Studies on the Electrochemical Behaviour of Cephalosporins at Different Types of Working Electrodes

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Cephalosporins are β-lactam antibiotics which can be considered to be one of the most important and most frequently used groups of the antibiotics applied in medicine, divided in four generations based on the time of their discovery and their antimicrobial properties. The aim of the present study consists in establishing the best conditions for electrochemical behaviour of cephalosporins in the view to develop a release profile for the antiobitics imobilized on a mesoporous support (MCM-41). The electrochemical studies were performed by cyclic voltammetry and square wave voltammetry using different working electrodes, such as glassy carbon and gold and various electrolytes, Britton–Robinson buffers, acetate, acetic acid, phosporic acid.

Keywords: cephalosporins, cyclic voltammetry, glassy carbon electrode, gold electrode, square wave voltammetry

Cephalosporins are the second major group of β-lactam antibiotics classified into four generations according to the period of their discovery and their therapeutic properties. These are widely used in clinical therapy for the treatment of severe infections because of their antibacterial and pharmacokinetic properties. Pharmaceuticals, identified as emerging contaminants, are used in large quantities in human and veterinary medicine for treatment of different diseases. Also, it is very important to perform studies concerning antibiotics concentration in hospital sewage water. In veterinary practice, antibiotics are utilized at therapeutic levels primarily to treat diseases and to prevent infections. The frequent, sometimes illegal, use of antibiotics may result in residues being found at different concentration levels in products of animal origin, such as milk. β-lactams comprise some of the antibiotics most frequently used for the treatment of sick animals in Europe in order to control such veterinary drugs in animals producing food, the European Union (EU) has established strict regulations, including specific maximum residue limits (MRLs) for each of these substances. Many EU Directives include legislation for this particular group of antibiotics in medicated feed and animal-derived food.
The cephalosporins determination has an importance not only in the field of human health for pharmacokinetic analysis but also for quality control in food and industry.
For clinical and pharmaceutical studies, development of rapid, sensitive and selective analytical methods for the determination of drugs in biological fluids is required.
Thus, to determine cephalosporins in pure form, in pharmaceutical preparations and biological fluids have been reported in the literature several analytical methods such as spectrophotometric methods [1-3], fluorimetric [4-6], chromatographic (HPLC) coupled with different detection systems [7-8], and capillary electrophoresis [9]. Electrochemical methods, due to their rapidity, simplicity and high sensitivity in analysis, have been favored to study compounds such as cephalosporins [10-13].

In this study we used the electrochemical method of analysis due to the fact that most of the interactions in the biological fluids or mucous membrane are electrochemical (van der Waals forces, hydrogen bonds, ionic bonds) and because cephalosporins have electrochemically active groups. The electrochemical behaviour of 3 cephalosporins, cefuroxime (2nd generation), cefotaxime (3rd generation) and cefepime (4th generation) (fig. 1) were studied using different types of working electrodes and different electrolytes medium.

Cefuroxime sodium is a semisynthetic cephalosporin with good activity against Klebsiella pneumoniae and almost all strains of Escherichia coli.

Cefotaxime sodium is a cephalosporin antibiotic widely used against gram-negative bacteria. It is a beta-lactam antibiotic with broad spectrum and treat many kinds of infections, including those of skin, bone, stomach, brain, blood, respiratory tract, sinuses, ear and urinary tract.

Cefepime sodium is used to treat infections caused by gram-negative bacteria, including Escherichia coli, Proteus, and Klebsiella; gram-positive organisms, including Streptococcus pneumoniae, S. pyogenes, and Staphylococcus aureus; and infections of the lower respiratory tract, urinary tract, skin, and bone.

Only the results obtained for electrochemical behaviour of cefotaxime will be presented.

Experimental part

Cefotaxime sodium, Cefuroxime sodium and Cefepime sodium were supplied by Sigma Aldrich. The stock solutions ($c = 1$ mmol·L^{-1}) were prepared by dissolving the exact amount of the respective substance in Milli Q water and stored in the dark and under refrigeration in order to minimize the decomposition. Standard solutions of these antibiotics with lower concentration were prepared daily by diluting the stock solutions with distilled water.

