

New Electrochemical Sensors Based on Clay and Carbon Micro and Nanoparticles for Pharmaceutical and Environmental Analysis

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ABSTRACT

Particle size separation and refinement, followed by physico-chemical characterization studies of two Romanian bentonites were performed. The electrochemical behavior of several pharmaceuticals and heavy metals was tested on clay-modified carbon paste electrodes (CPEs) and glassy carbon electrodes (GCEs) with different clay particle sizes using cyclic voltammetry (CV), square wave voltammetry (SWV) and amperometry. A biosensor based on horseradish peroxidase (HRP) immobilization in a polyetylenimine (PEI) and clay porous gel film for acetaminophen detection was successfully obtained. GCEs modified with clay and PEI or polypyrrole films were also tested for the trace metal analysis using CV and SWV with good results. Polypyrrole films, polystyrene spheres and carboxylated MWCNTs were deposited on graphite electrodes and characterized in order to develop a nitrate reductase biosensor.

Keywords: clay-modified electrodes, polymeric films, biosensors, heavy metals, pharmaceuticals

1 INTRODUCTION

Due to their high content of montmorillonite, two Romanian clays from Răzoare and Valea Chioarului were investigated for the development of new composite electrochemical materials with application in the fabrication of electrochemical sensors.

Due to their adsorbent properties and ion exchange capacity, clays have attracted the interest of electrochemists and their analytical applications have been exploited in the last two decades [1 - 4].

Adsorption of proteins on clay mineral surfaces represents an important application in fields related to the agricultural and environmental sciences, but also in the development of biosensors [5]. Organic molecules, macromolecules and biomolecules can be easily intercalated in solids with a 2D structural arrangement that have an open structure. Therefore, clay minerals based on three smectites (laponite, montmorillonite and nontronite) and on layered double hydroxides are likely to be exploited to improve the analytical characteristics of electrochemical

sensors [6]. Several clay-modified carbon based electrodes were developed in order to elaborate new electrochemical methods applied in pharmaceutical and biomedical analysis. They are also a good alternative to biocomponent immobilization at the electrode surface, offering in the same time a hydrated environment, very important for the activity of the enzyme. A horseradish peroxidase (HRP)/clay/polyetylenimine (PEI) film biosensor for acetaminophen detection and a nitrate reductase/polypyrrole film biosensor for nitrate detection are described.

Based on the remarkable ion exchange capacity of montmorillonite, several clay-polymeric film modified electrodes were tested for heavy metal detection.

2 MATERIALS AND METHODS

For the structural characterization of Răzoare and Valea Chioarului bentonites, it was resorted to their impurities removal by washing and decantation, obtaining a more homogeneous product with montmorillonite as main component. Several fractions, below 20 μm , 8 μm , 5 μm , 2 μm , 1 μm and below 0.2 μm were separated by sedimentation, decantation, centrifugation and ultracentrifugation after the procedures reported in the literature [7, 8].

The chemical composition, the ion exchange capacity, the surface area and the structural characteristics, *e.g.* particle size and shape, of each separated fraction were determined by transmission electron microscopy (TEM), X-ray diffraction and FTIR spectroscopy. Thermal behavior was also studied using differential thermal analysis and their electrochemical behavior was investigated [14].

CPEs were modified by mixing different Răzoare montmorillonite concentrations (1%, 2.5%, 5% and 10%) with "homemade" carbon paste (99-95%) prepared with solid paraffin [9].

Composite film electrodes (PEI/clay/GCE) were prepared by stirring PEI (5 mg) in absolute ethanol (125 μL) and distilled water (120 μL) for 15 minutes, then a nanoporous clay gel (6.5 μL) was added and stirred again for 15 minutes. Two different suspensions (20 μL) containing Valea Chioarului montmorillonite particles with diameter below 20 μm (I) and below 0.2 μm (II) were

deposited on the surface of two different GCEs BAS Inc, West Lafayette, USA) and left to dry for 4 hours at 4°C. HRP was entrapped into a clay and PEI porous gel at the surface of GCE.

A 5% clay suspension (50 μL) in 0.1M pyrrole and 0.1 M LiClO_4 was electropolymerized on GCE by CV (5 scans from -1.0 V to +1.0 V, scan rate 20 mVs^{-1}) vs Ag/AgCl pseudoreference. Another method of increasing electrode surface was achieved by deposition of polystyrene beads with 900 nm diameter on the GCE surface [10, 11]. The spheres were covered with a polypyrrolic film deposited by CV from an acetonitril solution followed by their removal in tetrahydrofuran. The surfaces were characterized by microscopy, AFM and Raman spectra.

