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Voltammetric study of the interaction between oxacillin sodium and cysteine in the presence and absence of Mn(II) ions in neutral buffer solution

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Abstract: In this study, the voltammetric behaviour of the interaction of oxacillin sodium (OXA) and OXA–cysteine (RSH) was studied by square-wave voltammetry, cyclic voltammetry in Britton–Robinson (B–R) buffer (pH 7.0). OXA gave two peaks at –0.248 and –1.224 V. For the interaction, the peak of mercurous cysteine thiolate ($Hg_2(SR)_2$) was selected. It was found that the peak currents corresponding to $Hg_2(SR)_2$ significantly decreased, while the peak potential shifted to more positive potentials upon the addition of OXA. The observed phenomena are due to the interaction of OXA with RSH on the surface of the mercury electrode. When OXA was added to the electrochemical cell along with Mn(II), new peaks at –0.146 and –0.608 V were observed. These peaks were due to the catalytic activity of OXA on the reduction of Mn(II) and could be attributed to the formation of Mn(II) complexes with different metal/ligand ratios. On the other hand, in the presence of RSH, the peak at –0.608 V vanished and a reduction peak was observed at –0.662 V. The catalytic reduction peak potential of Mn(II) at –0.662 V indicated that RSH slightly prevented the catalysis process of OXA due to their mutual interaction.

Keywords: voltammetry, oxacillin, cysteine, interaction.

INTRODUCTION

Oxacillin sodium, OXA, (Scheme 1) is a semi-synthetic penicillin belonging to the general class of drugs called antibiotics. OXA prevents bacteria from making their cell walls and hence the cells die. It is used to treat gram-positive infections and bacteria that are resistant to penicillin.[1]

The β-lactam ring of penicillins shows susceptibility towards attack by nucleophilic reagents in water, such as amines, alcohols and thiols, in competition with that by hydroxide ions.[2] The reaction of penicillin with proteins has been extensively reported in the literature.[3–5] The most rapid reaction with low-molecular-weight compounds occurs when thiol groups are present.[6] Penicillin–pro-

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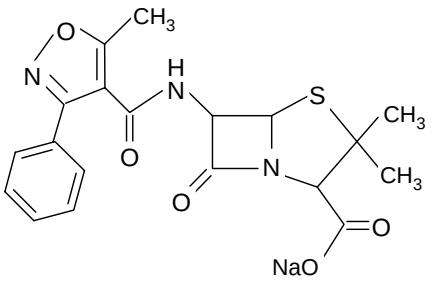
tein adducts produce penicilin allergy in sensitive individuals.[7] In addition, penicillin is thought to inactivate irreversibly carboxypeptidase by forming a penicilloyl–enzyme intermediate.[8] Mn(II) ions function as cofactors for some enzymes (enolase, isocitrate dehydrogenases, mitochondrial superoxide dismutase, etc.).[8] Enzymes, which are all proteins, are catalysts that enhance the rates of biological reactions.[8] Thus, the interaction between penicillin derivative drugs and amino acids in the presence and absence of cofactor metal ions is very important.



Scheme 1. The molecular structure of OXA.

Although studies on the binding mechanism of oxacillin were performed,[9–13] no voltammetric study on the interaction of OXA with RSH could be traced in the literature. Therefore, the aim of this study was to examine the voltammetric behaviour of OXA and its interaction with RSH in the presence and absence of Mn(II) ions.

EXPERIMENTAL

Chemicals

Oxacillin, cysteine and $MnSO_4 \cdot H_2O$ were purchased from Fluka, Merck and Sigma, respecttively. All chemicals were of analytical grade. The stock solutions were prepared in triply distilled and deionized water and used immediately.

Apparatus

Voltammetric measurements were obtained with an EG&G PAR 384B Polarographic Analyzer. An EG&G PARC 303A SMDE stand was used in the hanging mercury drop electrode (HMDE) mode. The three-electrode system was completed by means of an $Ag \mid AgCl \mid KCl_{sat}$ reference electrode and a platinum auxiliary electrode. The current–potential curves were recorded on a Houston Instrument DMP-40 plotter connected to the polarograph. The instrumental settings were: drop size, medium; equilibrium time, 5 s; scan rate, 200 mV s^{-1} (500 mV s^{-1} for CV).

