

*Dedicated to prof. dr. I. C. Popescu
on the occasion of his 70th anniversary*

MELDOLA BLUE IMMOBILISED ON MESOPOROUS CARBON AEROGEL - NEW ELECTRODE MATERIAL FOR NADH ELECTROCATALYTIC OXIDATION

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ABSTRACT. The new modified electrode based on Meldola Blue (MB) adsorbed onto carbon aerogel (CA) incorporated into chitosan matrix and deposited onto glassy carbon (GC) disc were used for NADH electrocatalytic oxidation. MB was immobilized by simple adsorption on mesoporous CA (MB-CA) and was immobilized on a GC disc using chitosan matrix. The morphological and structural characteristics of the new electrode material such as specific surface area, pore size distribution, pore volume, were obtained from N₂ adsorption - desorption isotherms and TEM measurements. The basic electrochemistry of adsorbed MB onto CA and its ability to catalyze NADH electrooxidation have been investigated by cyclic voltammetry and rotating disc electrode performed in different experimental conditions (potential scan rate, rotation speed, applied potential, NADH concentration, MB coverage). The electrocatalytic parameters of the investigated MB-CA/GC modified electrodes were estimated.

Keywords: *electrocatalysis, NADH electrooxidation, carbon aerogel, mesoporous materials, redox dyes*

INTRODUCTION

The dehydrogenase subgroup, the majority of redox enzymes, required for operation the presence of NAD(P)⁺/NAD(P)H coenzyme. The key problem to solve for electrochemical applications (biosensors, biofuel cells, bioorganic synthesis) is the electrochemical regeneration of the soluble

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redox cofactor. Many studies for cofactor electrochemical regeneration were focused on the NADH electrooxidation and it was concluded that in order to run this process on different conventional electrode materials relatively high overpotentials are required [1] or the presence of a suitable redox mediator is necessary [2].

Meldola Blue (MB), a phenoxazine compound involving two electrons/two protons in the redox process [3], is one of the best mediators for NADH catalytic oxidation for its high reaction rate [3]. Many electronically conducting materials (such as graphite [3], amorphous homemade zirconium phosphate (ZP) [2, 4] crystalline zirconium phosphate (α -ZP) [4 - 7] or crystalline titanium phosphate (α -TP) [5,6,8] were used in order to shift formal standard potential of MB (E°) towards more positive potentials, at a value allowing the operation of the modified electrode within the potential window essentially free of interfering reactions [9]. Also, an increase of this E° value will indirectly induce an increase of the rate constant for NADH electrocatalytic oxidation [10].

Moreover, great efforts have been made to find materials onto or into which the mediators can be strongly bound to avoid their leaching under experimental conditions [2, 4-8], and in order to improve the sensors characteristics (*i.e.* stability, sensitivity and reproducibility).

Recently, porous carbon materials (aerogels and xerogels) were used as support material for the immobilization of different chemical species with catalytic properties [11, 12]. Carbon aerogels (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 [11].

Thanks to its properties, which include excellent membrane-forming ability, high permeability toward water, good adhesion, biocompatibility, nontoxicity, and high mechanical strength, chitosan (Chi), is one of the most preferred materials used for immobilization of the active electrode materials [13].

In the present work, carbon aerogel (CA), a high mesoporous material, was used as support material for MB immobilization. The morphological and structural characteristics of the new electrode material such as specific surface area, pore size distribution, pore volume, were obtained from N_2 adsorption - desorption isotherms and TEM measurements. The resulted modified material MB-CA was deposited on the glassy carbon electrode (GCE) surface using chitosan matrix, and MB-CA/GCE was obtained. Aiming to evaluate the electrochemical behavior of the obtained modified electrodes, MB-CA/GCE, and their ability to catalyze the electrooxidation of

NADH cyclic voltammetry (CV) and rotating disc electrode (RDE) techniques were performed in different experimental conditions (potential scan rate, rotation speed, applied potential, NADH concentration, MB coverage). The obtained results (electrochemical and electrocatalytic parameters) allowed the estimation of the kinetic parameters of the new modified electrodes.