Amperometry, cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) were performed in a conventional three-electrode cell: clay-modified carbon based electrodes vs. Ag/AgCl and a Pt auxiliary electrode, under stirring conditions, at room temperature, with AUTOLAB PGSTAT 30 (EcoChemie, Netherlands) equipped with GPES and FRA2 software (100 mVs^{-1}). The pH of the solution was measured using a ChemCadet pH-meter.

All solutions were prepared by using high-purity water obtained from a Millipore Milli-Q water purification system. All reagents were of analytical grade, used as received.

3 RESULTS AND DISCUSSIONS

3.1 Clays' physico-chemical characterization

The chemical composition performed on the separated clay micro and nanoparticles revealed a high content of SiO_2 and Al_2O_3 and also a significant water content. Elements such as Mg^{2+} and Fe^{3+} act as substitutes of Al^{3+} in the octahedral configuration. Alkaline metals and Ca^{2+} can fix by adsorption means in the spaces between the structural packages of the clay [12].

The FTIR spectra of both Răzoare and Valea Chioarului bentonites revealed the characteristic bands of montmorillonite. The broad band at 3447 cm^{-1} was attributed to the stretching vibration of the interlayer adsorbed OH with the specific peak of montmorillonite at 3625 cm^{-1} [12]. The bands at 1000-1200 cm^{-1} and 466 cm^{-1} were assigned to the Si-O stretching vibration and the bands at 793 cm^{-1} and 520 cm^{-1} to the Si-O-Al group [13].

TEM images of the studied clays at higher magnification showed a diffusive, irregular and opalescent surface [12].

The X-ray spectra of the two clays displayed the characteristic diffraction peaks of montmorillonite at 20 (7.12° ; 19.68° ; 21.57° ; 28.14° ; 36.04° ; 61.66°) and also the presence in smaller quantities of other minerals, such as quartz, feldspat *etc.* [14]. The highest montmorillonite content was found in the Valea Chioarului bentonite sample with particle size below 0.2 μm .

Thermodifferential analysis data revealed the characteristic curves of montmorillonite for both Romanian clays [12]

The specific surface of the sample below 50 μm from Valea Chioarului bentonite was 190.86 m^2/g . The ion exchange capacity was determined by replacing the compensatory ions with NH_4^+ ions and was 68.32 $\text{mE}/100\text{g}$ for Răzoare bentonite and 78.03 $\text{mE}/100\text{g}$ for Valea Chioarului bentonite.

3.2 Clay films CPEs in pharmaceutical electroanalysis

Several clay-modified CPEs and GCEs were tested in the presence of different pharmaceuticals using CV, SWV and DPV.

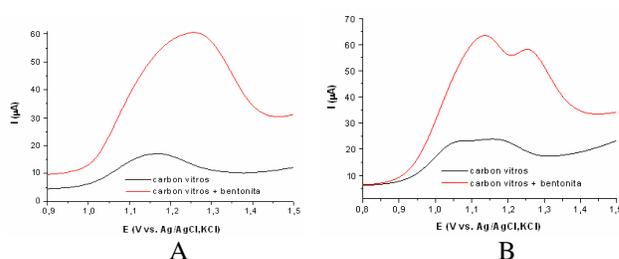


Figure 1: SWVs of 10^{-3} M theophylline solution (A); (step potential 40 mV; ampl. 50 mV, 70 Hz;) and 10^{-3} M codeine phosphate solution (B) in acetate buffer pH 4.5 (40 mV s^{-1} ; ampl. 0.1 V) at clay-modified GCE (red line) and bare GCE (black line).

Thus, higher current values could be obtained for some alcaloids, namely caffeine, theophylline (Figure 1A), aminophylline, papaverine hydrochloride and codeine phosphate (Figure 1B), by using DPV and SWV on Valea Chioarului clay-modified GCEs. The results were comparable with those already published for the same alcaloids at electrochemically activated GCEs [15, 16].

