Study of Electrochemically Modified Electrode with Synthesized N-benzyl-4,4′-bipyridine with Anti-Fouling Properties for Oxygen and Hydrogen Peroxide Detection

Ana Chira[1,2], Bogdan Bucur[1,*], Maria-Cristina Radulescu[1], Toma Galaon[3], Gabriel-Lucian Radu[1,2]

[1] Centre of Bioanalysis, National Institute of Research and Development for Biological Sciences, 296, Splaiul Independentei, Bucharest 060031, Romania;
[2] Faculty of Applied Chemistry and Materials Science, Politehnica University of Bucharest, 1-7, Polizu, Bucharest 011061, Romania;
[3] Faculty of Chemistry, Department of Analytical Chemistry, University of Bucharest, Sos. Panduri No. 90, Sect. 5, Bucharest 050663, Romania
[*]E-mail: bucurica@yahoo.com

1-[(4-nitrophenyl)methyl]-4,4'-bipyridinium (N-PMB) was synthesized by refluxing 4,4′-bipyridine and 4-nitrobenzylchloride. A stable film of 1-phenylmethyl-4,4′-bipyridine (PMB) was successfully electrografted on glassy carbon electrodes by the reduction of the diazonium moieties generated in situ. High antifouling ability was obtained for the PMB modified electrode which makes it a useful sensor device for environmental waters monitoring. The modified electrode was used as an amperometric sensor for mediated hydrogen peroxide detection at -0.5 V. The calibration curve was linear from 5 to 60 μM H_2O_2 (R^2=0.9978, *n*=7) with a detection limit of 0.25 μM. PMB modified electrodes was able to detect oxygen in river water with good sensitivity, selectivity and reproducibility.

Keywords: phenylmethyl-4,4′-bipyridinium modified electrode, hydrogen peroxide detection, oxygen detection, antifouling ability

1. INTRODUCTION

The chemical modification of electrodes allows to significantly improve their overall analytical performances [1]. The electrode modification protocols are based on: adsorption, covalent bonding, electrostatic interaction [2], bulk inclusion, precipitation, electropolymerization [3], etc. The chemically covalent modification either by chemical [4] or electrochemical reduction [5] of diazonium salts to carbon surfaces has been used for the development of novel interfaces with applications in electrocatalysis [6], in pH measurement [7], as a mediator for metallic ions determination [8], in

electrode kinetic investigations [9], and as a adhesion promotion in carbon fiber composites [11]. The simplicity of the modification procedures [11], long time stability of covalent attachment to the surface [12], and the wide substrate compatibility [11] are important advantages of the diazonium electrodes modification methods. Some of the electrode surfaces modified with aryl diazonium salts are: gold [13], platinum [14], ITO [15], glassy carbon [8], or carbon nanotubes [16]. Derivatives of 4,4'-dipyridine are used for the development of herbicides, electrochromism, molecular electronics, or supramolecular chemistry [17]. Various bipyridine derivatives have been used for development of modified electrodes for stripping of silver ions [8], for reductive detection of peroxide [18] or oxygen [19], and for detection of ascorbic acid [20], primary amines [21], alpha-fetoprotein [22] or dopamine in combination with ferrocyanide [23].

This study presents a method for the modification of glassy carbon electrodes (GCE) with a synthesized derivative of 4,4′-bipyridine that is electrodeposited using diazonium chemistry. The procedure for modifying the GCE surface is based on a newly synthesized compound: 1-[(4-nitrophenyl)methyl]-4,4'-bipyridinium (N-PMB) obtained from 4,4′-bipyridine and 4-nitrobenzylchloride (Fig. 1a). To the best of our knowledge, this is the first report of the 1-[(4-nitrophenyl)methyl]-4,4'-bipyridinium (N-PMB) use for electrochemical modification of electrodes with bipyridine based on diazonium chemistry. The electrochemical grafting of phenylmethyl-4,4′-bipyridinium (PMB) on the electrode surface has been achieved in only 11 min by two successive electrochemical steps carried out in the same solution: (1) reduction of nitro moieties of PMB to form amino groups and (2) electrografting of aryl diazonium salts that are produced in situ using nitrous acid (Fig. 1b). This proposed method is the improvement of a previous electrode modification protocol developed and characterized by our group for the determination of Ag^+ that required four different steps each one with its own solution [8]. Another method developed by Holm *et al.*, for the binding of 4,4′- bipyridine on the surface of GCE is based on the grafting of 4-(chloromethyl)phenyl on the electrode followed by a slow nucleophilic substitution reaction involving 1-ethyl- or 1-benzyl-4-(4′-pyridyl)pyridinium, but it requires 5 days for completion [24]. Our proposed method based on diazonium chemistry is versatile and has the advantage that it can be applied to various electrode materials unlike the thiolic containing dipyridine derivatives that form SAMs only on gold surfaces [25]. Our modified electrodes have good antifouling properties and were used for the detection of oxygen and hydrogen peroxide.

2. EXPERIMENTAL SECTION

2.1. Reagents

1-[(4-nitrophenyl)methyl]-4,4'-bipyridinium (N-PMB) was synthesized by refluxing 4,4′-bipyridine (from Acros Organics) and 4-nitrobenzylchloride (NBC) (from Aldrich). All other reagents: hydrogen peroxide 30%, acetonitrile (ACN, for HPLC), potassium ferrocyanide, potassium ferricyanide, potassium phosphate monobasic, sodium phosphate dibasic, potassium chloride, and sodium acetate were produced by Sigma. Aqueous solutions were prepared with purified water (18 MΩ cm^{-1}, Millipore). Phosphate buffer saline PBS (0.1 M, pH 7.0, supplemented with 0.1 M

KCl) was used for electrode characterization by cyclic voltammetry and electrochemical impedance spectroscopy. Solutions were deoxygenated with ultrapure argon (Siad, Romania). Environmental water was collected from Dambovita River (Bucharest downtown) during an algal bloom event.

2.2. Apparatus

All electrochemical measurements (cyclic voltammetry, electrochemical impedance spectroscopy, chronoamperometry, and chronopotentiometry) were carried out using a PGSTAT302N potentiostat/galvanostat (Metrohm-Autolab) equipped with a conventional three-electrode cell and controlled using Nova 1.8 software. The working electrode was a glassy carbon (GCE) with 3 mm diameter from Metrohm-Autolab, reference electrode was Ag/AgCl//3M KCl (Metrohm-Autolab) and platinum foil was used as an auxiliary electrode. The filter of the ECD module set to 1 s was used for the reduction of the electromagnetic noise produced by magnetic stirring during amperometric measurements. An Autolab pX1000 module was used for pH measurements. The HPLC analysis was carried out with an Agilent 1100 series LC (Agilent) consisting of: vacuum degasser (G1379A), binary pump (G1312A), autosampler (G1313A), column thermostated compartment (G1316A), and variable wavelength detector (G1314A). Data acquisition and analysis were performed using Agilent Chemstation software, revision B.03.02. Dissolved oxygen (DO) was measured using a commercial DOMeter CyberScan PCD 6500 (Eutech Instruments). Static contact angles measurements were performed with water using a CAM 101 goniometer (KSV Instruments).

2.3. Synthesis of 1-[(4-nitrophenyl)methyl]-4,4'-bipyridinium (N-PMB)

N-PMB was synthesized by refluxing a solution containing 0.0702 g of 4,4′-bipyridine and 0.0193 g of 4-nitrobenzylchloride NBC (4:1 molar ratio) in 45 mL of acetonitrile and water (2:1) for 22 h (Fig. 1a). The refluxed solution was stored at +4 ^{o}C for maximum 2 months. The reaction was investigated by HPLC using a Zorbax Eclipse XDB-Phenyl (150 x 4.6 mm, 5 µm particle size) column (Agilent Technologies) thermostated at 25 °C. The mobile phase consisted of aqueous solvent (0.1% H_3PO_4 and 10 mM heptane-1-sulfonic acid sodium salt) and ACN in the ratio 75:25 and was pumped at a constant flow rate of 1 mL/min. A 3%/min gradient elution program was applied from 25% to 55% ACN in 10 min followed by an isocratic plateau of 5 min to separate the reaction mixture. Column re-equilibration with initial composition of the mobile phase was done in an additional 5 min. UV detector wavelength was set at 254 nm. The chromatogram recorded for refluxed solution presents 3 major peaks: excess 4,4′-bipyridine (2.4 min), unreacted NBC (11.3 min) identified by injecting standard solutions of the two reagents, and the third peak attributed to N-PMB (5.2 min) (Supplementary Material Fig. S-1). The decrease of both 4,4′-bipyridine and p-nitrobenzyl chloride concentrations was in equimolar ratio suggesting the synthesis of a monosubstituted bipyridine compound. Refluxed solutions containing only one reagent were also analyzed and no modification was observed. In MS spectrum an intense line corresponding to the molecular ion m/z = 292 is

observed (Supplementary Material Fig. S-2). This confirms the formation of the reaction product between 4,4′-bipyridine and 4-nitrobenzylchloride.