RESULTS AND DISCUSSIONS

Morphological and structural characterization of mesoporous CA

According to the IUPAC classification, the shape of the adsorption - desorption isotherm recorded for the mesoporous CA (Figure 1) corresponds to the type IV isotherm, which is typically associated with the “ink bottle” mesopores. Ordered mesoporous CA (median pore radius 5.15 nm) with high specific surface area ($881 \text{ m}^2\text{g}^{-1}$) and high pore volume ($1.518 \text{ cm}^3\text{g}^{-1}$) were obtained.

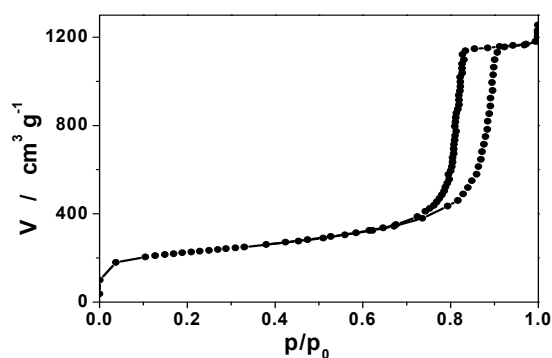


Figure 1. Adsorption-desorption isotherm for mesoporous CA

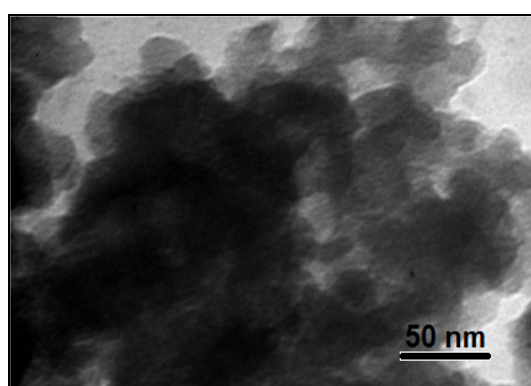


Figure 2. TEM image of mesoporous CA.

These properties cause special characteristics of carbon aerogel material, concerning both, the dispersion of the mediator on the carbon material surface (CA) and the easy access of the substrate to the mediator.

TEM image (Figure 2) confirms the porous three-dimensional structure of CA framework, build from interconnected nanoparticles, with an average diameter of about tens nm. The nanoparticles delimit mesopores with an average diameter of about few nm. The obtained results are in a good correlation with those obtained from BET analysis.

Electrochemical behavior of the MB-CA modified GC electrodes

A comparison between the cyclic voltammograms (CVs) recorded at MB-CA modified electrodes with similar measurements performed at bare GCE, show that MB-CA/GCE present one voltammetric peak pair, which is due to the oxido-reduction process involving the mediator redox couple (inset Figure 3).

The background current observed on the recorded CVs was higher for MB-CA modified electrode than that evidenced for the corresponding unmodified electrodes (GCE) (inset Figure 3). A plausible explanation of this fact should take into consideration that CA has high specific surface area, conferring a high electrochemical active surface to the modified electrodes [2].

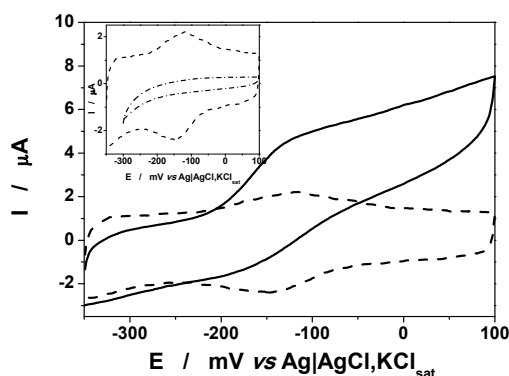


Figure 3. Electrocatalytic oxidation of NADH at MB-CA/GCE in the presence of 5 mM NADH and in its absence. Inset: Cyclic voltammogram recorded at MB-CA/GCE (---) and GCE (----). Experimental conditions: scan rate, 20 mV/s; supporting electrolyte, 0.1 M Tris buffer (pH 7); starting potential, -350 mV vs. Ag|AgCl, KCl_{sat}.

By continuous voltammetric cycling, within the potential window of practical interest, revealed that the peak current and the peak potential corresponding to the MB-CA redox wave are independent of the time evolution, proving a good short-term stability of MB-CA/GCE modified electrode. Thus, by using the relation: $((I_{pa})_{100th\ cycles}/(I_{pa})_{1st\ cycle}) * 100\%$, it was found a current increase up to ~105 % (results not shown). These results

show an increase of the electrode activity due to its activation induced by the continuous potential cycling.