Procedure

A 10 ml volume of B–R buffer (pH 7.0) solution was added to the voltammetric cell and the solution was purged with nitrogen gas for 5 min. The blank voltammogram was recorded. Then, the required aliquot of the stock solution of OXA was added to the cell by means of a micropipette and the sample voltammograms were recorded. For the interaction of OXA with RSH, OXA solution was added to B–R buffer (pH 7.0) containing 1.0×10^{-5} M RSH. The interaction between OXA and RSH was monitored by the changes of the peak potential and peak current of $Hg_2(SR)_2$. In the presence of 1.0×10^{-4} M Mn(II) ions, the voltammetric behaviour of both OXA and RSH was studied. Finally, the interaction of oxacillin with RSH in the presence of 1.0×10^{-4} M Mn(II) was monitored by the voltammetric techniques.

RESULTS AND DISCUSSION

Voltammetric behaviour of OXA

The voltammetric behaviour of OXA in B–R buffer (pH 7.0) is shown in Fig. 1. On the less negative potential side, a small reduction peak is observed at –0.248 V (Fig. 1, 1U). On the more negative potential side, a broad reduction peak (main peak) occurs at –1.224 V (Fig. 1, 2U). The peak current i_{p1}, of the small peak increased with increasing frequency, f, (from 10 to 100 Hz) and the linear equation of i_{p1} *vs.* f relationship was i_{p1} (nA) = 1.251×f (Hz) + 11.758 (r = = 0.995). According to this result, the peak at –0.248 V can be inferred as the adsorption of OXA molecules on the surface of the mercury electrode. For the characterization of main peak, cyclic voltammetry was also used.

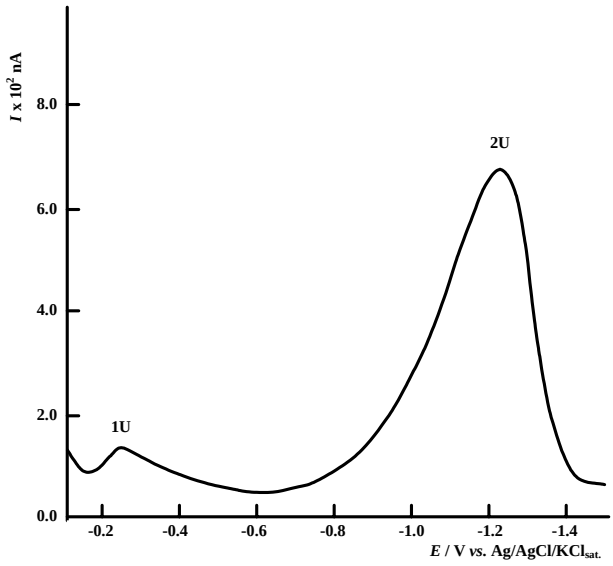


Fig. 1. Square-wave voltammogram of 5×10^{-5} M OXA in B–R buffer (pH 7.0) (Experimental conditions: scan rate, 200 mV s^{-1}; drop size, medium and equilibrium time, 5 s). 1U, Adsorption peak; 2U, reduction of heterocyclic isoxazol ring of OXA.

The cyclic voltammogram of OXA in the potential range of –0.60 to –1.6 V is given in Fig. 2. As can be seen, the main reduction peak of OXA (Fig. 2, 2U) has an anodic counterpart. The currents (i_{p2}) of the main reduction peak for OXA are proportional to scan rates (v) in the range of 50 to 500 mV s^{-1}; the linear equation of the log i_{p2} *vs.* log v relationship was log i_{p2} = 0.8495 log v + 0.0812 (r = 0.994). From the value of the slope, 0.8495, of the log i_{p2} *vs.* log v relationship of the main peak, it can be deduced that the main reduction peak of OXA is diffusion-controlled with an adsorption contribution. According to the potential difference $\Delta E_p = E_{pa} - E_{pc}$ for the main peak of OXA, αn is calculated to be 0.72 (where α and n denote the transfer coefficient and the number of electron transferred, respectively). The electrochemical redox reaction of OXA at a mercury electrode in B–R buffer (pH 7.0) solution is a quasi-reversible process with an anodic counterpart and αn = 0.72, as shown in Fig. 2 (2U).