2.4. Phenylmethyl-4,4'-bipyridine (PMB) layer formation on glassy carbon electrode (GCE)

Prior to modification, the GCE surface was cleaned by polishing with alumina (0.3 µm, Metrohm-Autolab), thoroughly rinsed with Milli-Q water and dried with argon. The GCE modification was carried out in 25 mL aqueous solution freshly prepared containing 2 mL of the refluxed solution, 5 mM sodium nitrite and 40 mM hydrochloric acid under ice. Due to the instability of nitrous acid, this solution may be used for modification of up to 4 GCE. The carbon surface was grafted with PMB in two steps: (1) transformation of N-PMB into 1-[(4-aminophenyl)methyl]-4,4'-bipyridinium (A-PMB) by electrochemical reduction of nitro groups to amino moieties using chronoamperometry at -0.6 V for 600 s and (2) electrographting of diazonium
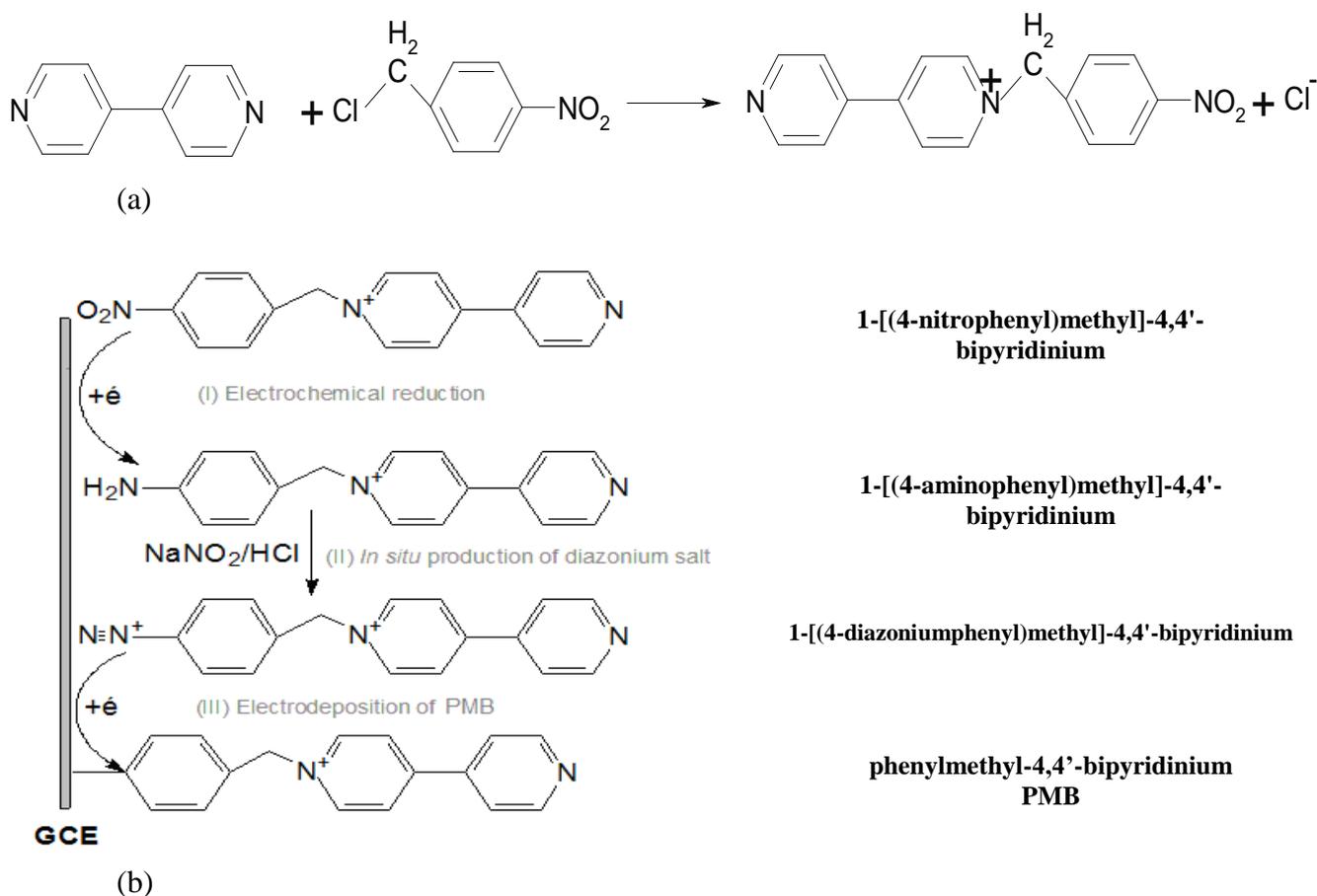

(a)

(b)

Figure 1. (a) Structure of synthesized compound N-PMB**; (b)** Schematic representation of the PMB electrodeposition on the GCE surface. salt formed in situ by chronopotentiometry at -0.25 µA for 600 s. The unstable diazonium groups were generated in situ by reaction of PMB with freshly prepared nitrous acid. Finally, the electrode was washed with ACN and water and dried with argon stream. The fabrication process of the modified electrode is presented in Fig. 1b. For comparison, a similar electrodeposition protocol was employed to obtain benzylchloride modified GCE using a solution containing NBC instead of N-PMB.

2.5. Surface characterization

The modified electrodes were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Five cyclic voltamogranms were recorded using as redox probe a solution of 1 mM potassium ferricyanide in PBS at 0.1 V/s scan rate in the domain of 0.6 to –0.2 V (with 0.6 V stating potential) or in a PBS deoxygenated solution in the domain 0.1 to -0.8 V (0.1 V stating potential). The EIS measurements were made in PBS containing 1 mM ferri/ferrocyanide in equimolar ratio at open circuit potential. Impedance spectra were recorded at 40 frequencies logarithmically distributed between the frequency range 9.5 kHz and 0.1 Hz. The results were represented as Nyquist plots and interpolated using Randles equivalent circuit. The experimental data of EIS were fitted with the classic Randles equivalent circuit where R_s is the electrolyte resistance, R_{ct} is the charge transfer resistance at the electrode interface, Q is the constant phase element related to the double layer capacitance, W is the Warburg impedance used to simulate the mass-transport effects in solution bulk. The charge transfer resistance (R_{ct}) was used for the surface characterization.

2.6. Detection of H_2O_2

Detection of H_2O_2 was evaluated by electrocatalytic reduction using PMB-GCE in deoxygenated PBS. As a first step, the response of the modified electrode to the H_2O_2 was investigated by CV at 0.1 V/s scan rate in the domain 0.1 to -0.8 V; 0.1 V stating potential. The PMB-GCE was used for the detection of peroxide by amperometry in stirred argon saturated PBS solution at -0.5 V. The current was measured as the difference between the baseline and the plateau after signal stabilization.

2.7. Detection of O_2

The electrochemical oxygen reduction reaction was investigated in PBS or in river water at room temperature by CV carried out within 0.1 to -0.8 V potential ranges at 0.1 V/s scan rate. The decrease of the oxygen concentration was studied by purging the solution with argon for up to 10 min.