Britton–Robinson (B–R) buffers and acetate buffer, phosphoric acid and acetic acid served as supporting

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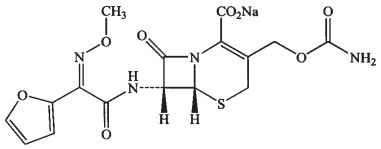
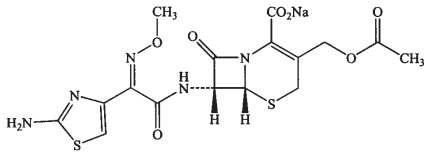
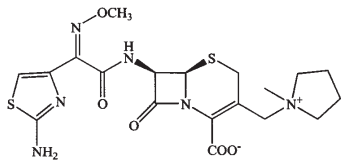


Cefuroxime sodium salt



Cefotaxime sodium salt



Cefepime sodium salt

Fig. 1.
Cephalosporins structure

electrolytes for voltammetric measurements. Britton – Robinson buffers were prepared in a usual way (i.e. by mixing a solution of 0.04 mol · L^{-1} phosphoric acid, 0.04 mol · L^{-1} acetic acid, and 0.04 mol · L^{-1} boric acid with the appropriate amount of 0.2 mol · L^{-1} sodium hydroxide solution).

All chemicals used for buffer preparation were of analytical grade purity. Voltammetric measurements were carried out using EG & G Princeton Applied Research with a galvanostat / potentiostat model 2273 A.

Cyclic and square wave voltammetry (SWV) was employed with: gold and glassy carbon working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (3 M KCl) reference electrode to which all the potential values are referred.

The pH of the solutions was measured with a pH meter Eutech Instruments, PCD 6500, *p*H/Ion/ Conductivity/DO Meter with a combined glass electrode.

Calibration dependences were evaluated by least squares linear regression method. All measurements were made in triplicate.

Results and discussions

During the last decades, modern voltammetric techniques have been used to determine organic compounds in different types of samples, especially in the pharmaceutical field. Due to the fact that all cephalosporins have electroactive groups which can be easily reduced at working electrodes, the electrochemical techniques were used for with good results for quantitative determinations.

From the literature it was observed that the electrode HMDE is used as working electrode, due to sensitivity and wide field of potential that can be used. The current regulations on environmental protection requires the use of electrodes "free of mercury", so seeking for replacing HMDE with other electrodes such as glassy carbon electrode (GCE), solid electrodes, the Pt, Au, screen printed electrodes, carbon paste electrode (CPE), chemically modified carbon nanotubes (CNT) (single wall-SWCNT) and multi-walled (MWCNT) unmodified or modified with different compounds was important. The choice of the working electrode is very important for the sensitivity and reproducibility of electrochemical analysis. Electro-reduction of cefotaxime was studied in different electrolytes (acetate buffer, Britton-Robinson buffer, acetic acid and phosphoric acid) at solid working electrodes by cyclic voltammetry (CV) and square wave voltammetry (SWV). Different parameters were tested to optimize the conditions for cefotaxime determination. The dependence of current intensities and potentials on *p*H, concentration, scan rate, nature of the buffer was investigated. According to the linear relationship between the peak current and the concentration square wave voltammetric methods for cefotaxime, a drug release study were performed.

Additional applications of electrochemistry includes the determination of electrode mechanisms. Redox properties of organic molecules can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity.

The presence of methoxyimino group in cephalosporin molecule is very important for its chemical and electrochemical behavior [7]:

$$R_2C{=}NOCH_3 + 2H^+ + 2e^- \leftrightarrow R_2C{=}NH + CH_3OH \quad (1)$$

$$R_2C{=}NH + 2e^- + 2H^+ \leftrightarrow R_2CHNH_2 \quad (2)$$

$$R_2C{=}NOCH_3 + 4H^+ + 4e^- \leftrightarrow R_2CHNH_2 + CH_3OH \quad (3)$$

The effect of the *p*H on the peak currents and peak potentials was examined using different electrolyte systems (*p*H between 3 and 6) showing the peak current maximum at pH 4.5 and the involvement of the H^+ ions in the electron transfer process. The obtained dependencies of Ip vs.$\nu^{1/2}$ (y=1.10x – 3.76, R^2=0.9939) (fig. 2) and Ip vs. ν showed the irreversible, diffusion-controlled process which is strongly influenced by the adsorption of cephalosporins on the working electrode surface.

To increase the sensitivity of determination square wave voltammetry was used.

The effect of pH on peak current intensity and peak potential was studied and the results are presented in table 1.

It has been observed that with increasing of pH the peak potential moved to more negative potential while the peak height decreased.