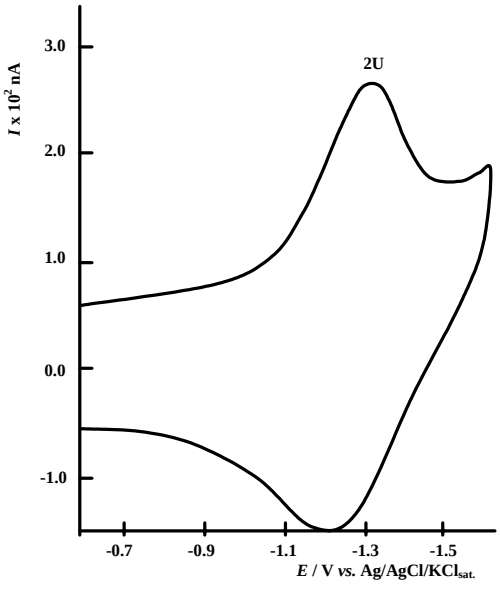


Fig. 2. Cyclic voltammogram of 1×10^{-4} M OXA in B–R buffer (pH 7.0) (Experimental conditions: scan rate, 500 mV s^{-1}; drop size, medium and equilibrium time, 5 s). 2U, Reduction of heterocyclic isoxazol ring of OXA.

It is well known that ampicillin and amoxycillin are electrochemically inactive at a dropping mercury electrode (DME) in aqueous solution.[14] The molecular structure of OXA is different from these owing to the presence of the electroactive isoxazol derivative group. In the literature, it was reported that the reduction of the heterocyclic isoxazol ring occurs at approximately –1.0 V.[15] Based on this fact, the main peak at –1.224 V can probably be attributed to the cathodic reduction of the heterocyclic isoxazol ring of OXA. However, the exact mechanism of the electrode reaction of OXA will be the subject of a further study.

Interaction of OXA with RSH

The square-wave voltammograms of RSH in the absence and presence of OXA are shown in Fig. 3. Under these experimental conditions, 1.0×10^{-5} M RSH had a well-developed cathodic peak at –0.536 V (Fig. 3, 2U) which corresponds to $Hg_2(SR)_2$ and after the addition of OXA to the RSH solution, the peak current of $Hg_2(SR)_2$ decreased, its peak potential shifted to more positive potentials (–0.354 V) and a new small, more positive peak appeared at –0.160 V (Fig. 3, 1U). The main reduction peak of OXA also appeared at a more positive potential (–1.114 V (Fig. 3, 3U)) in the presence of RSH. The peak currents of the peaks at –0.160 and –1.114 V increased with increasing concentration of OXA. According to the obtained voltammetric data, it can be stated that these peaks originate from the electrode reaction of OXA in the presence of RSH.

A typical labelled antigen–antibody binding curve for $Hg_2(SR)_2$ is shown in Fig. 4. A similar curve was observed by Heineman *et al.*[16] for 4-mercuric acetate estriol with estriol antibody. In addition, similar curves were also obtained for the

interaction of RSH with some monosaccharides.[17] Immunochemical reactions are among the most selective reactions known.[18] They are based on shape recognition of the antigen by the antibody binding site.[18] A voltammetric immunoassay relies on the monitoring of the binding *via* the decrease in the current response for the redox reaction of the labelled antigen in the presence of antibody.[18] A similar principle applies to the labelled antibody current which decreases in the presence of antigen.[18] In this first report on a voltammetric immunoassay, Heineman *et al.*[16] labelled estriol with mercuric acetate (as the electroactive moiety) and monitored the reaction of this labelled antigen with estriol antibody.