3. RESULTS AND DISCUSSIONS

3.1. Characterization of PMB-GCE

The electrodeposition protocol of PMB on GCE is based on the electrochemical reduction of the nitro functions of N-PMB to amino groups that are further transformed into diazonium. The 4-nitrobenzyl chloride reagent, used for the synthesis of N-PMB, can also be electrodeposited on the GCE surface by the same protocol. The electrodeposited layers on the GCE surface were characterized by CV. A comparison of the CV recorded in deoxygenated PBS for GCE modified with PMB, benzyl chloride or polished GCE is presented in Fig. 2 and demonstrates the presence of bipyridine groups on GCE surface. It can be observed that PMB- GCE has a reduction peak of the electrodeposited organic

layer at -0.5 V and an oxidation peak is observed at +0.05 V (*vs.* Ag/AgCl), while the benzylchloride modified electrode has only a capacitive current as opposed to polished GCE. The presence of the peaks in cyclic voltammogram was attributed to redox properties of dipyridine derivative immobilized on the GCE surface.
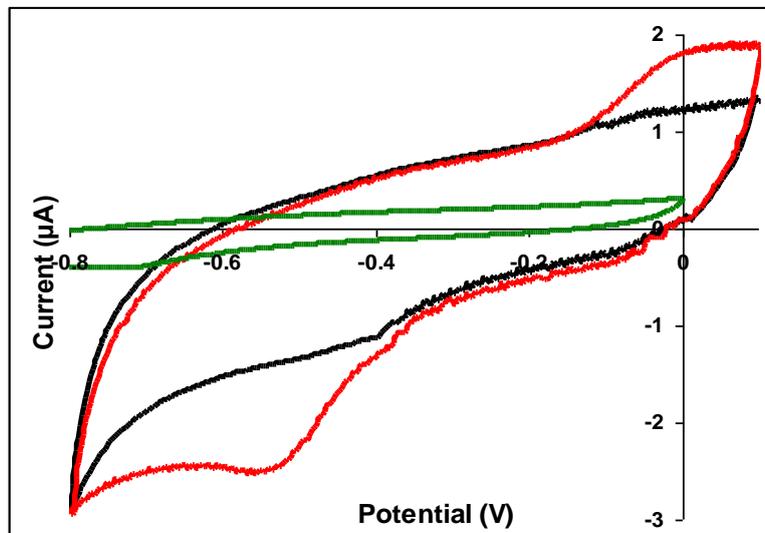

Figure 2. The CV recorded for a PMB-GCE (red), benzylchloride-GCE (black) and polished GCE (green) in deoxygenated PBS pH 7.0.

The influence of the pH on the CV peak of PMB- GCE was studied between 2 and 10. The cathodic peak potential shifts to the cathodic direction with a slope - 46 mV/pH (*E*pc (V) = - 0.0457×pH - 0.2658, (R^2=0.9738, *n*=5). This behavior indicates the involvement of one proton exchange on the modifed electrode surface [26]. Unlike the self-assembled monolayers (SAMs) formed on gold surfaces, the electrodeposition of diazonium compounds leads to a thick multilayer on the electrode [27]. The effect of potential scan rate on the peak currents was also investigated. Both anodic and cathodic peak currents are proportional to scan rate up to 250 mV s^{-1} this being characteristic for an adsorbed substance. The relationship between the peak currents and the scan rate are expressed by the equations: I_{pa} (μA)=2.1815×*v*+0.082 (R=0.996, n=6) and I_{pc} (μA)=-4.503×*v*-0.382 (R^2=0.9906, *n*=6). The modified electrode has a quasi-reversible behavior. It is interesting to note that the electrodes modified with monosubstituted and bisubstituted 4,4′-bipyridine derivatives have different electrochemical characteristics. The monosubstituted derivative presents only a single peak while the bisubstituted derivatives have two peaks; the peak potentials are also different [24].

The total surface coverage was determined by CV recorded for PMB-GCE in deoxygenated PBS. It was used the area under anodic peak presented in figure 2 in order to avoid the eventual overestimation due to the contaminant oxygen reduction. The value for the surface coverage of electroactive species in the modified layer (*Γ*), given in mol cm^{-2}, was obtained from the integrated charges (*Q*) of the anodic peaks (0.1 V/s), from the Eq. 1:

$$\Gamma = \frac{Q}{n_e FA} = \frac{I_{pa} 4RT}{n^2 F^2 vA} \quad (1)$$

where F is Faraday's constant, n_e the number of electrons transferred per molecule of redox active species, and A is the geometric electrode area, I_{pa} is the anodic peak current, R is the ideal gas constant, v is the potential scan rate and T is the temperature. For a modified electrode obtained under optimum conditions (600 s electrodeposition time) it was obtained a total surface coverage of 1.1×10^{-10} mol/cm^2. Similar values were reported by Holm *et al.* using the 5 days modification protocol [24].

A passivation of the electrode was observed for deposition times longer than 600 s. We attribute this passivation to the formation of multilayers. The passivation of the modified GCE surface was investigated by EIS and amperometry (Supplementary Material Fig. S-3). The increase of the R_{ct} is correlated with the surface coverage of GCE with a more passivating organic layer. An optimum electrodeposition of organic layer on the GCE aims to achieve a high sensitivity of the analytical measurements and it is a compromise between a minimum surface passivation (thin layer) and high local concentration of electrochemical mediator (thick layer). In figure S-3 are presented the EIS recorded for PMB-GCE modified using the optimum protocol in comparison with a passivated PMB-GCE obtained by electrodeposition for 900 s and a PMB-GCE covered using a shorter electrodeposition time of 300 s. We have observed that the optimum surface coverage was obtained for PMB-GCE that has a R_{ct} value between 3 and 4 kΩ. The sensitivity of PMB-GCE for hydrogen peroxide detected from amperometric measurements is optimum for electrodes obtained with an electrodeposition time of 600 s.

The hydrophilic properties of the PMB-GCE were assessed by contact angle measurements. Contact angle goniometry demonstrated an important modification of surface properties induced by electrode modification. The contact angle (θ) obtained for clean GCE indicate a hydrophobic surface (74±4.6º), while contact angles measured for PMB-GCE are characteristic for a hydrophilic surface. Thus, the contact angles for PMB-GCE were: 46±3.7º for electrodes obtained under optimum conditions and 49±4.7º for electrodes covered by passivation layers obtained by electrodeposition during 900 s. The optimum and passivized PMB-GCE have similar contact angles proving that both electrodes are covered by organic layers with similar interfacial properties (and different thickness as indicated by EIS measurements). For comparison, the contact angle for benzylchloride modified GCE is 58±2.1º, a value that demonstrate the deposition of an organic layer with different surface properties.

The spontaneous adsorption by non-covalent bonding interactions on the surface of polished GCE of the synthesized N-PMB and 4,4′-bipyridine or NBC reagents was studied at open circuit potential by immersing the GCE in standard solutions for 30 min, 2 h, and 20 h. The GCE were washed and characterized by EIS and CV. Both 4,4′-bipyridine and NBC reagents did not adsorb on the GCE surface. For N-PMB were observed only minimum changes for 30 min and 2 h immersion time. Thus, the values of charge transfer resistance (R_{ct}) obtained by fitting the EIS data increases from 96 Ω (for an initial state of bare GCE) to 206 Ω (for 30 min) and 260 Ω (for 2 h immersion time in synthesized N-PMB), respectively. For comparison the R_{ct} increase obtained for PMB-GCE using the optimum procedure is 3.5 kΩ. For 20 h, the results suggest a high adsorption of N-PMB on the surface of the GCE; the R_{ct} is about 2.3 kΩ (Supplementary Material Fig. S-4), but the electrodeposition takes place in only 11 min.

3.2. Stability of the electrodeposited PMB film on GCE

The stability of the PMB-GCE was tested by CV. Two cycles at a scan rate of 0.1 V/s were carried out in PBS at three potential ranges: 0.6 V to -0.6 V, 0.4 to -0.8 V and 1.0 V to -1.0 V. The electrodes were characterized by CV and EIS and compared with the initial modified electrode (Supplementary Material Fig. S-5A and S-5B). The PMB-GCE was stable after potential scan 0.6 V to -0.6 V and 0.4 to -0.8 V. Only a partial removal of the electrodeposited layer was observed after 2 cycles in the wider potential domain between 1.0 and -1.0 V. For comparison, a benzylchloride layer was partially removed after CV was carried out in the ranges from 0.6V to -0.6V and 0.4 to -0.8 V. The benzylchloride layer was almost completely removed by CV which was carried out between 1.0 and -1.0 V (Supplementary Material Fig. S-5C and S-5D). Our results suggest that the synthesized N-PMB allows the electrodeposition of a PMB layer on the GCE surface with a high stability that is significantly better in comparison with a benzylchloride layer obtained using only the NBC reagent. Both electrodes modified with a PMB or benzylchloride layer have the same covalent link between the GCE and the organic layer obtained using diazonium chemistry. We attribute this observed difference to a supplementary stabilization by lateral interactions between 4,4' dipyridine (similar with the higher stability of the SAM obtain with long alkyl chain in comparison with the stability shorter thiolic chain compunds). The developed PMB-GCE are stable in a wider potential range in comparison with the stability domain reported in the literature for other amperometric peroxide sensors: -0.1 to-0.7 V for stainless steel electrode [28], 0.4 to -0.6 V for carboxylated graphene modified graphite electrode [29] or for Rh–Prussian blue modified carbon paste electrode [30].

