STUDIA UBB CHEMIA, LVII, 2, 2012 (p. 59 - 69)

SPECTROELECTROCHEMICAL STUDIES OF CARBON-BASED SCREEN-PRINTED ELECTRODES MODIFIED WITH POROUS GELS OF ZIRCONIUM, CLAYS AND CALIXARENES

CECILIA CRISTEA[a], NICOLAE BONCIOCAT[b],
IULIU O. MARIAN[b, *], ROBERT SĂNDULESCU[a]

ABSTRACT. Electrochemical impedance spectroscopy (EIS) was applied in order to analyze the properties of carbon based screen-printed electrodes modified with clay, Zr porous gel. Parametric equations for Nyquist diagrams applicable in extreme domain of frequencies were used in the case of clay or Zr porous gel modifiers. The equations lead to an analysis of the curves around the frequency of 0.1 Hz. The terms expressing diffusion and charge transfer respectively, were calculated for the proposed electrodes. For comparison Nova 1.6 AUTOLAB soft was used to determining the electrochemical terms involved in Warburg classical circuits.

Keywords: *screen-printed electrodes, electrochemical impedance spectroscopy, clay, zirconium oxide nanoporous gel*

INTRODUCTION

Electrochemical impedance spectroscopy (EIS) is a powerful tool for monitoring the chemical and physical changes of interfacial properties on electrode surface [1]. Nyquist plot of EIS measurements are composed by a semicircle in the high – frequency region associated with resistance and capacitance and a straight line in the low-frequency region associated with the mass transfer [2]. Electrochemical impedance spectroscopy is a sensitive indicator of a wide variety of chemical and physical properties. An increasing trend towards the development of impedimetric biosensors is being currently observed. Impedimetric techniques have been performed to characterize the

---

[a] "Iuliu Hatieganu" University, Faculty of Pharmacy, Analytical Chemistry Dept., 4 Pasteur St. Cluj-Napoca, Romania

[b] Babeş-Bolyai University, Faculty of Chemistry, Physical Chemistry Dept., 11 Arany Janos St. Cluj-Napoca, Romania, * iomar@chem.ubbcluj.ro

fabrication of the biosensors and to monitor the catalyzed reactions of enzymes or the biomolecular recognition events of specific binding proteins, receptors, nucleic acids, whole cells, antibodies or antibody-related substances [3].

Nowadays there is considerable interest in the development of electroanalytical sensors (i.e., potentiometric, amperometric, electrochemical biosensors) for the detection of a wide range of analytes. Consequently, electrochemical impedance spectroscopy plays an important role in the characterization of many types of sensors. EIS has been used to provide information on various fundamental processes (i.e., adsorption/film formation, rate of charge transfer, ion exchange, diffusion, etc.) that occur at the electrode–electrolyte interface. [4].

Electrochemical impedance spectroscopy results were modeled by appropriate equivalent circuits for the aim of elucidating electrical properties of functionalized carbon-based electrodes [5].

To improve the sensibility and selectivity of the electrodes several modifiers could be used.

In this paper will be presented the electrochemical behavior studied with EIS of the clays and zirconia porous gel (ZPG) modifiers dispersed in thin films deposited on the surfaces of carbon-based screen printed electrodes (SPE). As reported in literature, the use of SPEs is a great simplification in the design and operation of analytical determinations, in accordance with the requirements of a decentralized assay [6-8]. Recently, the SPEs have demonstrated a great interest especially in the development of rapid analytical analysis and biosensor’s fabrication.

