

PHYSICO-CHEMICAL AND ELECTROANALYTICAL CHARACTERIZATION OF TWO ROMANIAN CLAYS WITH POSSIBLE APPLICATIONS IN PHARMACEUTICAL ANALYSIS

ADELA MAGHEAR¹, CECILIA CRISTEA^{1,*}, ANA MARIAN², IULIU O. MARIAN², ROBERT SĂNDULESCU¹

¹*Iuliu Hațieganu University of Medicine and Pharmacy, Faculty of Pharmacy, Analytical Chemistry Department, 4 Pasteur St, 400349 Cluj-Napoca, Romania,*

²*Babeș-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos St, 400021, Cluj-Napoca, Romania*

**corresponding author: ccristea@umfcluj.ro*

Abstract

The high demand for simple, fast, accurate, and sensitive detection methods in pharmaceutical analysis has driven the development of novel electrochemical sensors. Clay modified electrodes are likely to be used for this application. In this study, clays were used as electrode modifiers to improve the electrochemical properties of the existing electrodes. Bentonites obtained from Răzoare and Valea Chioarului deposits (Maramureș County, Romania) were refined and characterized by X-ray diffraction and transmission electron microscopy. The ion exchange capacity of purified clays was determined by replacing the compensatory ions with NH_4^+ ions. These clays were used to modify carbon paste electrodes (CPEs). The electrochemical behavior of acetaminophen, ascorbic acid, and riboflavin phosphate was tested by using cyclic voltammetry on clay-modified CPEs with different clay particle sizes. Resulting CPEs revealed either better electroanalytical signals or oxidation at lower potential. The development of new clay-modified sensors using such composite materials based on micro and nanoparticles could be applied in pharmaceutical analysis.

Rezumat

Cerința tot mai crescută pentru metode de detecție simple, rapide, precise și sensibile în analiza farmaceutică a condus la dezvoltarea unor noi senzori electrochimici. Electrozii modificați cu argilă pot fi o alternativă viabilă la alte metode de detecție. În acest studiu s-au utilizat argile ca modificatori ai electrozilor cu scopul de a ameliora proprietățile electrochimice ale acestora. Bentonitele obținute din depozitele de la Răzoare și Valea Chioarului (Maramureș, România) au fost purificate și caracterizate prin difracție de raze X și microscopie cu transmisie electronică. Capacitatea de schimb ionic a argilelor purificate s-a determinat prin înlocuirea ionilor compensatori cu ioni NH_4^+ . Argilele astfel obținute s-au utilizat la modificarea unor electrozi pastă de carbon. Comportamentul electrochimic al paracetamolului, acidului ascorbic și al fosfatului de riboflavină a fost testat prin voltametrie ciclică pe electrozii pastă de carbon modificați cu argilă cu particule de diferite dimensiuni. Electrozii rezultați au prezentat fie semnale electroanalitice mai bune, fie procese de oxidare la valori mai scăzute de potențial. Dezvoltarea unor noi senzori modificați cu argile având la bază aceste micro și nanomateriale compozite poate fi astfel aplicată în analiza farmaceutică.

Keywords: bentonites, clay-modified electrodes, montmorillonite, pharmaceuticals

Introduction

Bentonites are clay materials and represent secondary rocks formed from the devitrification, hydration and hydrolysis of other underlying rocks (*e.g.* volcanic tuffs, pegmatite *etc.*). An important feature of all bentonites is their important content of montmorillonite with its cryptocrystalline aggregate structure, at which low fractions of quartz, feldspar, volcanic glass, amphibole, pyroxene, chlorite, limonite, halloysit *etc.* are added. Montmorillonite has been used for centuries to produce ceramics. Furthermore, its applications in pharmacy, adsorbents and ion exchangers are also reported in the literature [1, 2]. These last applications are particularly useful for the development of electrochemical sensors [3].

A "chemical sensor is a small device that, as the result of a chemical interaction or process between the analyte and the sensor device, transforms chemical, or biochemical information of a quantitative or qualitative type into an analytically useful signal" [4]. Electrochemical sensors are reported in the literature as the most attractive because of their great sensitivity, experimental simplicity, and low cost [3]. Clay modified electrodes have attracted considerable attention attempting to control the path and scale of electrode reactions [5-10]. The composition of the carbon paste electrode (CPE) modified with clay was defined as a complex heterogeneous system consisting of conductive solids, semiconductors, and insulators, including a clay-induced aqueous phase. Phenomena of charge and mass transfer in such mixtures are extremely complicated and require a thorough characterization, moreover because the clays included in the electrode material are natural compounds whose composition and structure are subject to their place of origin. In spite of the wide range of electrode modifiers, clays have attracted the interest of electrochemists, in particular for their analytical applications [9, 11- 13, 17].