3.3. Antifouling ability

One major difficulty in the analysis of complex biological, food, or environmental samples with complex matrix using electrochemical sensors is the surface fouling that limits the applicability range, leads to signal drift, and decrease the sensor usage time and sensitivity. The antifouling ability is influenced by the surface properties of electrode surface and the physicochemical properties of the fouling substances. There are many compounds which can be present in real samples and can cause electrode surface fouling during measurement. The antifouling ability of the modified PMB-GCE in comparison with bare GCE was tested by immersion for 20 min in standard solutions of 1 mg/mL BSA, phenol, casein, riboflavin, pectin, sodium dodecyl sulfate, linoleic acid and pepsin. EIS measurements using ferri/ferrocyanide redox probe are sensitive enough to detect modifications on the electrode surface produced by both chemical reactions with small molecules and proteins at different overall electric charges obtained by change of pH [14]. The fouling of the electrode surface was evaluated by EIS as the difference of R_{ct} between initial state and after immersion in each solution. We have observed that all tested substances induce a significant increase in the ΔR_{ct} measured for bare GCE that is much higher in comparison with variation obtained for PMB-GCE. Thus the ΔR_{ct} increase for bare GCE is 14.7 times higher for linoleic acid in comparison with PMB-GCE and the ΔR_{ct} for BSA or casein is ~11 times higher for bare GCE. For pectin it was obtained a complete passivation for

bare GCE while for PMB-GCE it was measured a ΔR_{ct} of only 1.2 kΩ. The EIS measurements confirmed the significantly antifouling capacity of the PMB-GCE in comparison with bare GCE, indicating their possibility to be used in complex real samples (Fig. 3).
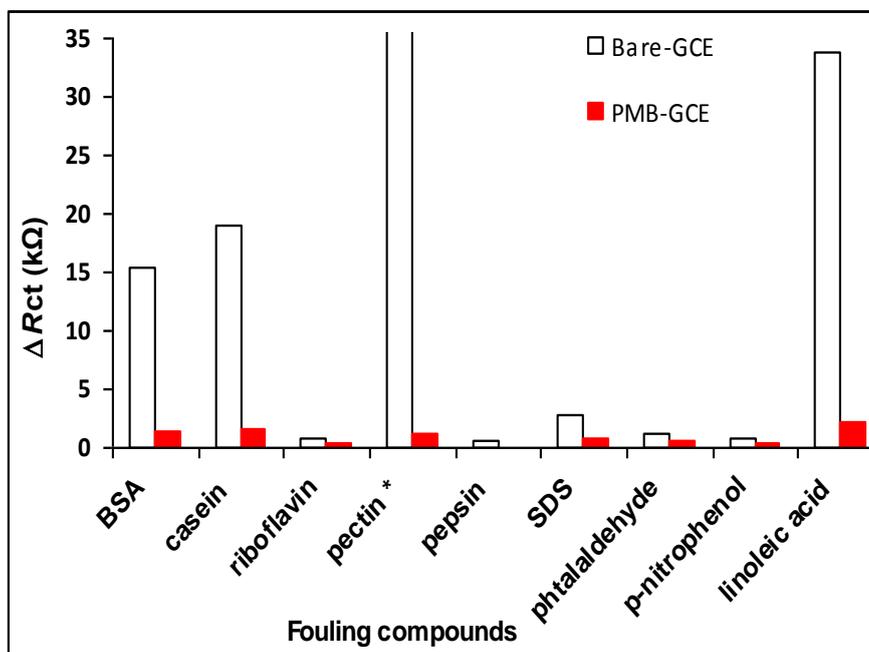

Figure 3. Variation of the R_{ct} (ΔR_{ct}) for bare GCE (white columns) and PMB-GCE (red columns) obtained for different compounds.
* ΔR_{ct} (R_{ct}initial - R_{ct}final) calculated for bare GCE after immersion in 1 mg/mL pectin solution is very high and cannot be represented.

The antifouling effect of the PMB layer on GCE was also tested using river water collected during an algal bloom event. The PMB-GCE was immersed in river water with algae under light. After 1 and 24 h, the characteristics of the electrode were controlled by EIS and compared with the initial state (Fig. 4). The results obtained with the PMB-GCE indicate that electrode characteristics remain almost unchanged during 1 or 24 h. The Nyquist diagrams for PMB-GCE do not show an important change after electrode immersion, the R_{ct} increases from 3.24 kΩ (initial) to 3.61 kΩ for 1 h, respectively 4.17 kΩ after 24 h. A significant non-specific adsorption was observed for bare GCE used for comparison in similar conditions. The Nyquist plot obtained for a bare GC electrode showed a linear shape. The semicircle shape with a large diameter was observed for bare GC electrode which indicates a large increase of the R_{ct}. After immersion the modified electrode in river water the R_{ct} increased from ~0.1 kΩ (initial) to 2.99 kΩ for 1h and 25.1 kΩ after 24 h immersion in water river sample. In consequence, the obtained PMB-GCE presents good antifouling ability that it is useful for long term *in situ* environmental water monitoring or for management of urban wastewater treatment plants. Deposition of neutral hydrophilic layer of polyethylene glycol is a popular route to obtain surfaces with antifouling properties, but other alternatives are also available: e. g. hydrophobic boron doped diamond is substantially better in comparison with GCE due to the absence of oxygen functional

groups and other surface sites that are responsible for the adsorption of surface-active agents [31]. The sparsely covering of the surface with polymeric chains protruding into the solution gives rise to steric repulsion and prevents further approach of the protein [32]. We attribute the remarkable antifouling abilities of our PMB-GCE to a combination of effects including: the reduction of the surface hydrophobic character, the covering of the functional groups from the GCE surface, the formation of organic chains protruding into solution that are deformed by proteins and gives rise to steric repulsion.

Figure 4. The characterization by CV and EIS of a bare-GCE [A, B] and PMB-GCE [C, D] after their immersion in river water during 1 h (red) and 24 h (yellow). For comparison it is presented the initial state (black). Nyquist plots: points are the experimental data and the lines are the curves obtained by fitting using the Randles equivalent circuit.

3.4. O_2 detection

The O_2 measuring in aqueous solutions is carried out using different methods like Clark electrode or optical detection systems [33]. We have investigated the electrocatalytic reduction of

oxygen using PMB modified and bare GCE by CV in potential range 0.1 to -0.8 V with a scan rate of 0.1 V/s. A large reduction peak was recorded by CV for O_2 using PMB-GCE in PBS or river water collected during algal bloom. Lower O_2 concentrations were obtained by Ar purging. The analytical signals recorded for PMB-GCE were significantly higher in comparison with a bare GCE and in consequence the PMB-GCE is able to detect lower O_2 concentrations. Thus, the analytical signals obtained in PBS for 1.8 mg/L DO using bare GCE were at the limit of detection while PMB-GCE still recorded ~20% of the current obtained before Ar purging. At 0.1 mg/L DO, the obtained analytical signals using PMB-GCE were at the limit of detection while no significant signal was obtained using a bare GCE. For longer purging times the signal recorded using PMB-GCE was below the limit of detection. The measurements made using river water lead to similar results with the difference of the higher initial signal due to oxygen production by algae exposed to light (Supplementary Material Fig. S-6).

3.5. Performance of the PMB-GCE for H_2O_2 detection

The reduction of H_2O_2 was initially studied by CV using a PMB-GCE and compared with a bare GCE. The CV recorded at scan rate of 100 mV s^{-1} in the presence of 0.1 mM H_2O_2 in deoxygenated PBS is irreversible. The optimum response for H_2O_2 detection was studied in electrolyte solutions at different pH values (2.0 to 10.0) and the best analytical signal was obtained in deoxygenated PBS at pH 7.0. The magnitude of current recorded for the reduction of 0.1 mM H_2O_2 at a PMB-GCE is significantly higher in comparison with a bare GCE (Fig. 5). Thus, at a potential of −0.5 V, it was recorded a signal of -14.8 µA for the PMB-GCE in comparison with - 5.1 µA for bare GCE.
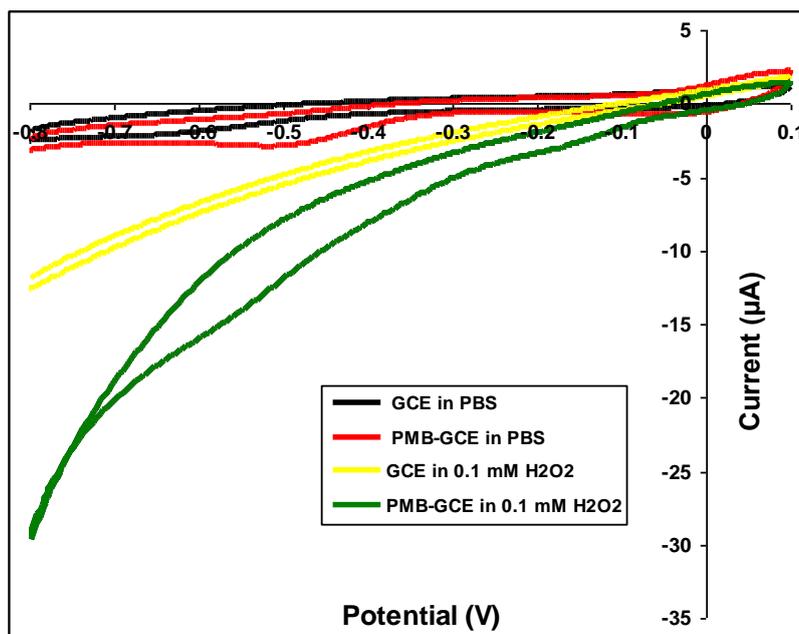