Among different versions of composite electrodes for pharmaceutical compounds detection (acetaminophen, purine alkaloids etc.) the clay modified electrodes are interesting because they combine the adsorbent properties with ion exchange ability and with the hydration layer. The clays, collected from our geographical area (Valea Chioarului, Romania), were enriched, completely characterized (chemical composition, diffractometry, specific surface, ionic exchange capacity and thermal analysis) and separated by sedimentation, centrifugation and ultracentrifugation in samples with different particle sizes. The surface electrode was modified by adding a thin film of a mixture of polymer polyethyleneimine (PEI) and different amounts of clays. Polyethyleneimine is a linear polymeric cation and kept during several weeks a good permeability. It was used by various authors as entrapment material in the development of biosensors [9] and showed a good rate of electron transfer between the biocomponent and the electrode [10, 11].

The same procedure was used for the fabrication of the screen-printed electrodes modified with PEI and zirconia porous gel (ZPG). The advantage of using zirconium oxide nanoporous gels was exploited in the construction of biosensors due to their biocompatibility [12, 13]. Lately many approaches used zirconia porous gel to immobilize enzyme during polymerization or electrodeposition [14]. According to the literature, zirconium gel or thin film were used to immobilize hemoglobin, DNA, myoglobin and HRP at gold or carbon electrode. Zirconia porous gels were used to entrap the biomolecules due to their biocompatibility. (ZPG) was recently used for the entrapment of hemoglobin and myoglobin and the protein ZrO_2 film preserved their bioactivity and showed a good electrocatalytic behavior towards H_2O_2 reduction. The analytical characteristics of the developed biosensor proved that the nanogel preserved the catalytic activity and a good hydration microenvironment for the enzymes. Due to its lack of toxicity, good conductivity, affinity for groups containing oxygen, the ZrO_2 nanogels became attractive for the construction of biosensors [15]. In order to fully characterize the sensors besides cyclic voltammetry and amperometry, EIS studies were performed.

EIS method was applied in order to analyze the properties of mentioned above modified carbon based screen-printed electrodes. Parametric equations for Nyquist diagrams applicable in extreme domain of frequencies were used. The equations lead to an analysis of the curves around the frequency of 0.1 Hz. The terms expressing diffusion and charge transfer respectively, were calculated for some analyzed modified electrodes.

RESULTS AND DISCUSSIONS

In some previous papers, Bonciocat *et al.* [7, 8] have developed a new approach to the Electrochemical Impedance Spectroscopy (EIS), when only charge transfer and diffusion limitations are present. By using this approach some new results, concerning the parametric equations of Nyquist plots corresponding to redox multielectrodes, have been obtained. Thus in the domain of very small frequencies (around 0.1 Hz) these equations are:

$$\mathrm{Re}(t) = R_{sol} + A + \frac{J_1[\omega(t-\tau)]}{\sqrt{2\pi}} B\omega^{-1/2} \tag{1}$$

$$-\mathrm{Im}(t) = \frac{J_1^2[\omega(t-\tau)] + J_2^2[\omega(t-\tau)]}{2\pi} B^2 C_d + \frac{J_2[\omega(t-\tau)]}{\sqrt{2\pi}} B\omega^{-1/2} \tag{1'}$$

where $Z_{cell} = \mathrm{Re} + j\,\mathrm{Im}$, $\omega = 2\pi\upsilon$ represent the radial frequency of the alternating current, τ the moment of time when the alternating overtension $\tilde{\eta}$ is superimposed over the constant overtension η applied at t = 0, and t is the time when the Nyquist plot recording ends. C_d is the double layer capacity and J_1 and J_2 are the Fresnel integrals.

$$J_1[\omega(t-\tau)] = \int_0^{\omega(t-\tau)} \frac{\cos x}{x^{1/2}}dx \text{ respectively } J_2[\omega(t-\tau)] = \int_0^{\omega(t-\tau)} \frac{\sin x}{x^{1/2}}dx \quad (2)$$

whose values tend to $\sqrt{\pi/2} = 1.253$ for sufficiently great values of the product $\omega(t-\tau)$.

A and B express the charge transfer, respective diffusion limitations of the interface and have the meanings

$$1/A = \sum_i 1/A_i \qquad 1/B = \sum_i 1/B_i \quad (3)$$

where the terms on the right sides of eqs. (3) refer to the individual electrode reactions occurring simultaneously at the interface.