The peak separation value, $\Delta E_p = 40$ mV ($\Delta E_p = E_{pa} - E_{pc}$, where E_{pa} and E_{pc} are the cathodic and anodic peak potentials), the width at half maximum current intensity ($W_{1/2 \text{ anodic}} = 110$ mV and $W_{1/2 \text{ cathodic}} = 100$ mV), and the ratio between the intensity of the cathodic and anodic peaks ($I_{pa}/I_{pc} = 1.1$) point out a quasi-reversible redox process [2, 6, 7].

The values of the formal standard potential ($E^{\circ'}$) for MB-CA modified GCEs (evaluated as the average of the potentials for the oxidation and the reduction peaks) are in the optimal potential range for electroanalytical applications, and ensure an efficient production of enzyme active NAD^+ [9]. The value of $E^{\circ'}$ obtained for MB adsorbed onto CA presents a positive potential shift (-140 mV vs. $\text{Ag|AgCl, KCl}_{\text{sat}}$), compared to the $E^{\circ'}$ value observed for MB adsorbed directly onto graphite (-175 mV vs. SCE) [3].

Compared with the literature value of $E^{\circ'}$ obtained for MB adsorbed onto α -ZP [6, 7], and onto α -TP [6, 8] (-100 mV vs. $\text{Ag|AgCl, NaCl}_{3\text{M}}$, Tris buffer, pH 7), the $E^{\circ'}$ for MB-CA redox couple, presents a negative potential shift, which increases in comparison with the $E^{\circ'}$ for MB adsorbed onto amorphous ZP [2, 6] (+ 40 mV vs. $\text{Ag|AgCl, NaCl}_{3\text{M}}$, Tris buffer, pH 7). This behaviour can be assigned to the negative charge density of the compared support materials, moreover, the increase of negative charge density corresponds to the increase of $E^{\circ'}$ values [7].

$E^{\circ'}$ value, for MB-CA/GC electrodes, depends on the pH of the surrounding electrolyte, in the same manner as that reported for MB dissolved in aqueous solution [12], MB adsorbed on spectrographic graphite [3], on α -ZP [6,7] or on α -TP [6, 8]. In good agreement with previously published results [3], the $E^{\circ'}$ vs. pH dependence shows two linear regions: one with the slope of ~ 60 mV/pH (equivalent to a $2 e^- + 2 H^+$ redox process), and the second having the slope of ~ 30 mV/pH (equivalent to a $2 e^- + 1 H^+$ redox process) (Figure 4).

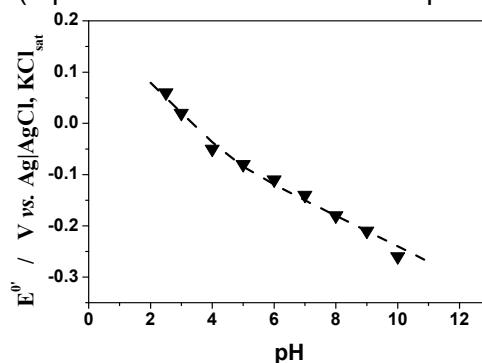


Figure 4. pH influence on the $E^{\circ'}$ value of MB-CA/GC electrode. Experimental conditions: see Figure 3.

This behavior indicates that, irrespective of the support material, the pK_a value for the adsorbed MB is around 4 [3]. 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 value for MB-CA/GCE is 4.7 ± 0.3 ($\chi^2 = 0.0001$, $R^2 = 0.9928$, $N = 9$), and is in good agreement with those reported for MB adsorbed on spectrographic graphite ($pK_a = 5$) [3].

The influence of a wide range of potential scan rates ($0.001 - 1 \text{ V s}^{-1}$) at pH 7, on the electrochemical behavior of the immobilized redox mediator MB on the mesoporous CA was studied by recording the cyclic voltammograms. The dependence of the peak currents (I_{pa}) on the scan rate (ν) is linear, with the slopes of $\log(I_{pa})$ vs. $\log(\nu)$ dependencies of 0.9019 ± 0.0238 ($R^2 = 0.9945$, $N = 19$), close to the theoretical value (*i.e.*, 1), confirming the immobilization of the redox mediator MB on the mesoporous CA (Figure 5).