After the most suitable chemical conditions and instrumental parameters for square wave voltammetry determination were established, a calibration plot for the

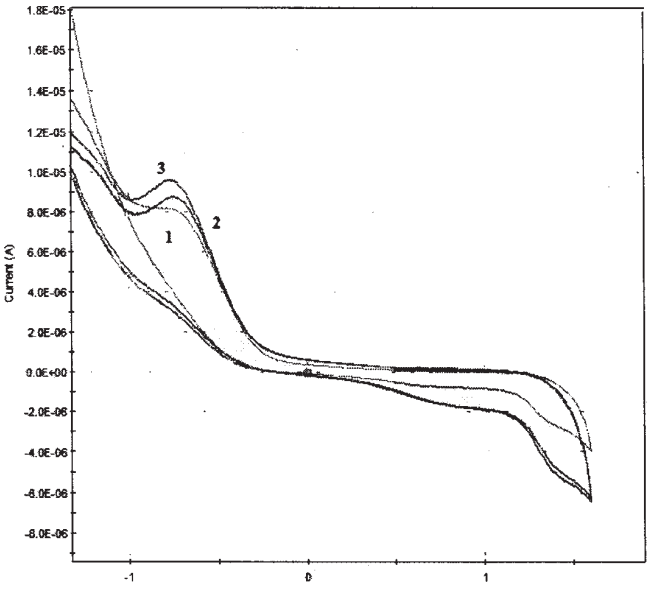


Fig. 2. Cyclic Voltamogram obtained for cefotaxime $2.3 \cdot 10^{-4}$ μmol·L^{-1} on GCE in acetate buffer (pH = 5.00) at different scan rates: (1) 20 mV·s^{-1}, (2) 50 mV·s^{-1}, (3) 100 mV·s^{-1}

pH	I_p (μA)	E (mV)
3.00	0.42	-900
4.00	0.63	-960
5.00	0.72	-1050
6.00	0.24	-1100

Table 1
THE EFFECT OF THE *p*H ON THE PEAK CURRENTS AND PEAK POTENTIALS IN THE CASE OF SW VOLTAMMETRIC DETERMINATION OF CEFOTAXIME 40 μmol·L^{-1}

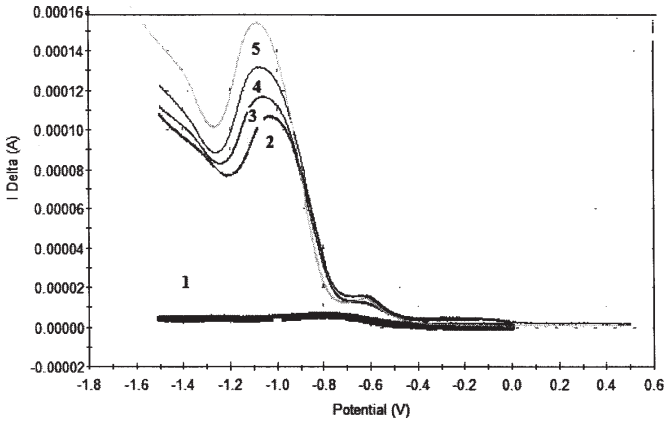


Fig. 3. SW Voltammograms of cefotaxime in acetate buffer (pH=5.00), scan rate 50 mV·s^{-1} on GCE, (1) 0, (2) 2, (3) 4, (4) 6 mmol·L^{-1}

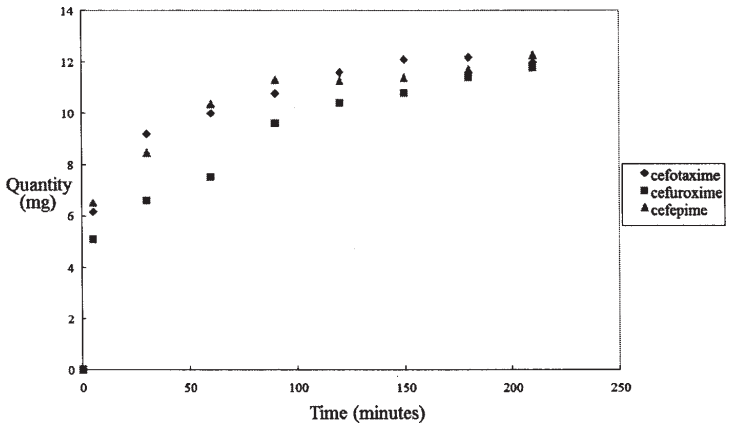


Fig. 4. Time-dependent cephalosporins release from MCM-41 in acidic media

Compound	Linearity range (μmol·L^{-1})	Calibration equation	Correlation coeficient (n=5)	LOD (μmol·L^{-1})
Cefotaxime	0.1-1	0.1109x+0.3023	0.9988	0.05
Cefuroxime	1-10	0.2623x+0.2031	0.9984	0.80
Cefepime	1-10	0.1340x+0.2054	0.9944	0.50

Table 2
CALIBRATION CURVE PARAMETERS

analyzed drug was recorded to estimate the analytical characteristics of the developed method (fig. 3).