Figure 5. The CV recorded for a PMB-GCE in deoxygenated PBS pH 7.0: in the absence (red) and in the presence of 0.1 mM H_2O_2 (green). For comparison it is presented a bare GCE in the absence (black) and in the presence of 0.1 mM H_2O_2 (yellow).

The analytical performances of modified electrodes for the reduction of H_2O_2 were investigated by amperometry. Amperometric measurements were carried out in stirred deoxygenated PBS at −0.5 V. The current–time responses and the calibration graphs using both PMB modified and bare GCE for successive addition of H_2O_2 from 5 µM to 60 µM are presented in Fig. 6. It can be seen that the current was proportional to the H_2O_2 concentration. For the modified electrode a sharp increase of current was observed after each addition of H_2O_2 that is substantially larger in comparison with the analytical signals recorded for bare or benzylchloride modified GCE. The proposed sensor exhibited good amperometric sensitivity of 2.68 µA/µM compared with only 0.18 µA/µM for bare GCE and 0.17 µA/µM for benzylchloride modified GCE. The relative standard deviations (RSD) for 6 repeated measurements with the same modified electrode for 20 µM H_2O_2 and 40 µM H_2O_2 were 1.12% and 1.25%, respectively. These results indicate that the proposed sensor has an excellent reproducibility.

The detection limit was estimated to be 0.25 µM H_2O_2 (defined as 3 times the standard deviation of the noise). This limit of detection obtained with the proposed PMB-GCE for H_2O_2 detection is better than those obtained with other modified non-enzymatic electrodes.
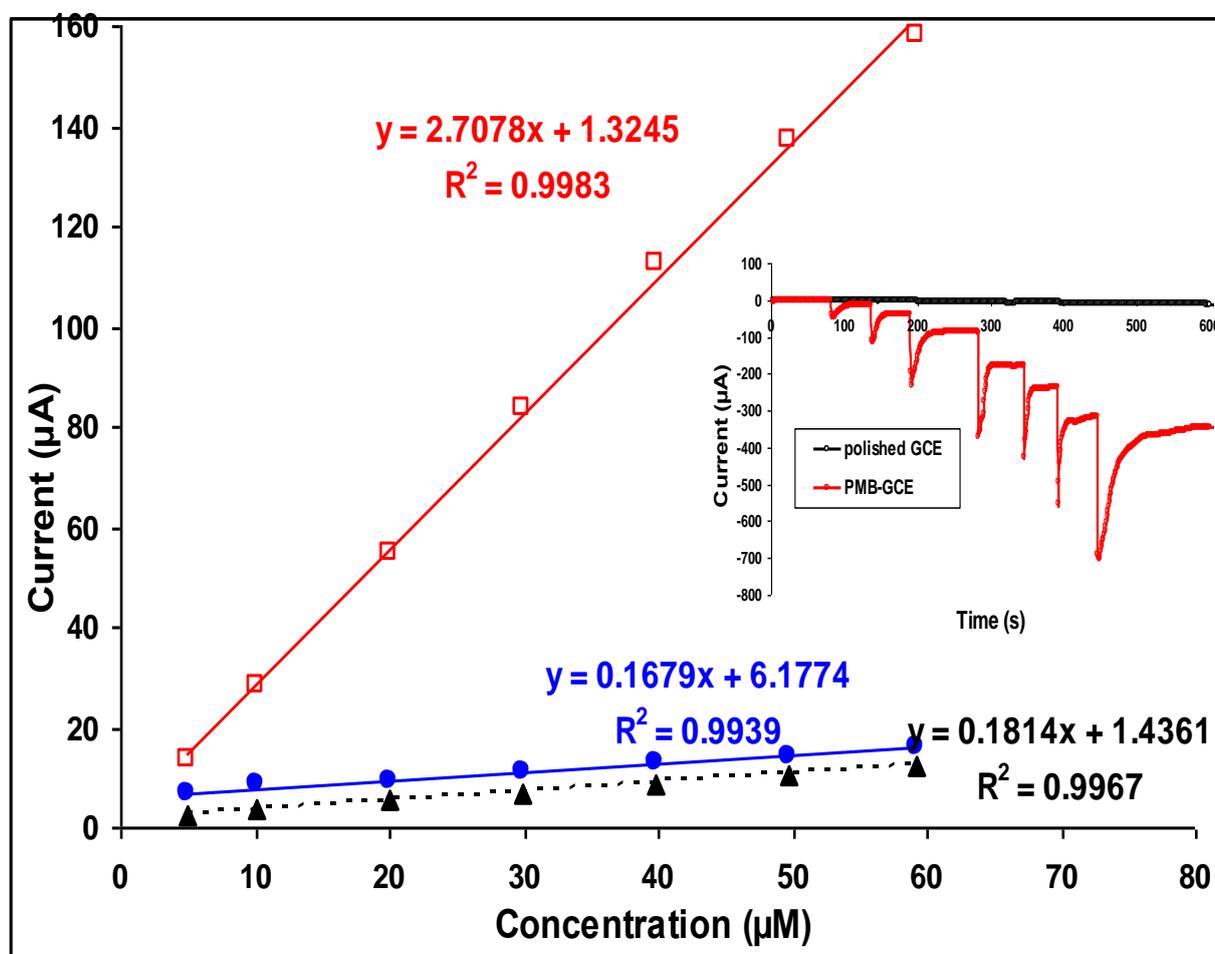

Figure 6. The calibration for H_2O_2 detection using PMB modified GCE (red). For comparison are presented the results obtained using a benzylchloride modified GCE (blue) and bare GCE (black). Insert: The recorded analytical signals.

There are reported numerous electrochemical methods for H_2O_2 analysis wit different limits of detection: i.e. 5 μM for a sand-blasted stainless steel electrode, applied potential -0.5 V [28], 1 μM for carboxylated graphene modified graphite electrode at 3.26 at -0.18 V [29], 28 μM for a Rh – Prussian Blue carbon paste modified electrode at -0.04 V [30] or 0.5 μM for a GCE modified with organic–inorganic hybrid material at -0.4 V [18].

3.6. Interferences study

Several possible interfering compounds potentially coexisting with H_2O_2 such as ascorbic acid, citric acid, glucose, uric acid, dopamine, catechol, and sodium iodide were investigated. The interference experiments were performed in 0.1 M PBS (pH 7.0) by amperometry at −0.5 V. We compared the analytical signal obtained for 10 μM H_2O_2 with the response recorded for H_2O_2 in the presence of the same concentration of each interfering substance. The obtained results indicated that the interferences were weak under the optimum conditions (Supplementary Material Fig. S-7). Therefore, the PMB-GCE is selective and, coupled with its antifouling abilities, can be used in complex samples.

4. CONCLUSIONS

GCE was electrochemically modified using a newly synthesized 1-[(4-nitrophenyl) methyl]-4,4'-bipyridinium (N-PMB) based on diazonium chemistry. The electrodes were readily prepared by two electrochemical steps carried out in the same solution. The proposed phenylmethyl-4,4'-bipyridinium (PMB) modified GCE had excellent electrocatalytic activity for H_2O_2 or O_2 detection due to the electrocatalytic properties. The calibration graph showed a linear dependence from 5 μM to 60 μM H_2O_2, with a detection limit of 0.25 μM. We demonstrate high antifouling ability of the PMB modified GCE which makes it a useful sensor device for environmental waters monitoring.