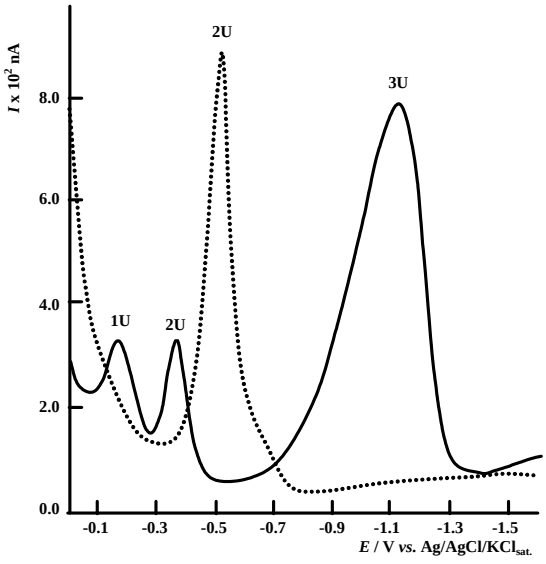


Fig. 3. Square-wave voltammograms of 1×10^{-5} M RSH in the presence of 0 M (·····) and 5×10^{-5} M OXA (—) in B–R buffer pH 7.0 (Experimental conditions as in Fig. 1). 1U, Adsorption peak of OXA; 2U, reduction of $Hg_2(SR)_2$; 3U, reduction of heterocyclic isoxazol ring of OXA.

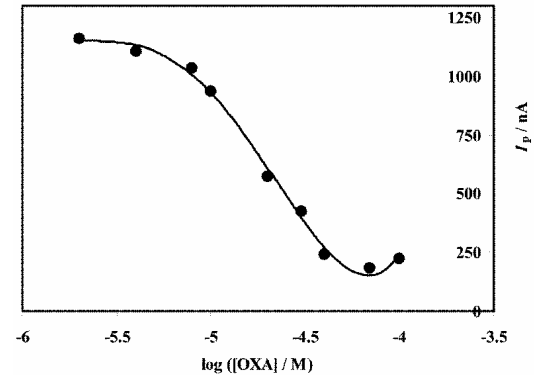


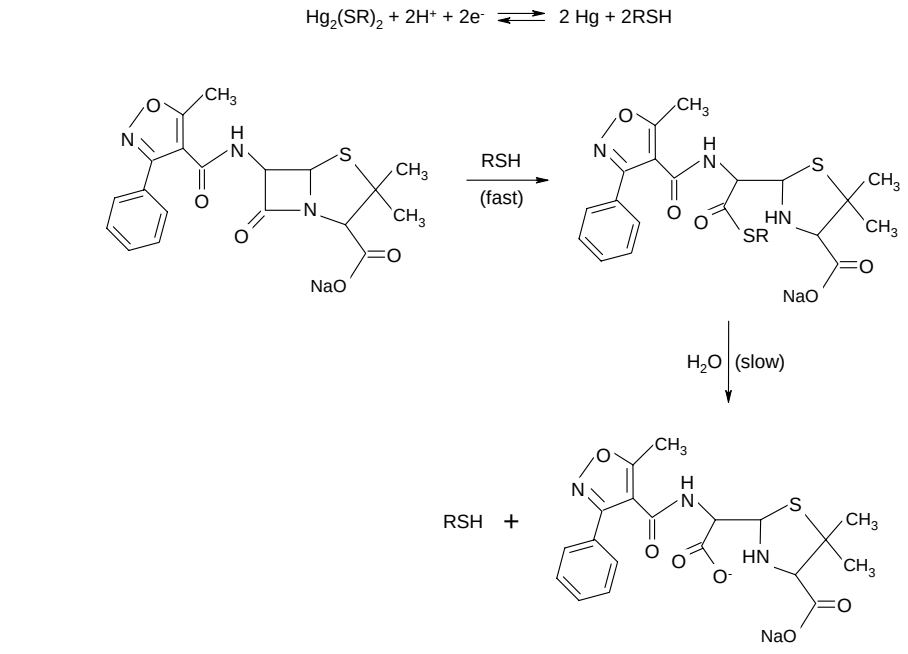
Fig. 4. Plot of peak current of $Hg_2(SR)_2$ *vs.* log [OXA].