The EIS allows the estimation of the total quantities A and B, characterizing the multielectrode as a whole, but doesn't permit the estimation of the individual quantities A_i, respective B_i.

If the product $\omega(t-\tau)$ is sufficiently great, we may accept the approximation

$$J_1[\omega(t-\tau)] \cong J_2[\omega(t-\tau)] \cong 1.253$$

even for the smallest radial frequency used, say $\omega_1 = 2\pi\upsilon_1 = 0.628 s^{-1}$.

Of course, the above condition may be fulfilled, because the interval of time $t-\tau$ is at our disposal.

Further, the double layer specific capacity is about 20μF/cm^2, if an indifferent electrolyte is present in excess in the solution. Therefore, the double layer capacity is $C_d = 0.2$ S, if the electrode surface S is given in m^2. Consequently, the first term on the right side of eq. (1') may be neglected if:

$$B \langle 1/0.2S\omega^{1/2} \quad (4)$$

For $\omega_1 = 0.628 s^{-1}$ and S=12.56.10^{-6} m^2, we get B < 500·10^3 Ωs$^{-½}$, condition that generally, holds true. In these conditions the system of eqs. (1, and 1') becomes:

$$\mathrm{Re}(t) \cong R_{sol} + A + \frac{1}{2}B\omega^{-1/2} \quad (5) \quad \text{and} \quad -\mathrm{Im}(t) \cong \frac{1}{2}B\omega^{-1/2} \quad (5')$$

The latest term on the right side hand of eq. (5) represent the *Warburg diffusion resistance* of the interface

$$R_W(\omega) = \frac{1}{2}B\omega^{-1/2} \tag{6}$$

Both terms *A* and $R_W(\omega)$ are ohmic terms, which don't introduce a phase difference. As for the term $\frac{1}{2}B\omega^{-1/2}$ of eq. (5'), it represents *the capacitive reactance* of the interface

$$X_{C_W}(\omega) = \frac{1}{2}B\omega^{-1/2} \tag{7}$$

As we see, $R_W(\omega) = X_{C_W}(\omega)$ but, in the complex plane, the Warburg diffusion resistance is represented on the real axis, while $X_{C_W}(\omega)$, being negative, is represented downwards on the imaginary axis. The capacitive reactance is due to *the Warburg pseudo-capacitance* of the interface.

$$X_{C_W}(\omega) = \frac{1}{\omega C_W(\omega)} \tag{8}$$

and consequently:

$$R_W(\omega)C_W(\omega) = 1/\omega \tag{9}$$

Finally, writing eqs. (5 and 5') for $\omega = \omega_1 = 0.628s^{-1}$

$$B = -\mathrm{Im}(\omega_1)\frac{2}{\omega_1^{-1/2}} = 1{,}58.[-\mathrm{Im}(\omega_1)]\,(\ \Omega s^{-1/2}\ ) \tag{10}$$

$$R_{sol} + A = \mathrm{Re}(\omega_1) - [-\mathrm{Im}(\omega_1)] \tag{11}$$

Using a proposed theoretical model based on Volterra equation, charge transfer and diffusion terms were calculated for each modified electrode.

For comparison the electrochemical terms R_{ts}, R_s, C_d, and W of classical Warburg circuit was calculated with Nova 1.6 Autolab software.

Electrodes modified with clays thin films

The electrochemical behavior of the home made SPE modified with thin films of clays was tested in the presence of some pharmaceutical compounds as ascorbic acid, acetylsalicylic acid and acetaminophen. Chemical composition studies as well as diffractometry, IR and X ray studies established the properties of several clays originated from the northern part of our country [16]. After some preliminary electrochemical studies it was decided to work with montmorillonite with under 0.2 μm particle dimensions. Thin films of PEI and clay were deposited on the surface of the working electrode in order to change the electrochemical characteristics of it. The electrochemical behavior of the clay modified electrodes was tested in the presence of potassium ferro/ferricyanide (Figure 1) and the charge transfer and diffusion terms are presented in Table 1.

