl or KOH (Merck).

All experiments were performed in deoxygenated electrolytes by bubbling argon for 20 min before each measurement. Al reagents were used as received.

For each prepared modified glassy carbon, the surface coverage (Γ, mol cm^{-2}) was estimated through integration of the area of the wave registered with cyclic voltammetry.

NADH electro-oxidation study. The electrocatalytic oxidation of NADH using MB adsorbed onto MB-αZP, MB-αTP or CA modified glassy carbon electrodes were investigated through addition of freshly prepared NADH (Sigma) solution to the electrolyte solution. Investigations on the electrocatalytic oxidation of NADH were performed using cyclic voltammetry. For cyclic voltammetry measurements, the initial potential was -300 mV *versus* Ag|AgCl,KCl$_{sat}$ for MB-αTP and MB-CA and -250 mV *versus* Ag|AgCl,KCl$_{sat}$ for MB-αZP. The experiments were performed in the potential range: -300 to 100 mV *versus* Ag|AgCl,KCl$_{sat}$ for MB-αTP and MB-CA and -250 to 100 mV *versus* Ag|AgCl,KCl$_{sat}$ for MB-αZP.

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