Shifts of the peak potential of $Hg_2(SR)_2$ to more positive values was also observed for the interaction of thiols and folates on the surface of mercury.[19,20] The effect of a chemical reaction following a reversible electron transfer is the facilitation of the redox process, *i.e.*, a shift of electroreduction towards positive po-

tentials.[19] The faster is the chemical reaction, the more positive is the potential of the cathodic peaks.[20] With very fast follow-up reactions, the peak can shift (at a constant scan rate) by as much as 120 mV.[19] On the other hand, an added adsorbable substance usually acts as an inhibitor of a reversible electrode reaction and shifts the reduction processes to negative potentials.[21] However, the positive shift of the reduction potential of $Hg_2(SR)_2$ in the presence of OXA is 182 mV. Hence, the interaction of OXA with RSH can be inferred from the follow-up chemical reaction between them. As a result, the anodic shifts of the reduction potential and the decrease in the current of $Hg_2(SR)_2$ may be due to the binding of RSH with OXA on the mercury electrode.

Llinás *et al.*[2,22] studied the thiol-catalysed hydrolysis of cephalosporins[2] and benzylpenicillin.[22] They reported that thiols catalyse the hydrolysis through the formation of a thioester intermediate and the catalytically reactive form of the thiol is the thiolate anion.[2,22] Thiolysis of penicillins occurs with the rate-limiting breakdown of the tetrahedral intermediate facilitated by proton transfer from the solvent water to the departing amine.[22] In a similar manner to the reaction of thiols with cephaloridine in water,[2] the reaction between RSH and OXA can be as given in Scheme 2.

Scheme 2 represents a fast follow up reaction. In addition, the fixed current of $Hg_2(SR)_2$ in the presence of excess OXA is probably due to the reproduction of RSH according to Scheme 2.



Scheme 2. Proposed mechanism for interaction of OXA with RSH.

Interaction of OXA with Mn(II) ions

The square-wave voltammogram of 1.0×10^{-4} M Mn(II) ions in B–R buffer (pH 7.0) in the absence of OXA has a peak at a potential of –1.650 V (Fig. 5, 5U). This cathodic peak was attributed to the irreversible reduction of Mn(II) ions to Mn(0), because no anodic counterpart was observed on the cyclic voltammogram of Mn(II) ions in B–R buffer (pH 7.0). The addition of OXA (Fig. 5) to the electrolyte containing Mn(II) ions strongly modified the square-wave voltammogram and two new peaks appeared at the potentials of –0.152 and –0.630 V (1U and 3U). These peaks are at more positive potentials than the peak produced by Mn(II) ions in the absence of OXA. With increasing OXA concentration, the peak currents of the new peaks increased and their peak potentials shifted to slightly positive values (–0.146 and –0.608 V) while the peak current of free Mn(II) ions was decreased (Fig. 5). These peaks can be attributed to the formation of Mn(II) complexes with different metal/ligand ratios on the mercury surface. OXA has catalytic activity on the reduction of Mn(II). It may be concluded that the voltammetric process is the reduction of Mn(II) catalysed by the formation of a complex between Mn(II) and OXA adsorbed on the electrode surface (Scheme 3). Similar results were obtained with cephalexin and Ni(II).[23] However, the peaks at –0.146 and –0.608 V can be attributed to the formation of Mn(II) complexes with different metal/ligand ratios.

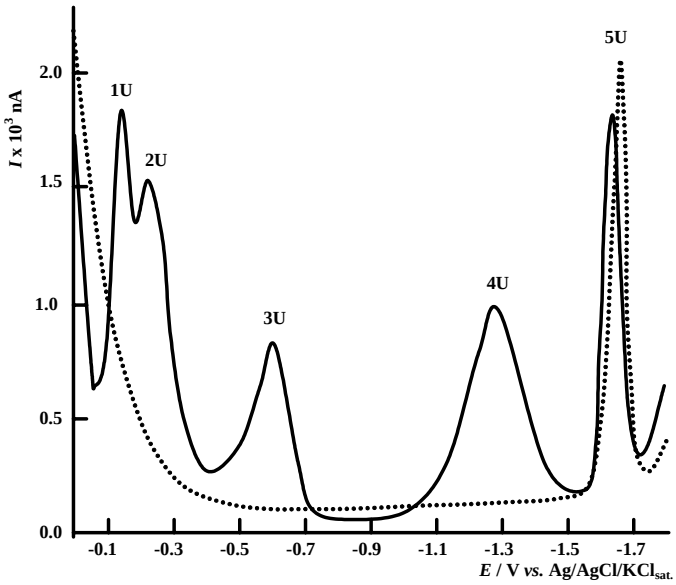
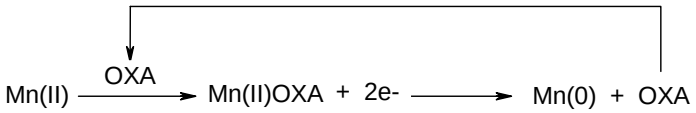