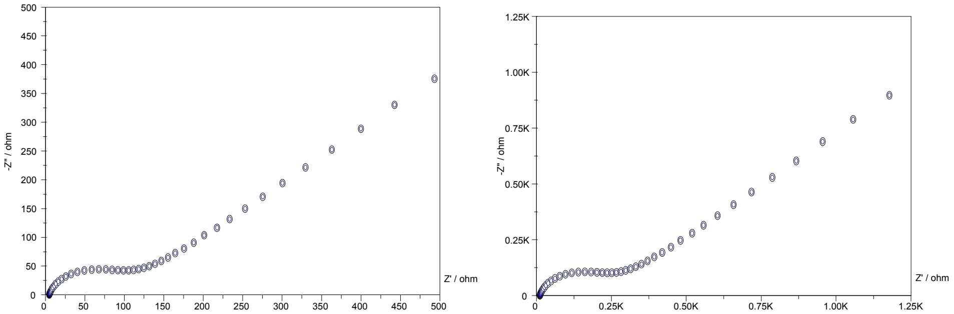

Figure 1. Impedance spectra of $0.5 \cdot 10^{-3}$ M potassium ferro/ferricyanide at unmodified (left) and clay modified SPE (right) in 0.25M KCl

Table 1. Charge transfer and diffusion terms for clay modified electrodes

Type of electrode	**Re(ω_1) (Ω)**	**-Im(ω_1) (Ω)**	**R_{sol} + A (Ω)**	**B (Ω $s^{-1/2}$)**
Unmodified SPE	493	375	118	592.5
Clay modified SPE	1180	897	283	1417.3

The terms R_{sol}+A and B was calculated considering the point situated at 0.1Hz in impedance spectra.

The experimental data was fitted by AUTOLAB soft and the electrical parameters of classical Warburg circuits are presented in Table 2

Table 2. Calculated values with Nova 1.6 Autolab for Warburg circuit

Type of electrode	**R_{ts} (Ω)**	**C_d (μF)**	**R_{sol} (Ω)**	**W (Ω^{-1} $s^{1/2}$)**	**W^{-1} (Ω $s^{-1/2}$)**
Unmodified SPE	80.2	5.91	5.2	$2095 . 10^{-2}$	477.3
Clay modified SPE	191.6	2.473	12.42	$877 . 10^{-3}$	1140.2

In Table 2 the term W^{-1} reflect the contribution of Warburg parameters at imaginary part of classical Warburg circuit. The W^{-1} term can be associated to the B term in accepted mathematical model. Without R_{sol}, R_{ts} and C_d terms contributions the differences between the calculated values in last columns in Table 1 and Table 2 are due probably to the proposed model.

However for unmodified SPE the relative error is 19.4% and for clay modified electrode the relative error is 19.5%.

Electrodes modified with ZPG thin films

The advantage of using zirconium oxide nanoporous gels was exploited in the construction of biosensors due to their biocompatibility [11]. Before developing the biosensors the characteristics of the new modified electrodes with thin films of PEI and ZPG were tested on the presence of a redox probe.

The electrochemical behavior of the ZPG modified electrodes was tested in the presence of potassium ferro/ferricyanide (Figure 2) and the charge transfer and diffusion terms are presented in Table 3.