Romania has many bentonite deposits, some in operation, others unexplored. Due to their high content of montmorillonite, Răzoare and Valea Chioarului bentonites (from the North-Western part of Romania) were investigated for the development of new composite electrode materials with applicability in the fabrication of electrochemical sensors.

The paper will present beside the structural characterization of several clays from Northern part of Romania their possible application in the development of new sensors for pharmaceutical analysis.

Materials and Methods

Physico-chemical composition studies, as well as X-ray diffraction (XRD) and transmission electron microscopy (TEM) established the properties of the two clays.

For the structural characterization of Răzoare and Valea Chioarului bentonites, it was resorted to their impurities removal, in order to obtain a higher content of montmorillonite in the resulting samples. The refinement was achieved by washing and decantation obtaining a more homogeneous product, rich in montmorillonite, the main component from their structure. All of the following characterization procedures and analytical experiments are based on these refined clay samples.

Separation was performed on different clay granulometric particle sizes by sedimentation, decantation, centrifugation, and ultracentrifugation after the procedures reported in the literature, according to Stokes' law [14, 15]. Several fractions, below 50 μm , 20 μm , 8 μm , 5 μm , 2 μm , 1 μm , and below 0.2 μm were separated and characterized.

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 XRD and TEM.

Chemical composition was achieved by gravimetry (Si), complexometry (Fe, Al, Ca, Mg), colorimetry (Ti at 436 nm), and flame photometry (Na at 589 nm, K at 768 nm). Ion exchange capacity was obtained by treating the clay sample with an ammonium chloride solution, followed by the filtration and determination of Ca and Mg (by complexometry), Na and K (by flame photometry).

For TEM studies, an aqueous suspension of clay was deposited on a thin layer of collodium, followed by evaporation. Examination of the samples was achieved using a JEOL JEM 1010 microscope.

Diffraction was achieved on fine powder material using a Shimadzu X-ray diffractometer XRD 6000 N, equipped with a monochromator and a position-sensitive detector. The X-ray source was a Cu anode (40kV, 30 mA). The diffractograms were recorded in the 0-90° 2 θ range, with a 0.02 step size and a collection of 0.2 s per point.

Surface area analyses were recorded with a Thermo analyzer Q Finnigan type SURF 9600 by single point method, on Răzoare clay fraction below 20 μm and on Valea Chioarului clay sample below 50 μm , respectively, without any previous thermal treatment.

The electrochemical behavior of clay-modified CPEs and of clay-modified thin films glassy carbon electrodes (GCEs) was tested in the

presence of some pharmaceutical compounds as ascorbic acid, riboflavin phosphate, and acetaminophen.

Clay water suspensions of 50 mg/mL were prepared for the fractions below 20 μm and 0.2 μm for Valea Chioarului clay and below 20 μm for Răzoare clay. Standard solutions of acetaminophen, riboflavin, and ascorbic acid were prepared to provide a final concentration of 10^{-3} M.

CPEs were modified by mixing different Răzoare clay concentrations (1%, 2.5%, 5%, and 10%) with "homemade" carbon paste prepared with solid paraffin [16].

Electrochemical studies like cyclic voltammetry (CV) were performed in a conventional three-electrode system: new modified carbon based electrodes (working electrodes), platinum (auxiliary electrode), Ag/AgCl 3M KCl (reference electrode), under stirring conditions. All the CV experiments were recorded at 100 mVs^{-1} . GCEs were provided by BAS Inc. (West Lafayette, USA) and were carefully washed with demineralized water and polished using diamond paste (BAS Inc.). The experiments were achieved with AUTOLAB PGSTAT 30 (EcoChemie, Netherlands) equipped with GPES and FRA2 software. 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.

Paraffin (Ph Eur, BP, NF), graphite powder, acetaminophen (minimum 99.0 %), L-ascorbic acid (99.0 %), and riboflavin (Ph Eur) were provided by Merck and KCl (analytical grade) from Chimopar București. All reagents were of analytical grade, used as received without further purification.