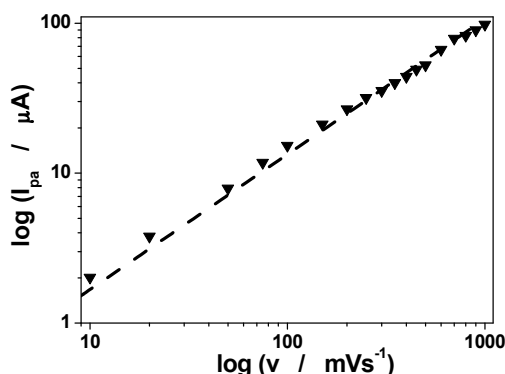


Figure 5. Influence of the potential scan rate on the anodic peak current recorded at MB-CA/GC electrode. Experimental conditions: supporting electrolyte, 0.1 M Tris buffer (pH 7).

Electrocatalytic NADH oxidation at MB-CA modified GC electrodes

MB-CA/GCE showed clear electrocatalytic activity for NADH oxidation. Thus, the cyclic voltammograms recorded at these electrodes in the presence of NADH (Figure 3, solid lines) present all the features of an electrocatalytic process: the oxidation peak is significantly increased, simultaneously with the disappearance of the reduction peak [2, 6 - 8].

The efficiency (E_{ff}) of the electrocatalytic oxidation of NADH at MB-CA / GCE was calculated according to the following relation:

$E_{ff} = (I_{cat} - I_0)/I_0$, 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. The calculated electrocatalytic efficiency was 2.2, when the electrode was tested at 20 mV/s.

In order to establish the optimum surface coverage ensuring the highest electrocatalytic efficiency, the dependence of the catalytic current intensity on the surface coverage was investigated (Figure 6).

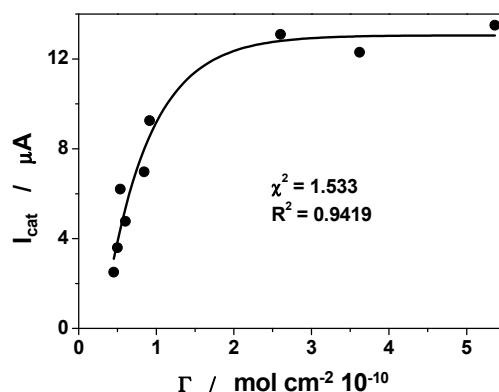


Figure 6. Dependence of NADH electrooxidation current on surface coverage of MB-CA/GCEs. Experimental conditions: scan rate, 2 mV/s; electrode rotation speed, 300 rpm; supporting electrolyte, 0.1 M Tris buffer (pH 7).

Therefore, the surface coverage (Γ) was estimated by using the equation: $\Gamma = Q/nFA$, 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.

Above of a critical value ($1.85 \times 10^{-10} \text{ mol/cm}^2$), the catalytic response becomes practically independent on the surface coverage. Below this value the catalytic current depends almost linearly on the surface coverage. This suggests that besides the reaction rate and the mass transfer of NADH, additional kinetic restrictions may prevail. As consequence, all RDE experiments were performed in the range of surface coverage, where the catalytic current intensity is linearly dependent on the MB surface concentration [2, 7, 8].

RDE measurements allow the estimation of the apparent second order rate constants (k_{obs}) corresponding to different NADH concentrations, by using the Koutecky-Levich approach [2, 3, 8]. Table 1 summarizes the calculated parameters for MB-CA/GCE. Comparing the kinetic parameters values (*i.e.* K_M , k_{obs} , k_{+2}) obtained for MB-CA/GCE, with those obtained for MB adsorbed onto ZP [2], α -ZP [6, 7], or α -TP [6, 8] and incorporated in carbon paste electrodes, it can be concluded that by using a very low amount of MB-CA immobilized onto GCE surface comparable values were obtained.

Table 1. Comparison of the kinetic and electrochemical parameters for NADH electrooxidation at different modified electrodes.