Fig. 5. Square-wave voltammograms of 1.0×10^{-4} M Mn(II) (·····) and 1.0×10^{-4} M Mn(II) + 8.0×10^{-4} M OXA (—) in B–R buffer pH 7.0 (Experimental conditions as in Fig. 1). 1U and 3U, catalytic peaks of Mn(II) in the presence of OXA; 2U, adsorption peak of OXA; 4U, reduction of heterocyclic isoxazol ring of OXA; 5U, the reduction of free Mn(II).



Scheme 3. The proposed pathway for the catalytic reduction of Mn(II).

Interaction of RSH with Mn(II) ions

The square-wave voltammogram of 1.0×10^{-4} M Mn(II) in the presence of RSH is shown in Fig. 6, from which it can be seen that with increasing RSH concentration, the peak current of free Mn(II) decreased, while reduction peaks of mercuric and mercurous cysteine thiolates ($Hg(SR)_2$) and $Hg_2(SR)_2$) were observed at –0.132 and –0.598 V (Fig. 6, 1U and 2U). In addition, a new small peak was observed at –0.704 V (Fig. 6, 3U) and its peak current increased with increasing RSH concentration. This additional peak can be attributed to the reduction of the adsorbed chelate. The appearance of the chelate peak depends on the molar ratio, [RSH]/[Mn(II)]. The chelate peak was observed at molar ratios ≥1:2.

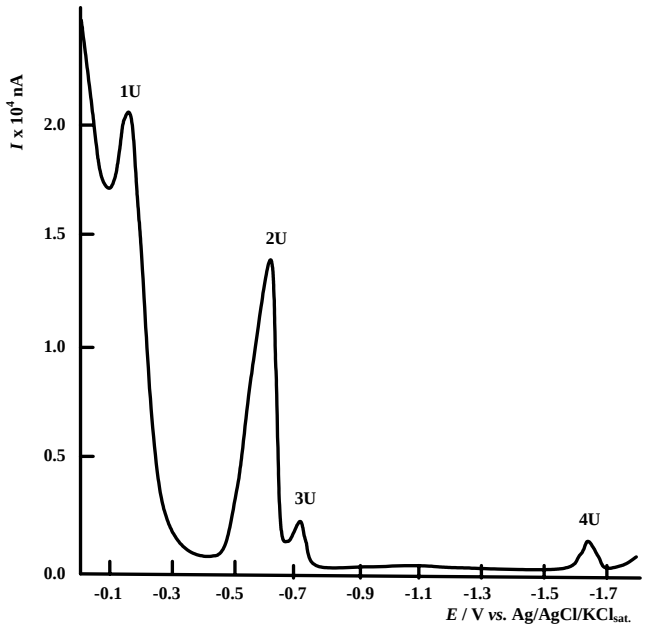


Fig. 6. Square-wave voltammogram of 4.0×10^{-4} M RSH in the presence of 1.0×10^{-4} M Mn(II) in B–R buffer pH 7.0 (Experimental conditions as in Fig. 1). 1U, Reduction of $Hg(SR)_2$; 2U, reduction of $Hg_2(SR)_2$; 3U, catalytic peak of Mn(II) in the presence of RSH; 4U, the reduction of free Mn(II).