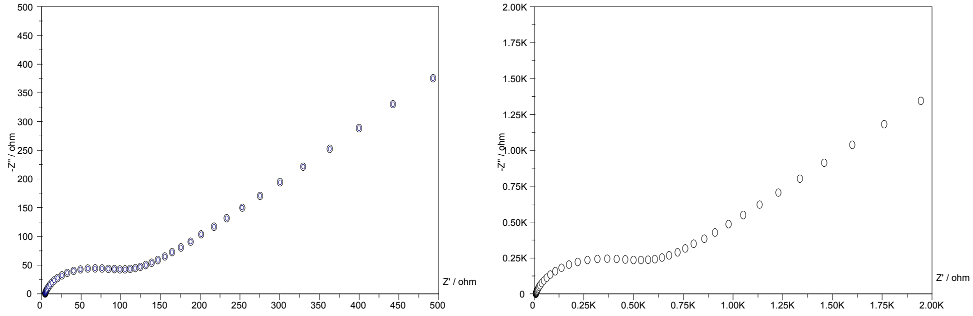

Figure 2. Impedance spectra of $0.5 \cdot 10^{-3}$ M potassium ferro/ferricyanide at unmodified SPE (left) and ZPG modified SPE (right) in 0.25 M KCl

Table 3. Charge transfer and diffusion terms for ZPG modified electrodes

Type of electrode	**Re(ω1) (Ω)**	**-Im(ω1) (Ω)**	**Rsol + A (Ω)**	**B ($\Omega\ s^{-1/2}$)**
unmodified SPE	493	375	118	592.5
ZPG modified SPE	1943	1340	603	2117.2

The terms R_{sol}+A and B was calculated considering the point situated at the 0.1Hz in impedance spectra.

The experimental data was fited by AUTOLAB soft and the electrical parameters of classical Warburg circuits are presented in Table 4

Table 4. Values calculated with Nova 1.6 Autolab soft for Warburg circuit

Type of electrode	**R_{ts} (Ω)**	**C_d (μF)**	**R_{sol} (Ω)**	**W ($\Omega^{-1}\ s^{1/2}$)**	**W^{-1} ($\Omega\ s^{-1/2}$)**
Unmodified SPE	80.2	5.91	5.2	$2905 . 10^{-6}$	477.3
ZPG modified SPE	400	2.634	9.38	$559 . 10^{-6}$	1788.9

Like in the previous experiment by comparing the last columns of Table 3 and Table 4 the calculated relative error in the case of ZPG modified SPE is 15.4%.

On the other hand the modified SPE have another C_d because of thin film modifiers added on the surface of working electrode.

The modeling with parametric equations was in agreement with the results obtained by Nova 1.6 Autolab software, both for clay modified and ZPG modified SPEs.

EXPERIMENTAL

Thin films of polymer (polyethyleneimine) with various additives (clays, zirconia porous gel) were deposited on the surface of the carbon based screen-printed electrodes (SPE).

The graphite based SPEs (composed by graphite working electrode and graphite counter electrode and a silver pseudo-reference electrode obtained by the screen-printed technique on an insulated plate) were purchased from Dropsens, Spain [11,15].

The clays, collected from our geographical area (Valea Chioarului, Romania), were enriched and completely characterized (chemical composition, diffractometry, specific surface, ionic exchange capacity and thermal analysis) and separated by sedimentation, centrifugation and ultracentrifugation of the different particle sizes [17].

The modified electrodes were prepared by mixing 5 mg polyethyleneimine (PEI) in125 μL dry ethanol and 120 μL distillate water with 6,5 μL (50 mg/mL) hydrated clays of 0.2 μm fraction.

A similar procedure was used for the fabrication of the SPE modified electrodes with PEI and ZPG.

The zirconia alcogel was prepared according to the literature [12]. The zirconia alcogel has been prepared, starting from 0.25M zirconium salt ($ZrO(NO_3)_2.xH_2O$) alcoholic solution by refluxing for 2 h at 90ºC then allowed to cool at room temperature. 5mg PEI with 125μl ethanol and 120μl distillated water were mixed for 15 min with vortex. 6.5μl porous gel were added and mixed another 15 min with vortex. Polyethyleneimine (MW 60000) from Aldrich was used without purification.