Results and Discussion

Clays' characterization

Elemental analyses confirmed clays chemical composition and revealed the differences between the two clays (Table I). Bentonites have a high content of SiO_2 and Al_2O_3 and also significant water content. The components present in small quantities and in varying proportions are: MgO, CaO, K_2O , Na_2O , Fe_2O_3 and TiO_2 . 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. The structural formulas of the clay minerals are: Răzoare $(\text{Ca}_{0.03} \text{Na}_{0.30} \text{K}_{0.06})_{\Sigma=0.39} (\text{Al}_{1.54} \text{Mg}_{0.37} \text{Fe}_{0.10})_{\Sigma=2.01} (\text{Si}_{3.84} \text{Al}_{0.16})_{\Sigma=4.00}$

$O_{10}(OH)_2 \cdot nH_2O$, and Valea Chioarului $(Ca_{0.06} Na_{0.27} K_{0.02})_{\Sigma=0.35} (Al_{1.43} Mg_{0.47} Fe_{0.10})_{\Sigma=2.00} (Si_{3.90} Al_{0.10})_{\Sigma=4.00} O_{10}(OH)_2 \cdot nH_2O$.

Table I
Chemical composition of Răzoare and Valea Chioarului bentonites

Sample	SiO ₂ %	TiO ₂ %	Al ₂ O ₃ %	Fe ₂ O ₃ %	CaO%	MgO%	Na ₂ O%	K ₂ O%	L.C.* %
Răzoare	68.60	0.22	13.89	1.36	0.30	3.38	1.50	0.45	11.30
Valea Chioarului	59.82	0.25	16.14	1.67	0.70	3.92	1.75	0.25	15.50

* Loss on calcination process at 1000°C

TEM images of Răzoare (Figure 1A) and Valea Chioarului clays (Figure 1C) at higher magnification showed a diffusive, irregular, and opalescent surface. The very fine dispersed montmorillonite (bellow 0.2 nm) formed extremely thin lamellar layers with nanometer dimensions (Figure 1B).

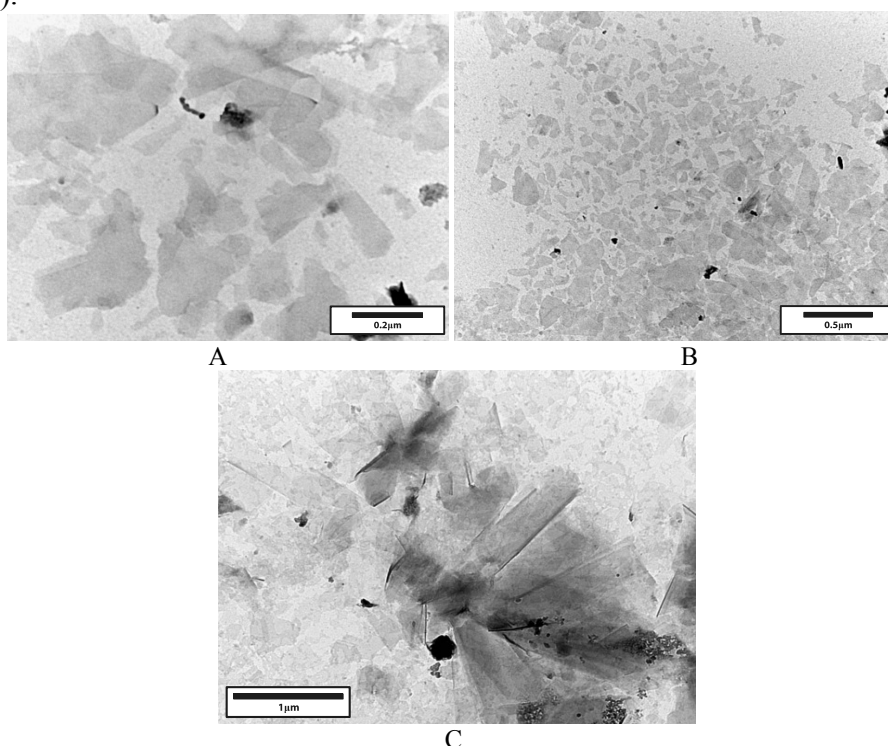


Figure 1

TEM characterization of Răzoare (A, B) and Valea Chioarului (C) clays

The XRD diffractogram of Razoare clay, fraction bellow 20 μm (Figure 2A) displayed the characteristic diffraction peaks of montmorillonite at 2θ (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 at 2θ (20.68° ; 26.50° ; 36.36° ; 42.10° ; 54.70° ; 59.67°), feldspat at 2θ (23.22° ; 24.10° ; 27.74° ; 35.08°) *etc.* [18].