References

1. R. C. Alkire, D. M. Kolb, J. Lipkowski and P. N. Ross *Chemically Modified Electrodes*, in Gooding JJ, Lai LMH, Goon IY (ed) Nanostructured Electrodes with Unique Properties for Biological and Other Applications, John Wiley & Sons, New York (2009)
2. W. Kutner, J. Wang, M. L′Her and R. P. Buck, *Pure. App. Chem.,* 70 (1998) 1301-1318
3. A. Vasilescu, T. Noguer, S. Andreescu, C. Calas-Blanchard, C. Bala and J. L. Marty, *Talanta,* 59 (2003) 751-765
4. M. Pandurangappa, T. Ramakrishnappa, *J. Solid State Electrochem.,* 12 (2008) 1411–1419

5. A. A. İsbir-Turan, E. Kılıç, Z. Üstündağ, H. Ekşi, A. O. Solak and B. Zorer, *J. Solid State Electrochem.*, 16 (2012) 235–245

6. M. Kullapere, G. Jurmann, T. T. Tenno, J. J. Paprotny, F. Mirkhalafand and K. Tammeveski, *J. Electroanal. Chem.*, 599 (2007) 183–193

7. X. H. Yang, S. B. Hall, A. K. Burrell and D. L. Officer, *Chem. Commun.,* 21 (2001) 2628-2629

8. M. C. Radulescu, A. Chira, M. Radulescu, B. Bucur, M. P. Bucur and G. L. Radu, *Sensors*, 10 (2010) 11340-11351

9. J. C. Harper, R. Polsky, D. R. Wheeler and S. M. Brozik, *Langmuir*, 24 (2008) 2206-2211

10. M. Delamar, G. Desarmot, O. Fagebaume, R. Hitmi, J. Pinson and J. M. Saveant, *Carbon*, 35 (1997) 801-807

11. D. J. Garrett, P. Jenkins, M. I. J. Polson, D. Leech and K. H. R. Baronian, *Electrochim. Acta*, 56 (2011) 2213-2220

12. P. Allongue, M. Delamar, B. Desbat, O. Fagebaume, R. Hitmi, J. Pinson and J. M. Saveant, *J. Am. Chem. Soc.*, 119 (1997) 201-207

13. M. Khoshroo, A. A. Rostami, *J. Electroanal. Chem.,* 647 (2010) 117-122

14. O. I. Covaci, B. Bucur, M. P. Bucur and G.L. Radu, *Microchim. Acta*, 169 (2010) 335-343

15. S. Maldonado, T. J. Smith, B. D. Williams, S. Morin, E. Barton and K. J. Stevenson, *Langmuir* 22 (2006) 2884-2891

16. P. Abiman, G G. Wildgoose and R G. Compton, *Int. J. Electrochem. Sci.*, 3 (2008) 104 - 117

17. P. M. S. Monk, *The viologens: Physicochemical Proprietes, Synthesis and Applications of the salts of 4, 4'-bipyridine*, John Wiley & Sons, New York (1998)

18. M. Ammam, E. B. Easton, *Sens. Actuators B*, 161 (2012) 520–527

19. C. D. Clark, J. D. Debad, E. H. Yonemoto, T. E. Mallouk and A. J. Bard *J. Am. Chem. Soc.*, 119 (1997) 10525-10531

20. E. K. Janghel, V. K. Gupta, M. K. Rai and J. K. Rai, *Talanta*, 72 (2007) 1013–1016

21. K. Uchikura, M. Kirisawa and A. Sugii, *Anal. Sci.*, 9 (1993) 121-123

22. J. Hou, N. Gan, F. Hu, L. Zheng, Y. Cao and T. Li, *Int. J. Electrochem. Sci.*, 6 (2011) 2845 - 2858

23. C. Y. Hsu, V. S. Vasantha, P. Y. Chen and K.C. Ho, *Sens. Actuators B*, 137 (2009) 313–319

24. A. H. Holm, R. Moller, K. H. Vase, M. Dong, K. Norrman, F. Besenbacher, S. U. Pedersen and K. Daasbjerg, *New J. Chem.*, 29 (2005) 659-666

25. P. Miller, G. Ramakrishna, E. Sinn, M. Joyce, M. Rebros, E. Rebrosova, G. Mezei and M. Z. Atashbar, *Sens. Actuators B*, 176 (2013) 768-774

26. J. Schreurs, P. Veugelers, A. Wonders and E. Barendrecht, *Recl. Trav. Chim. Pays-Bas*, 103 (1984) 263-269

27. M. M. Chehimi, *Aryl Diazonium salts: New Coupling Agents in Polymer and Surface Science*, John Wiley & Sons, New York (2012)

28. S. Patra, N. Munichandraiah, *J. Chem. Sci.*, 121 (2009) 675–683

29. S. Prasannakumar, R. Manjunatha, C. Nethravathi, G. S. Suresh, M. Rajamathib and T. V. Venkateshad, *Port. Electroch. Acta*, 30 (2012) 371-383

30. V. M. Ivama, S. H. P. Serrano, *J. Braz. Chem. Soc.*, 14 (2003) 551-555

31. D. Shin, A. D. Tryk, A. Fujishima, A. Merkoçi and J. Wang, *Electroanalysis*, 17 (2005) 305–311

32. A. M. Woodward, E. A. Davies, S. Denyer, C. Olliff and D. B. Kell, *Bioelectrochemistry*, 51 (2000) 13-20

33. R. Ramamoorthy, P. K. Dutta and S. A. Akbar, *J. Mater. Sci.*, 38 (2003) 4271 – 4282
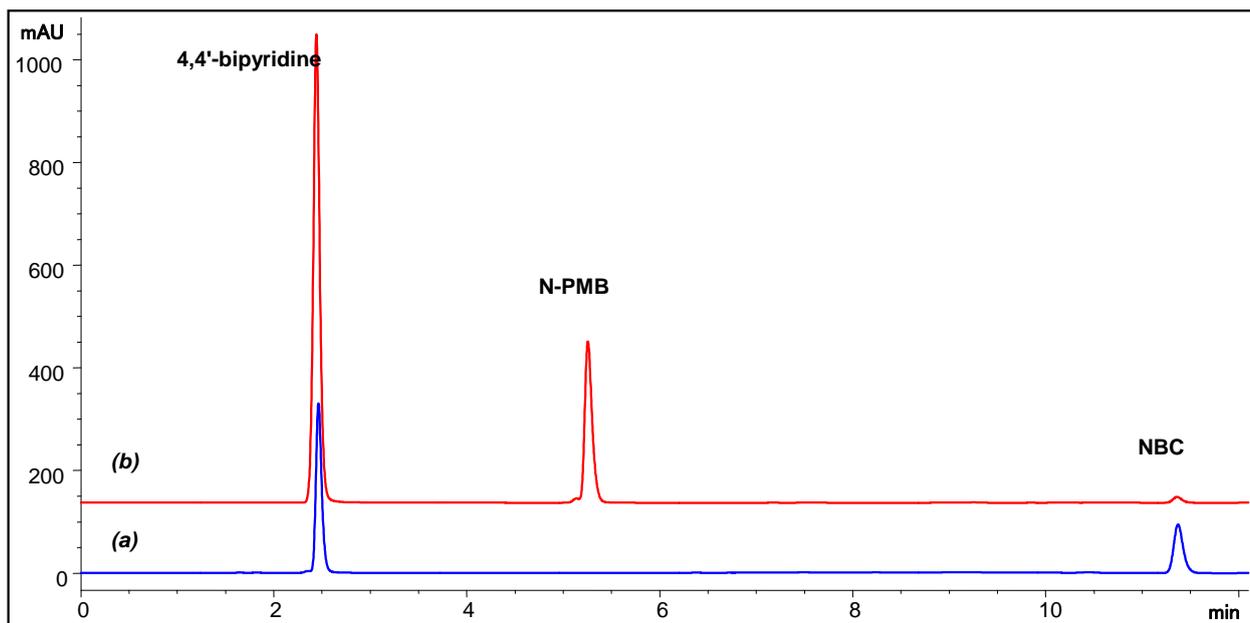
Electronic Supplementary Material:

Figure S-1. Overlaid chromatograms recorded for the injection of: (a) standard solution of 4,4'-dipyridine (460 µg/mL) and NBC (480 µg/mL) and (b) product mixture resulted after reaction synthesis that contains the reaction product (N-PMB).
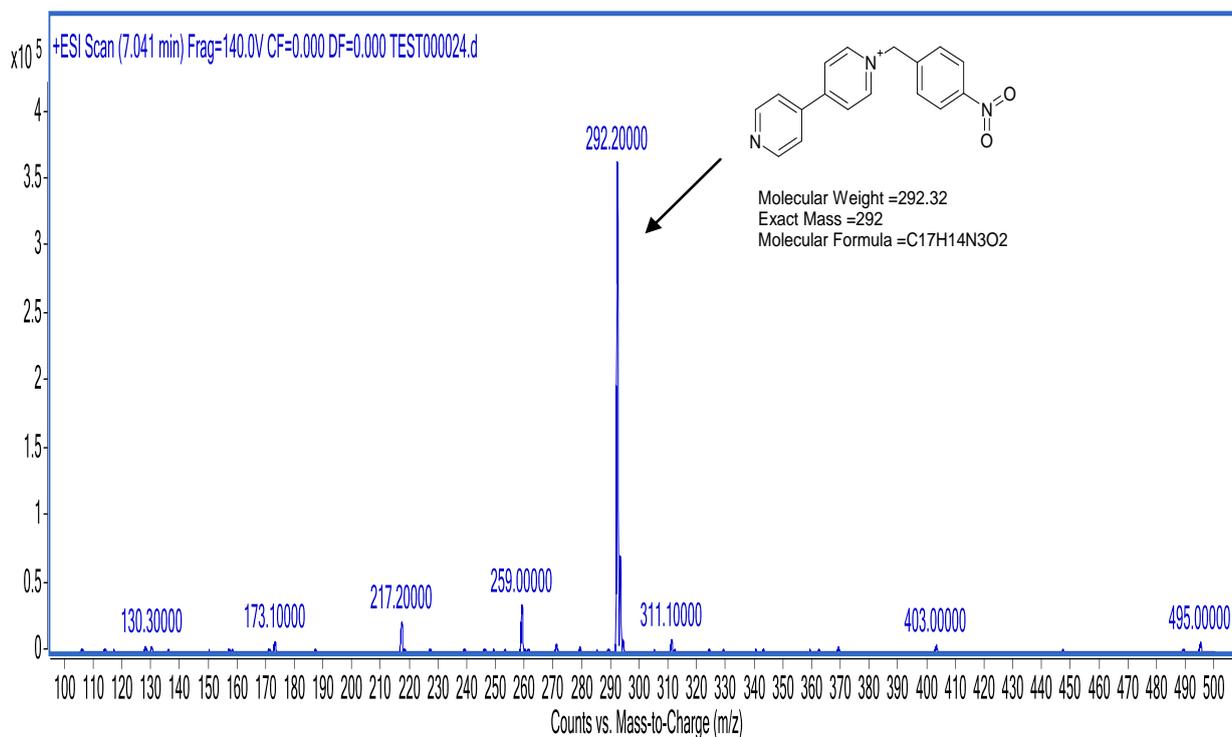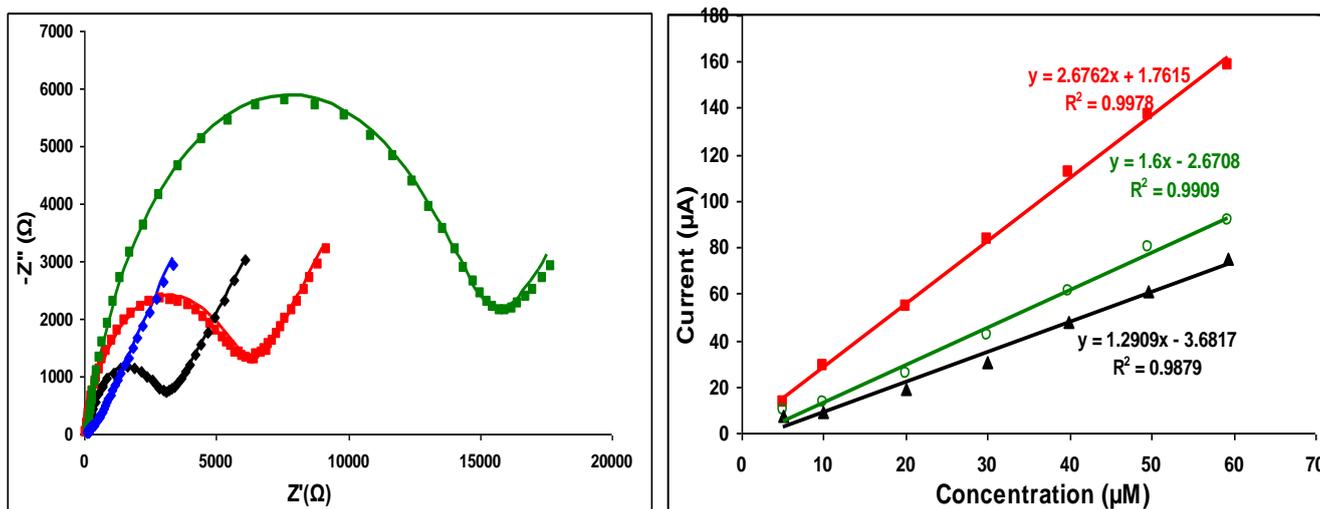

Figure S-2. MS spectrum recorded with a spectrometer Triple Quad LC/MS 6410B Agilent Technologies, under the following conditions: drying gas (N_2); vaporizer temperature: 350°C; drying gas flow: 9 L/min; pressure of the nebulizer gas: 40 psi; capillary voltage: 3000 V; high voltage end plate offset: 140 V; mass scanning 105 – 500 Da.

Figures S-3. EIS and calibration curves for PMB-GCE modified by electrodeposition at 300 s (black), 600 s (red) and 900 s (green). For comparison is presented EIS recorded for bare GCE (blue).
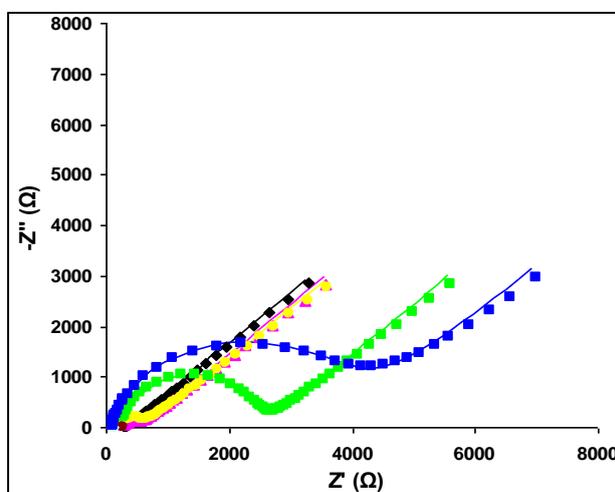