All the experiments were performed in the presence of electrolyte solutions (0.25M KCl, $0.5\cdot10^{-3}$ M potassium ferro/ferricyanide). The chemicals were provided by Sigma-Aldrich or Merck and they were of analytical grade. The electrochemical analysis was performed with AUTOLAB potentiostat PGSTAT 30 and FRA 2 module (frequency domain 0,1Hz to 100 kHz) by using unmodified and modified graphite screen-printed electrode as electrochemical cell. All impedance spectra were normalized to the surface area of working electrode. The working temperature was room temperature (25ºC).

CONCLUSIONS

Screen-printed electrodes modified with various compounds (clays and zirconia porous gel) included in a conductive polymer and directly deposited on the electrode surface were investigated. Two ways of investigations were proposed for clay or ZPG modified SPEs: parametric equations modeling and Nova 1.6 Autolab software.

By comparing the contribution of Warburg terms calculated in theoretical model proposed with contribution of Warburg terms calculated by Autolab soft for the same classical Warburg circuit reasonable relative errors results.

ACKNOWLEDGEMENTS

The financial supports of PN II IDEI 338/2011 grant program and UMF (young researchers grant) 27020/3/15.11.2011 is appreciated.

REFERENCES

1. S.M. Park, J.S. Yoo, *Anal. Chem.,* **2003**, *75*, 455A-461A;
2. J.Li, L. Xu, H. Huang, J. Zhou, E.S. Abdel-Halimb, J.R. Zhang, J.J. Zhu, *Talanta*, **2011**, *85*, 2113-2120;
3. J.G. Guana, Y.Q. Miao, Q.J. Zhanga**,** *Journal of Bioscience and Bioengineering,* **2004**, *97(4),* 219-226;
4. B. Pejcic, R. De Marco, *Electrochimica Acta*, **2006**, *51(18),* 6217-6229;
5. H. Saklya, R. Mlika, I. Bonnamour, F. Aouni, H. Ben Ouada, N. Jaffrezic Renault, *Electrochimica Acta*, **2007**, *52(11)*, 3697-3703;
6. J. Wang, *Analyst*, **1994**, *119,* 763-766;
7. N. Bonciocat, *Studia UBB Chemia*, **2008**, *LIII*, 31-42;
8. I.O. Marian, N. Bonciocat, C. Cristea, R. Săndulescu, M. Bucșa, M. Vlassa, *Electroanalysis,* **2010**, *22(5),* 542-548;
9. M.D. Rubianes, G.A. Rivas, *Eletrochem. Commun.* **2007**, *9*, 480-484;
10. E. Lojou, P. Bianco*, Electrochim. Acta*, **2007**, *52*, 7307-7314;
11. S. Laschi, E. Bulukin, I. Palchetti, C. Cristea, M. Mascini, *ITBM-RBM*, **2008**, *29*, 202-207;
12. S.Q. Liu, J.J. Xu, H.Y. Chen, *Colloid Surf. B*, **2004**, *36*, 155-159;
13. S.Q. Liu, J.J. Xu, H.Y. Chen, *Bioelectrochemistry,* **2002**, *57*, 149-154;
14. V. Sima, C. Cristea, F. Lăpăduș, I.O. Marian, A. Marian, R. Săndulescu, *J. Pharm. Biomed. Analysis*, **2008**, *48*, 1195-1200;

15. V. Sima, C. Cristea, E. Bodoki, G. Duţu, R. Săndulescu, *Central European Journal of Chemistry*, **2010**, *8(5),* 1034-1040;

16. A. Marian, I.O. Marian, C. Cristea, R. Săndulescu, Gh. Vasilie, *Studia UBB Ambientum*, **2009**, *LIV*, 81-90;

17. A. Maghear, C. Cristea, A. Marian, I.O. Marian, R. Săndulescu, *Clays and clay minerals,* **2012**, submitted.