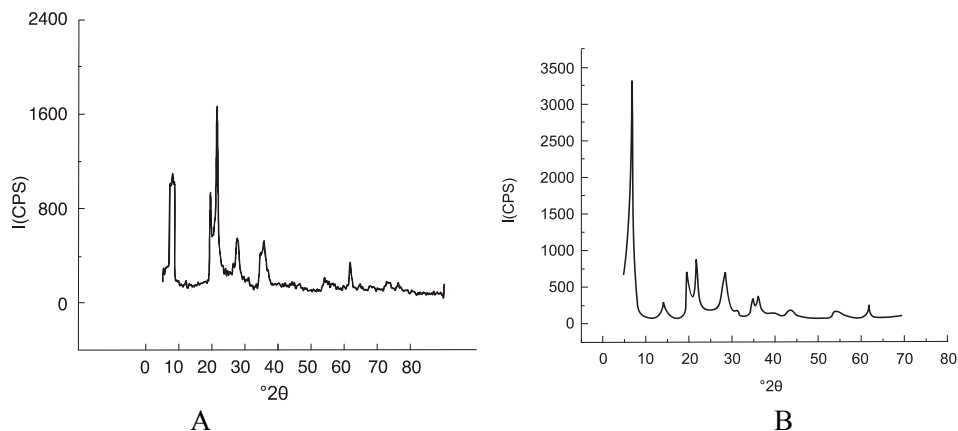


Figure 2

XRD diffractograms of Răzoare (A) and Valea Chioarului clays (B)

The XRD diffractogram obtained for Valea Chioarului clay using a sample with particle size below $0.2\mu\text{m}$ (Figure 2B) showed a high content of montmorillonite (with its characteristic peaks at 2θ : 6.94° ; 19.96° ; 21.82° ; 28.63° ; 36.14° ; 62.01°), which confirmed the position of the diffraction peaks, in agreement with literature data [18], and also the almost negligible presence of other minerals.

The presence of montmorillonite was also proved by comparing the XRD data of the below $0.2\mu\text{m}$ sample before and after treatment with ethyleneglycol. After adsorption of ethyleneglycol, it was noticed an increase in the reticular distance in c axis direction from 12.72 \AA to 17.18 \AA , corresponding to 6.9434° and to 5.137° peaks, respectively, characteristic for montmorillonite.

The value of the specific surface of the sample bellow $20\mu\text{m}$ of Răzoare clay was $50\text{ m}^2/\text{g}$. Due to the aggregation effect which occurred during the drying process of the fine granulation prepared clay, the specific surface of the Valea Chioarului clay was determined on the sample below $50\mu\text{m}$. The obtained value was $190.86\text{ m}^2/\text{g}$.

The ion exchange capacity of the analyzed clays was determined by replacing the compensatory ions with NH_4^+ ions, followed by their quantitative determination. Therefore, the ionic exchange capacity of Răzoare clay was $68.32\text{ mE}/100\text{g}$ and for Valea Chioarului clay it was estimated at $78.03\text{ mE}/100\text{g}$.

Electrochemical behavior of the refined clays

The electrochemical behavior of several clay-modified electrodes was tested in the presence of some pharmaceutical compounds: acetaminophen, ascorbic acid, and riboflavin phosphate (Figure 3). Taking into consideration the adsorbent properties of the investigated clays, the study aimed to improve the oxidation and reduction potentials obtained on unmodified CPEs.

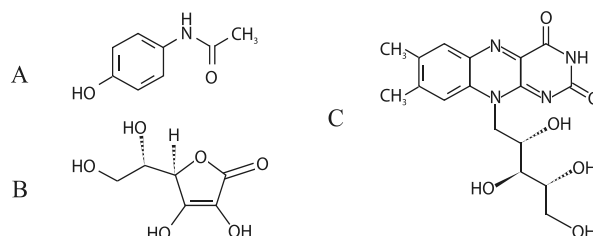


Figure 3

Chemical structures of investigated pharmaceutical substances: (A) acetaminophen, (B) ascorbic acid, and (C) riboflavin

Acetaminophen and ascorbic acid (Figure 4A and 4C) showed relatively similar electrochemical behavior in cyclic voltammetric investigations, one irreversible oxidation peak being obtained at 0.78 V and 150 μ A for acetaminophen and 200 μ A for ascorbic acid with 1% clay-modified CPEs. In both cases, the increase in the clay content was followed by an important shift of the oxidation potential towards lower values, 0.70 V for acetaminophen and 0.60 V for ascorbic acid, showing that the increasing clay concentration facilitates the oxidation process ($\Delta E = 100 - 150$ mV).