Figure S-4. The non-specific adsorption on a bare GCE studied by EIS after immersion in a N-PMB solution for different times: 0.5 h (pink); 1 h (yellow) and 20 h (green). For comparison are presented the signals recorded for bare GCE (black) and a PMB-GCE obtained using the optimum procedure (blue). Nyquist plots: points are the experimental data and the lines are the curves obtained by fitting using the Randles equivalent circuit.
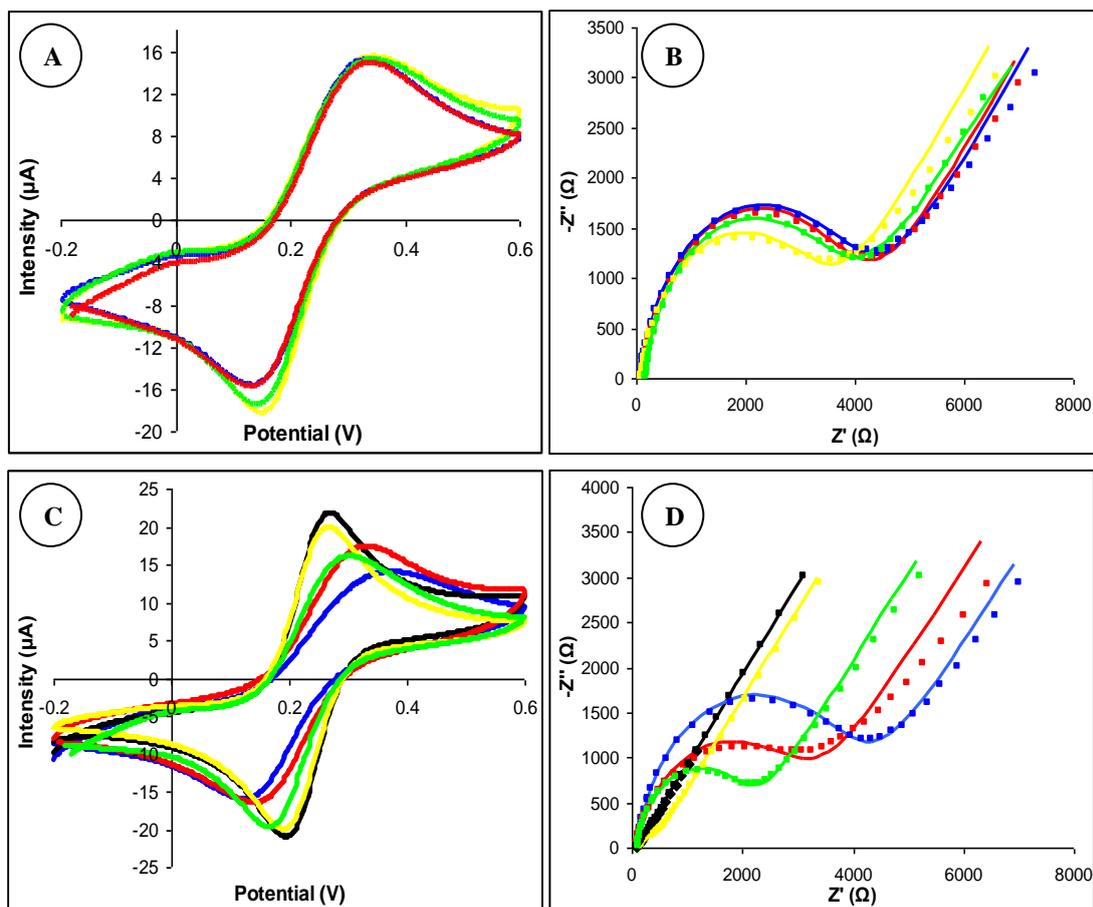
Figure S-5. The stability of PMB-GCE [A, B] and benzylchloride-GCE [C, D] studied by CV and EIS after two cycles in the potential ranges: –0.6 V to 0.6 V (red), -0.8 to 0.4 V (green), –1 V to 1 V (yellow). For comparison are presented the initial signals recorded for a bare GCE (black) and the initial modified electrode (blue). Nyquist plots: points are the experimental data and the lines are the curves obtained by fitting using the Randles equivalent circuit.
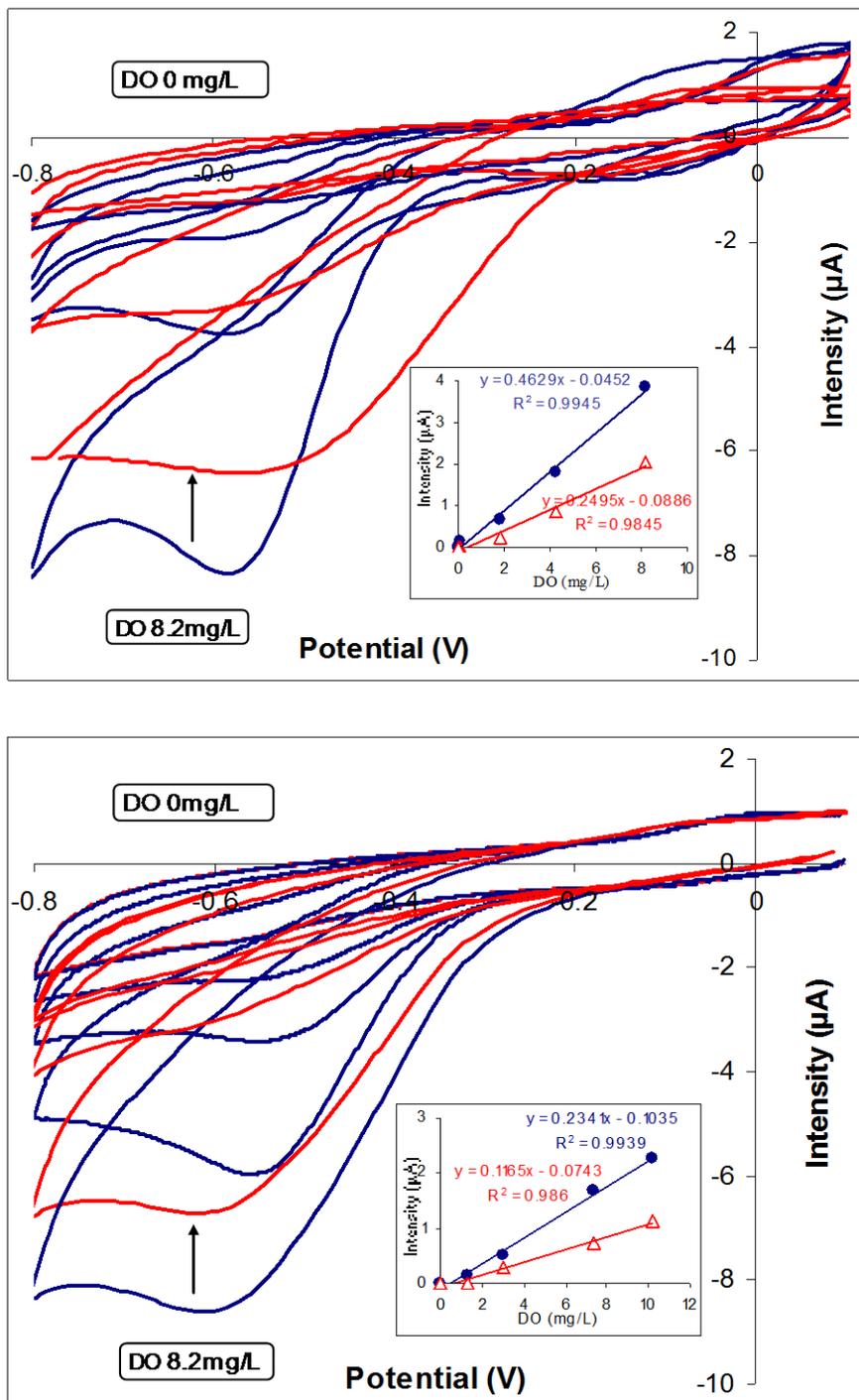
Figure S-6. The O_2 analysis by CV using a PMB-GCE (blue) and bare GCE (red) in river water [A] and PBS [B] for different concentration of DO: 0; 0.1; 1.8; 4.3 and 8.2 mg/L. Inserts: Plots of peak currents vs. oxygen concentration.
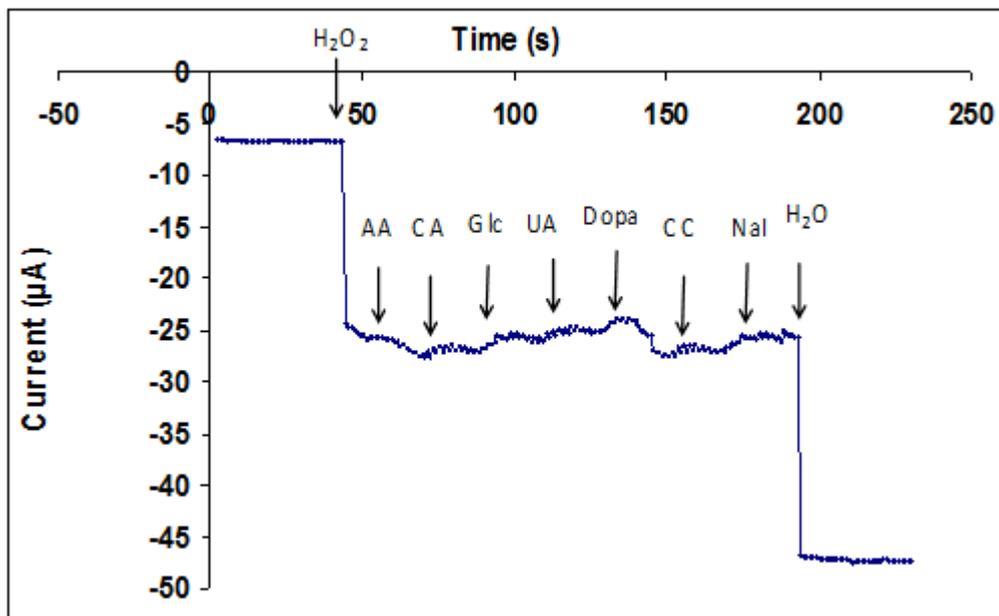
Figure S-7. Amperometric response of a PMB-GCE after successive addition of 10 µM H_2O_2, ascorbic acid (AA), citric acid (CA), glucose (Glc), uric acid (UA), dopamine (Dopa), catechol (CC), sodium iodide (NaI) and H_2O_2 in deoxygenated PBS pH 7.0. Working potential: -0.5 V (vs Ag/AgCl).