Interaction between OXA and RSH in the presence of Mn(II) ions

With addition of RSH solution to the cell containing 1.0×10^{-4} M Mn(II) and 8.0×10^{-4} M OXA, the peak at –0.608 V (Fig. 5, 3U) vanished and a reduction peak was observed at more negative potential (–0.662 V) (Fig. 7, 4U). The current of this reduction peak increased and its peak potential shifted towards more negative values with increasing RSH concentration. On the other hand, a new

peak was also seen at –0.490 V (Fig. 7, 3U). This peak (–0.490 V) belongs to the reduction of $Hg_2(SR)_2$. As its reduction was observed at more positive potentials in the presence of OXA (see above).

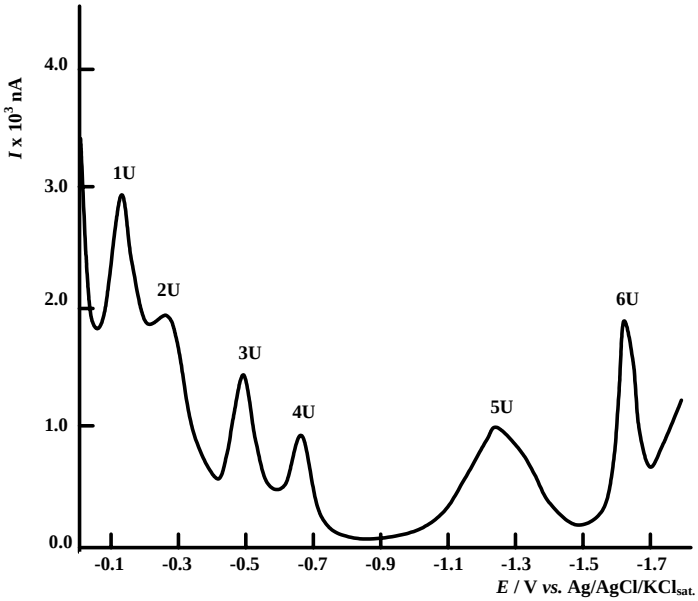


Fig. 7. Square-wave voltammogram of 1.0×10^{-4} M Mn(II) and 8.0×10^{-4} M OXA mixture in the presence of 1.0×10^{-4} M RSH in B–R buffer pH 7.0 (Experimental conditions as in Fig. 1). 1U, Reduction of $Hg(SR)_2$; 2U, adsorption peak of OXA; 3U, reduction of $Hg_2(SR)_2$ in the presence of OXA; 4U, catalytic peak of Mn(II) in the presence of RSH and OXA; 5U, reduction of heterocyclic isoxazol ring of OXA; 6U, the reduction of free Mn(II).

According to the data, it can be said that RSH interacts with OXA in the presence or absence of Mn(II) ions. Moreover, OXA catalyzes the reduction of Mn(II). However, this catalysis process is thermodynamically difficult in the presence of RSH because of the fact that the catalytic reduction peak potential of Mn(II) is observed at more negative potentials. Perhaps, Mn(II) forms a mixed ligand complex with adsorbed species of OXA and RSH on the surface of the mercury electrode.

Complex formation between Mn(II) and OXA or RSH and also the interaction of OXA with RSH may give valuable information concerning what occurs when administrating OXA drug. On the other hand, the voltammetric techniques could be used to determine these interactions. Consequently, the observed interactions *in vitro* may perhaps be employed as a model for the metabolic processes of OXA in the living matter.

И З В О Д

ВОЛТАМЕТРИЈСКО ИСПИТИВАЊЕ ИНТЕРАКЦИЈА ИЗМЕЂУ НАТРИЈУМ--ОКСАЦИЛИНА И ЦИСТЕИНА У ПРИСУСТВУ И ОДСУСТВУ Mn(II) ЈОНА У НЕУТРАЛНОМ ПУФЕРСКОМ РАСТВОРУ