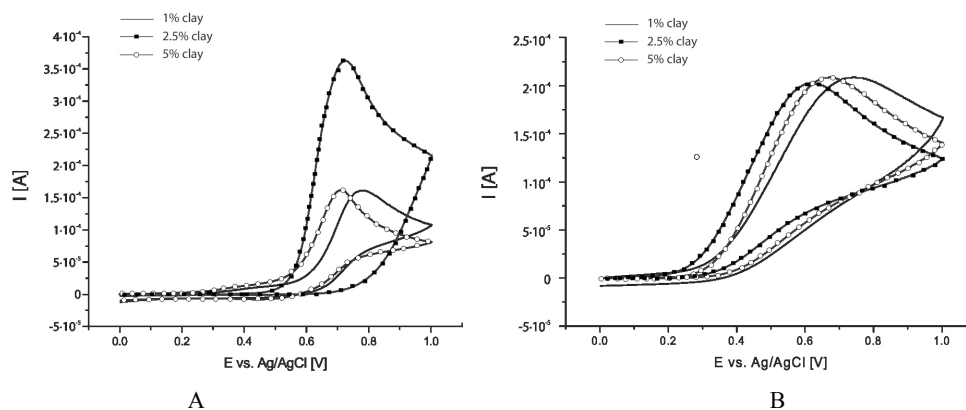


Figure 4

Cyclic voltammograms of 10^{-3} M acetaminophen (A) and 10^{-3} M ascorbic acid (B) on 1% (solid line), 2.5% (square line), and 5% (dot line) Răzoare clay-modified CPEs (KCl 0.1 M, 100 mVs^{-1})

In the case of acetaminophen, an increase in the current from 150 to 350 μA could be observed in the oxidation range at the 2.5 % clay-modified CPE, while for the 5 % clay-modified CPE the current had the same order of magnitude as the 1% clay-modified CPE (Figure 4A).

Ascorbic acid showed a different behavior, the increase in the clay content having no influence on the current range, but facilitating the oxidation reactions, proved by the above mentioned shift of the anodic potential towards lower values (Figure 4B).

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 as presented in Figure 5.

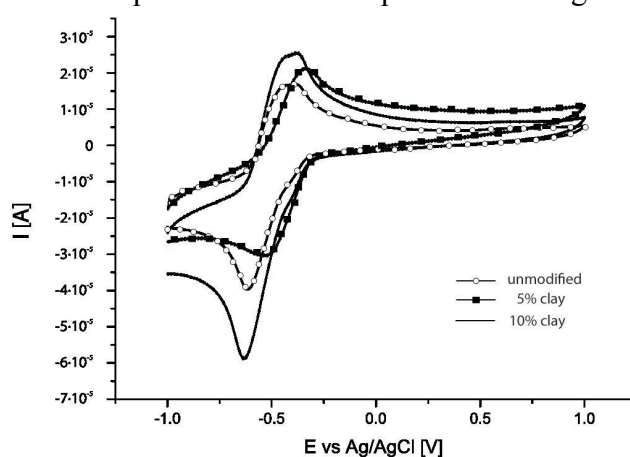


Figure 5

Cyclic voltammograms for 10^{-3} M riboflavin phosphate at unmodified CPE (dot line), at 5% (square line), and 10% (solid line) Răzoare clay-modified CPEs (KCl 0.1 M, 100 mVs^{-1})

Anodic currents increased proportionally with the clay content with about 5 nA and 10 nA for the 5% and the 10% clay-modified CPEs versus unmodified CPEs, respectively. The increase in the cathodic current was higher (20 nA) than the anodic current (10 nA) for the 10% clay-modified CPEs. Thus, it can be concluded that an increase in the clay concentration favors riboflavin detection. A significant difference could be observed when the 5% clay-modified CPE current was compared with the current of the unmodified CPE. In the oxidation range, the current was 5 nA higher than the current measured at the unmodified electrode, while in the reduction range the value was about 10 nA lower than the one measured at the unmodified electrode. This proved that a lower concentration of clay was not enough for riboflavin detection.