Electrode	^b Γ mol·cm ⁻²	^a K_M mM	^a k_{obs} [NADH]=0 M ⁻¹ ·s ⁻¹	^a k_{+2} s ⁻¹	^a no. of electrons transferred	Ref. No.
MB-ZP-CPE	6.2·10 ⁻¹⁰	2	8.5·10 ²	1.7	1.94	[2]
MB-ZP-SG-CPE	1.3·10 ⁻⁹	2.5	2.9·10 ²	0.73	1.96	[2]
MB-ZP-SK-CPE	5.6·10 ⁻¹¹	2.5	7.2·10 ³	18	1.6	[2]
MB- α -ZP-SK-CPE	3.2·10 ⁻¹¹	1.2	4.5·10 ³	5.6	-	[7]
MB- α -TP-SK-CPE	8·10 ⁻¹²	1.7	3.8·10 ³	6.5	2.1	[8]
MB-CA/GCE	1.8·10 ⁻¹⁰	6.3	1.27·10 ³	8.06	1.3	This work

^a determined from Koutecky-Levich treatment for RDE measurements.

^b determined from CV measurements.

CONCLUSIONS

A simple and effective method has been successfully used for the preparation of the modified MB-CA/GCEs. The electrochemical parameter of the modified electrode ($\Delta E_p = 40$ mV) points out the presence of the immobilized MB species on the electrode surface. The value of $E^{o'}_{MB-CA}$ is very close to the optimal potential range for the amperometric detection. The pH dependence of $E^{o'}_{MB-CA}$ was fitted by a nonlinear regression, allowing to estimate the pK_a of immobilized MB. The obtained kinetic parameters of the investigated electrode recommend it as a stable, sensitive and reproducible electrode for NADH electrooxidation.

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EXPERIMENTAL SECTION

CA synthesis. CA was prepared using a mixture of resorcinol (98% purity, Aldrich), formaldehyde (37% solution, Aldrich), Na₂CO₃ (99.9% purity, Aldrich), and deionized water, according to a previously reported sol-gel method [14]. Thus, resorcinol (0.29 moles) was dissolved in bidistilled water (R/W = 0.2 g/ml). Formaldehyde solution was added to resorcinol solution (R/F = 0.5 molar ratio) under vigorous stirring. Na₂CO₃ 0.1 M aqueous solution was added drop by drop to the previous mixture (R/C = 500 molar ratio). Resulted solution was placed into tightly closed glass moulds and cured 1 day at

room temperature and 4 days at 70°C. After 15 days of aging at room temperature, the obtained wet gel was washed with acetone. Then, the wet gel was dried with CO₂ in supercritical condition (90 – 100 atm and 32°C) when the organic aerogel was obtained. This sample was pyrolysed in Ar atmosphere for 2 h at 850°C, when carbon aerogel was obtained.

Morpho-structural characterization of CA. Transmission electron microscopy (TEM) and nitrogen adsorption-desorption analysis were used for morpho-structural characterization of mesoporous CA.

TEM investigation of CA was performed with a Hitachi H-7000 microscope operating at 125 keV.

Nitrogen adsorption-desorption analysis were performed with Sorptomatic ADP (Thermo Electron Corp.) equipment after degassing around 100 mg of CA for 20 h at 106°C in a vacuum (<1 mPa). From the carried out nitrogen adsorption analysis it was determined the specific surface areas by using the BET (Brunauer-Emmet-Teller) method and the pore size distribution and pore specific volume by the BJH (Barret-Joyner-Halenda) method.

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

Preparation of MB-CA/GCEs. The home-made glassy carbon disc 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 and rotating disc electrode measurements. The glassy carbon disc 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 (Sigma) 0.1 M. Then a suspension of 1 g/L MB-CA in chitosan solution was prepared. A volume of 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 cm².

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 and rotating disc electrode

measurements were 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. All reagents were used as received without further purification.

For each prepared modified glassy carbon, the surface coverage (Γ , mol cm⁻²) was estimated through integration of the area of the wave registered by cyclic voltammetry.

NADH electrocatalytic oxidation study. The electrocatalytic oxidation of NADH using MB-CA/GCEs were investigated through addition of freshly prepared NADH (Sigma) solution of adequate concentration, by cyclic voltammetry and rotating disc electrode techniques.

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