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У раду је испитивано волтаметријско понашање натријум-оксацилина (OXA) и интеракције OXA и цистеина (RSH) у Британ–Робинсоновом (Britton–Robinson, B–R) пуферу pH 7.0 коришћењем волтаметрије са правоугаоним таласом и цикличне волтаметрије. OXA је показала два волтаметријска пика: на –0.248 и –1.224 V. За испитивање интеракција изабран је пик цистеинтиолата живе ($Hg_2(SR)_2$). Запажено је да струјни пикови који потичу од $Hg_2(SR)_2$ након додатка OXA значајно опадају и да се потенцијал пика помера ка позитивнијим вредностима. То потиче од интеракција OXA са RSH на површини живине електроде. Када се у електрохемијску ћелију додају и OXA и Mn(II) јони, појављују се нови пикови на –0.146 и –0.608 V. Они потичу од каталитичке активности OXA за редукцију Mn(II) јона и могу се приписати формирању Mn(II) комплекса са различитим односима металног јона и лиганда. С друге стране, у присуству RSH пик на –0.608 V нестаје, а запажа се редукциони пик на –0.662 V. Потенцијал пика каталитичке редукције Mn(II) на –0.662 V указује да RSH у извесној мери умањује каталитичку активност услед њихове међусобне интеракције.

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REFERENCES

1. http://www.cancer.org/docroot/CDG/content/CDG_oxacillin_sodium.asp
2. A. Llinás, B. Vilanova, M. I. Page, *J. Phys. Org. Chem.* **17** (2004) 521
3. B. M. Beadle, R. A. Nicholas, B. K. Shoichet, *Protein Sci.* **10** (2001) 1254
4. K. Graves–Woodward, R. F. Pratt, *Biochem. J.* **332** (1998) 755
5. S. Barbosa, P. Taboada, V. Mosquera, *Chem. Phys.* **310** (2005) 51
6. K. F. Nakken, L. Eldjarn, A. Pihl, *Biochem. Pharmacol.* **3** (1960) 89
7. B. B. Levine, *J. Exp. Med.* **112** (1960) 1131
8. E. L. Smith, R. L. Hill, I. R. Lehman, R. J. Lefkowitz, P. Handler, A. White, *Principles of Biochemistry: General Aspects*, International Student Edition, Seventh Edition, McGraw–Hill Book Company, London, 1985, p.p. 474, 397, 355, 325, 179
9. S. Y. Bi, L. Ding, D. Q. Song, Y. Tian, H. Q. Zhang, *Acta Chim. Sinica* **63** (2005) 2169
10. A. M. Bressler, T. Williams, E. E. Culler, W. M. Zhu, D. Lonsway, J. B. Patel, F. S. Nolte, *J. Clin. Microbiol.* **43** (2005) 4541
11. M. A. Zeitlinger, R. Sauermann, F. Traunmuller, A. Georgopoulos, M. Muller, C. Joukhadar, *J. Antimicrob. Chemother.* **54** (2004) 876
12. D. Mihai, G. Mocanu, A. Carpov, *J. Bioact. Compat. Polym.* **15** (2000) 245
13. A. Dasgupta, A. Sperelakis, A. Mason, R. Dean, *Pharmacotherapy* **17** (1997) 375
14. S. J. Lyle, S. S. Yassin, *Anal. Chim. Acta* **274** (1993) 225
15. M. Al–Omar, A. Al–Majed, E. A. Gadkariem, F. Belal, *J. Pharm. Biomed. Anal.* **37** (2005) 199
16. W. R. Heineman, C. W. Anderson, H. B. Halsall, *Science* **204** (1979) 865
17. S. Çakır, E. Biçer, O. Çakır, *Glycoconjugate J.* **16** (1999) 579
18. J. Wang, Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine, 1988, VCH Publishers, Inc., New York, p. 42

19. M. Heyrovský, B. Prokopová, *Collect. Czech. Chem. Commun.* **62** (1997) 172
20. B. Prokopová, M. Heyrovský, *Bioelectrochem. Bioenerg.* **41** (1996) 209
21. J. Heyrovský, J. Kůta, *Principles of Polarography*, Academic Press, New York, 1966, p. 299
22. A. Llinás, J. Donoso, B. Vilanova, J. Frau, F. Muñoz, M. I. Page, *J. Chem. Soc., Perkin Trans.* **2** (2000) 1521
23. J. H. Méndez, A. S. Pérez, M. D. Zamerrreño, L. V. Laso, *Anal. Chim. Acta* **160** (1984) 335.