The linearity was confimed by calculating the important calibration curve parameters which are presented in table 2.

The reliability of the proposed SWV method for the determination of cephalosporins was investigated by a release study. Following the developed electroanalytical procedure described above, in the optimum conditions, a release study of cephalosporins deposited on a mesoporous support was conducted.

Thus, the same quantity of cephalosporins (Cefotaxime, Cefuroxime and Cefepime) was deposited on a mesoporous inert support, MCM-41. In vitro cefalosporins delivery studies were performed in a 50 mL glass reactor under magnetic stirring 200 rpm at 25 °C.The release profiles were obtained by using 12.3-27.5 mg of hybrids suspended in 10 mL acidic medium. The results obtained were presented in figure 4.

The applications in different fields, such as medicine, cosmetics, food preservatives, modified polymer synthesis explain the interest in the designed functionalized supports to incorporate bioactive molecule [13]. Ordered mesoporous silicas have been proposed for the first time as carriers for drug delivery in 2001 by Vallet-Regí et al. [14].

Drug delivery systems have a great impact on medical technology, improving the performance of many existing drugs and enabling the use of entirely new therapies.

Controlled drug delivery systems can achieve precisely spatial and temporal delivery of therapeutic agents to the target site. The controlled drug delivery systems can maintain the concentration of drugs in the precise sites of the body within the optimum range and under the toxicity threshold, which improve the therapeutic efficacy and reduce toxicity.

The most frequently applied technique for clinical and pharmaceutical studies is high-performance liquid chromatography (HPLC) using different detection systems.

Thus, the results obtained by voltammetric method were confirmed using also HPLC-UV technique. The chromatographic studies represent the aim of another paper.

Conclusions

The electrochemical behaviour of cephalosporins on different type of working electrodes in various media was studied. The method is rapid and simple and can be used for cephalosporins determination from different types of samples with a minimum sample preparation. The method was used for studies on cephalosporins delivery deposited on mesoporous supports.

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References

1. EL-SHABOURY, S. R., SALEH, G. A., MOHAMED, F. A., RAGEH, A. H., J. Pharm. Biomed. Anal., 45, nr. 1, 2007, , p. 1.
2. AYAD, M. M., SHALABY, A. A., ABDELLATEF, H. E., ELSAID, H. M., J. Pharm. Biomed. Anal., 20, nr. 3, 1999, p. 557.
3, PATEL, S. A., PATEL, N. M., PATEL. M. M., Ind. J. Pharm. Sci., 68, nr. 2, 2006, p. 278.
4. RAO, G. D., KUMAR, K. G., CHOWDARY, K. P. R., Ind. J. Pharm. Sci., 63, nr. 2, 2001, p. 161.
5. KAI, M., KINOSHITA, H., MORIZONO, M. Talanta, 60, nr. 2-3, 2003, p. 325.
6. BEBAWY, L. I., EL KELANI, K., ABDEL FATTAH, L., J. Pharm. Biomed. Anal., 32, nr. 6, 2003, p. 1219.
7. ALEKSIC, M. M., KAPETANOVIC, V., ATANACKOVIC, J., JOCIC, B., ZECEVI, M. Talanta 77, nr. 1, 2008, p. 131.
8. AKL, M. M., AHMED, A., AHMED, R., J. Pharm. Biomed. Anal., 55, nr. 2, 2011, p. 247.
9. GASPAR, A., ANDRASI, M., KARDOS S., J. Chromatogr. B, 775, nr. 2, 2002, p. 239.
10. NIGAM, P., MOHAN, S., KUNDU, S., PRAKASH, R., Talanta, 77, nr. 4, 2009, p. 1426.
11. JACOBSON, G. A., MARTINOD, S., CUNNINGHAM, C. P., J. Pharm. Biomed. Anal.,40, nr. 5, 2006, p. 1249.
12. REDDY, T. M., SREEDHAR, M., REDDY, S. J. J. Pharm. Biomed. Anal., 31, nr. 4, 2003, , p. 811.
13. JAIN, R., GUPTA, V. K., JADON, N., RADHAPYARI, K., Anal. Biochem., 407, nr. 1, 2010, , p. 79.
14. WANG, S., Micropor. Mesopor. Mat., 117, nr. 1-2, 2009, p. 1.
15.VALLET-REGI, M., RAMILA, A., DEL REAL, R. P., PEREZ-PARIENTE, J., Chem. Mater., 13, 2001, p. 308.

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