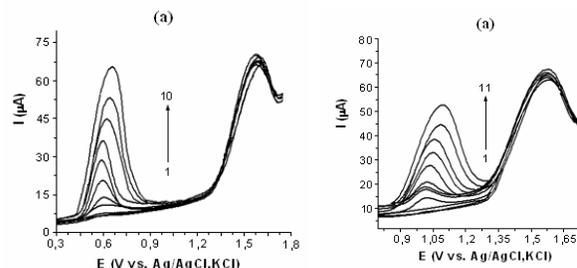


Figure 2: SWVs at electrochemically activated GCE for a 10^{-3} M caffeine solution and different concentrations: 1) 0; 2) 10^{-6} M; 3) $5 \cdot 10^{-6}$ M; 4) 10^{-5} M; 5) $5 \cdot 10^{-5}$ M; 6) $7.5 \cdot 10^{-5}$ M; 7) 10^{-4} M; 8) $2.5 \cdot 10^{-4}$ M; 9) $5 \cdot 10^{-4}$ M; 10) $7.5 \cdot 10^{-4}$ M; 11) 10^{-3} M, in acetate buffer pH 4.5 of paracetamol (a) and acetylsalicylic acid (b), (40 mV s^{-1} ; amplitude 100 mV; modulation time 5 ms).

Riboflavin phosphate exhibited a typical reversible cyclic voltammetric response at unmodified carbon paste electrode, with an oxidation peak at -0.45 V and a reduction peak at -0.60 V. The increase in the clay concentration in the carbon paste was followed by the shift towards lower values of the oxidation and reduction potentials.

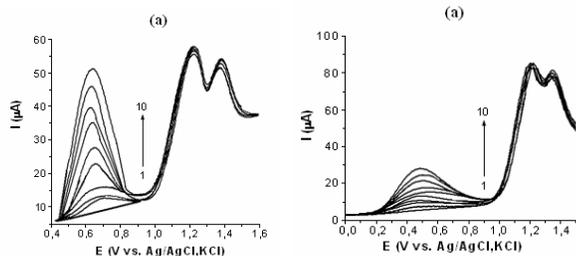


Figure 3: SWVs at electrochemically activated GCE for a 10^{-3} M codeine phosphate solution and different concentrations (1) 0, (2) 10^{-6} M, (3) $5 \cdot 10^{-6}$ M, (4) 10^{-5} M, (5) $5 \cdot 10^{-5}$ M, (6) $7.5 \cdot 10^{-5}$ M, (7) 10^{-4} M, (8) $2.5 \cdot 10^{-4}$ M, (9) $5 \cdot 10^{-4}$ M, (10) $7.5 \cdot 10^{-4}$ M (11) 10^{-3} M, in acetate buffer pH 4.5 of paracetamol (a) and ascorbic acid (b), (40 mV s^{-1} ; amplitude 100 mV; modulation time 5 ms).

3.3 Enzyme/clay/polymeric film biosensors

A biosensor for the detection of acetaminophen was developed by deposition of a thin PEI film on the surface of a GCE and its electrochemical behavior was compared with the doped CPEs. In spite of its relative water solubility, the PEI film was able to immobilize HRP offering an improved hydration layer essential for the activity of the enzyme.

In the case of PEI film GCEs both reversible oxidation and reduction processes of acetaminophen could be observed, while on the clay-modified CPEs only the oxidation process could be detected by CV (Figure 4).

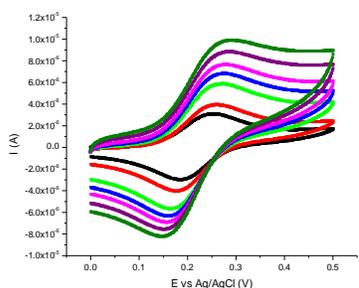


Figure 4: Cyclic voltammograms of 10^{-4} M acetaminophen solution in 0.1 M phosphate buffer pH 7.4, using $20 \mu\text{m}$ Valea Chioarului clay/ 1 mg/mL PEI film GCE; Scan rates: 10 (black), 20 (red), 50 (light green), 75 (blue), 100 (purple), 150 (violet) and 200 mVs^{-1} (dark green).

The amperometric studies were performed by recording the electrochemical reduction of the enzymatically generated electroactive oxidized species of acetaminophen (NAPQI) in the presence of hydrogen peroxide [17] after stepwise addition of 10^{-4} M acetaminophen solution.

The amperometric assays were realized at -0.2V. The HRP-clay-PEI/GCE had a sensitivity of $6.28 \cdot 10^{-7}$ M and a linear range between $5.25 \cdot 10^{-6}$ M and $4.95 \cdot 10^{-5}$ M [18, 19].

The microscopic image of the polystyrene beads covered with a polypyrrolic film (Figure 5), confirmed the augmentation of the surface and the nanopatterned structure.