Conclusions

Two Romanian clays from Răzoare and Valea Chioarului deposits (Maramureş County) were refined and characterized revealing montmorillonite as the main component of their structure. New composite materials based on clay micro and nanoparticles and polymeric films for the development of electrochemical sensors and biosensors were developed. The electrochemical behavior of acetaminophen and riboflavin phosphate was tested for the first time on clay-modified CPEs with different clay particle sizes using cyclic voltammetry and new electrochemical methods could be elaborated and applied in pharmaceutical analysis. The obtained results emphasized the great active surface, the adsorbent and ionic exchange properties and showed the advantages offered by Răzoare and Valea Chioarului clays for the development of novel modified electrodes applied in pharmaceutical analysis.

Acknowledgements

The authors are grateful for the financial support to the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project number PN-II-ID-PCE-2011-3-0355 and to UMF "Iuliu Hațieganu" for the research projects 27027/3/15.11.2011 and POS-DRU 88/1.5/S/58965.

References

1. Vaccari, A., Preparation and catalytic properties of cationic and anionic clays, *Catalysis Today* 1998, 41, 53-71.
2. Vaccari, A., Clays and catalysis: A promising future, *Applied Clay Science* 1999, 14, 161 - 198.
3. Mousty, C., Sensors and biosensors based on clay-modified electrodes - new trends, *Applied Clay Science* 2004, 27, 159-177.
4. Stetter, J.R., Penrose, W.R., Yao, S., Sensors, chemical sensors, electrochemical sensors, and ECS, *Journal of The Electrochemical Society* 2003, 150, S11-S16.
5. Fitch, A., Clay modified electrodes: A review, *Clays Clay Miner* 1990, 38, 391-400.
6. Bard, A.J. Mallouk, T., Electrodes modified with clays, zeolites, and related microporous solids, *Molecular design of electrode surfaces* 1992, 22.
7. Lipkowski, J. Ross, P.N., *The electrochemistry of novel materials*, 1994. VCH Publishers Inc., New York.
8. Macha, S.M. Fitch, A., Clays as architectural units at modified-electrodes, *Microchimica Acta* 1998, 128, 1-18.
9. Navrátilová, Z. Kula, P., Clay modified electrodes: Present applications and prospects., *Electroanalysis* 2003, 15, 837-846.
10. Tonlé, I.K., Ngameni, E., Walcarius, A., From clay- to organoclay-film modified electrodes: Tuning charge selectivity in ion exchange voltammetry. *Electrochimica acta* 2004, 49, 3435-3443.
11. Walcarius, A., Zeolite-modified electrodes: Analytical applications and prospects. *Electroanalysis* 1996, 8, 971-986.

12. Walcarius, A., Analytical applications of silica - modified electrodes – a comprehensive review. *Electroanalysis* 1998, 10, 1217-1235.
13. Cernat A., Tertis M., Griveau S., Bedioui F, Sandulescu R, New modified electrodes with HRP immobilized in polymeric films for paracetamol analysis, *Farmacia*, 2012, 60(1), 1-12
14. Tanner, C.B. Jackson, M.L., Nomographs of sedimentation times for soil particles under gravity or centrifugal acceleration. *Soil Science Society of America Proceedings* 1947, 12, 60-65.
15. Jackson, M.L., Whittig, L.D., Pennington, R.P., Segregation procedure for the mineralogical analysis of soils. *Proceedings. Soil Science Society of America* 1950, 1949, 14, 77-81.
16. Săndulescu, R., Mirel, S., Oprean, R., The development of spectrophotometric and electroanalytical methods for ascorbic acid and acetaminophen and their applications in the analysis of effervescent dosage forms. *Journal of pharmaceutical and biomedical analysis* 2000, 23, 77-87.
17. Duțu G, Cristea C, Ede B, Hârceagă V., Saponar A., Popovici E, Săndulescu R, The electrochemical behavior of some local anaesthetics on screen printed electrodes modified with calixarenes, *Farmacia*, 2011, 59(2), 147-160
18. Kaeble, E.F., *Handbook of X-rays*. 1967, 1-17. McGraw-Hill, New York.

Manuscript received: December 11th 2012