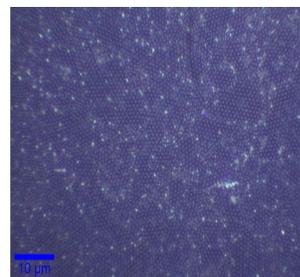


Figure 5: Microscopic image of the nanopatterned structure.

In addition, the Raman spectra performed after the removal of the polystyrene beads confirmed the presence of the conductive polymer and the absence of the polystyrene from the new obtained surface (Figure 6).

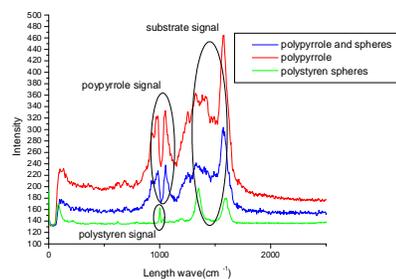


Figure 6: Raman spectra of polypyrrole films

3.4 Clay-modified electrochemical sensors for heavy metals detection

Based on the remarkable ion exchange and adsorbent properties, Răzoare and Valea Chioarului clays were employed for the development of several clay modified carbon based electrodes and Cd^{2+} , Cu^{2+} and Pb^{2+} were detected using square wave voltammetry [12].

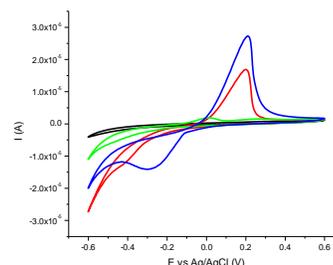


Figure 7: Cyclic voltammograms of $6.3 \cdot 10^{-4}$ M Cu^{2+} solution at GCE (red line), PEI/GCE (green line) and Valea Chioarului ($\text{Ø } 1 \mu\text{m}$) clay/PEI/GCE (blue line) (phosphate buffer pH 4, scan rate 50 mV/s).

It can be concluded that the polymeric film almost blocked the electrode, while the Valea Chioarului clay-modified electrode improved the signal of copper (Figure 7).

Polypyrrole films and carboxylated MWCNTs were deposited by CV on pencil graphite electrodes in order to immobilize nitrate reductase for nitrate detection.

The electrical permeability of the clay polypyrrole (PPy) modified GCEs was tested in 10^{-3} M potassium ferrocyanide, 10^{-3} M hexaamine ruthenium(III) chloride (Figure 8) and 10^{-3} M ferrocene dimethanol by CV.

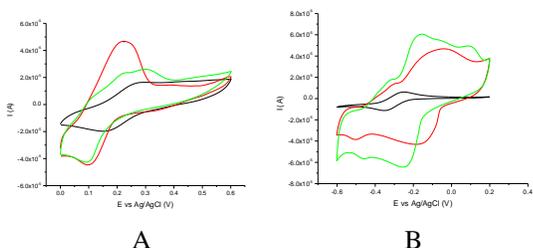


Figure 8: Cyclic voltammograms of 10^{-3} M potassium ferrocyanide (A) and 10^{-3} M $\text{Ru}(\text{NH}_3)_6^{3+}$ (B) solutions at bare GCE (black line), PPy/GCE (red line) and Valea Chioarului (Ø 0.2 μm) clay/PPy/GCE (green line).

The currents obtained for the clay/PPy and PPy modified electrodes were higher compared to the unmodified electrodes, while for ferrocene dimethanol the signals were inconvenient. The higher current for the clay/PPy/GCE in $\text{Ru}(\text{NH}_3)_6^{3+}$ emphasizes the cationic exchange capacity of the clay, while the signal decrease in $[\text{Fe}(\text{CN})_6]^{4-}$ solution is due to the repulsion forces that appear between both negatively charged complex and clay.

4 CONCLUSIONS

Several micro and nanoparticle fractions of two Romanian bentonites from Răzoare and Valea Chioarului were separated and characterized using thermal analysis, TEM, X-ray diffractometry and FTIR spectra. The electrochemical behavior of acetaminophen, ascorbic acid and riboflavin phosphate was tested on clay-modified CPEs with different clay particle sizes using CV. The development of a biosensor based on HRP immobilization in a PEI and clay porous gel film for acetaminophen amperometric detection was successfully achieved. The obtained results emphasized the great active surface, the adsorbent and ionic exchange properties of Răzoare and Valea Chioarului bentonites, showing the advantages offered by these clays in the development of modified electrodes applied in pharmaceutical analysis.

The nanopatterned polypyrrole surface was a good platform for biocomponent immobilization in the development of biosensors.

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