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MILKA L. AVRAMOV IVIĆ[1]
SLOBODAN D. PETROVIĆ[2,3]
PREDRAG M. ŽIVKOVIĆ[2]
DUŠAN Ž. MIJIN[2]
KATICA M. DRLJEVIĆ[4]

[1]ICTM - Institute of Electrochemistry, University of Belgrade, Belgrade, Serbia
[2]Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia
[3]Hemofarm, Stada, Pharmaceutical and Chemical Industry, Vršac, Serbia
[4]Medicines and Medical Devices Agency of Serbia, Belgrade, Serbia

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A STUDY OF THE CATALYTIC ROLE OF A GOLD ELECTRODE IN THE ELECTROCHEMICAL ACTIVATION OF FOUR MACROLIDE ANTIBIOTICS IN SODIUM BICARBONATE SOLUTION

Using the cyclic voltammetry, it has been shown that hydrogen evolution at a gold electrode is necessary in the electrochemical activation of azithromycin dihydrate and erythromycin A. After four hours of the potential holding at -1.2 V vs. SCE, the pH of the electrolyte has been changed from 8.40 to 8.96; from 8.40 to 8.77 in the presence of erythromycin A, and from 8.40 to 9.18 in the presence of azithromycin, indicating the reaction of the hydrogen species with antibiotics. This effect has been confirmed by using the phenolphthalein indicator and by analysing colours of the solutions by UV-Vis, as well as by FTIR spectroscopy. Under the identical experimental conditions at the gold electrode, in contrast to azithromycin dihydrate and erythromycin A, roxithromycin and midecamycin electroactivity promotion has been obtained during the first forward sweep starting from the area of a double layer region.

Key words: macrolide antibiotics; catalytic role; gold electrode; UV-Vis; spectroscopy.

The electrochemical activities of azithromycin, erythromycin A and clarithromycin at a gold electrode surface in 0.05 M $NaHCO_3$ were reported recently [1--5]. It was shown that the gold electrode could be successfully applied for the qualitative and quantitative electrochemical determination of azithromycin dihydrate and azithromycin from capsules (Hemomycin®) [1].

On reaching the potential value of -1.2 V *vs.* SCE, hydrogen evolution occurs at a gold electrode in 0.05 M $NaHCO_3$ and, consequently, the equivalent amount of OH^- species is evolved which act as the initial catalyst for the anodic oxidation of azithromycin [1]. It was observed that the electrochemical activities of erythromycin A and clarithromycin are also promoted by hydrogen evolution [2-5] under identical experimental conditions.

Purging of molecular hydrogen into the electrolyte and the addition of OH^- with the calculated amount of NaOH did not cause the electrochemical activity of azithromycin. The change of the pH value of electrolyte by the addition of calculated amount of sodium hydroxide and sulfuric acid and the use of carbonate buffer solution (pH 8.6) also had no effect. It was obvious that only the evolved electroactive species promote the anodic oxidation of azithromycin. It was noticed that the ionization of azithromycin dihydrate in 0.05 M $NaHCO_3$ was realized by its interaction with evolved hydrogen species and OH^- species [1]. All presented phenomena including figures in the Results and discussion develop the presentation of the catalytic role of the species evolved at the gold electrode surface. Several patent applications concerning the improvement of azithromycin solubility by ionization of the pure molecule in order to apply the antibiotic in ophthalmology support this assumption [6,7]. Erythromycin A and azithromycin dihydrate were selected for comparison in the study of the catalytic effect of hydrogen evolution because of their different structures (Fig. 1).

It was observed recently that it is not necessary to reach the potential of hydrogen evolution for the beginning of roxithromycin and midecamycin electroactivity. Their quite different structures from azithro-

Correspondening author: M.L. Avramov Ivić, ICTM - Institute of Electrochemistry, University of Belgrade, Njegoševa 12, Belgrade, Serbia.
E-mail: milka@tmf.bg.ac.rs
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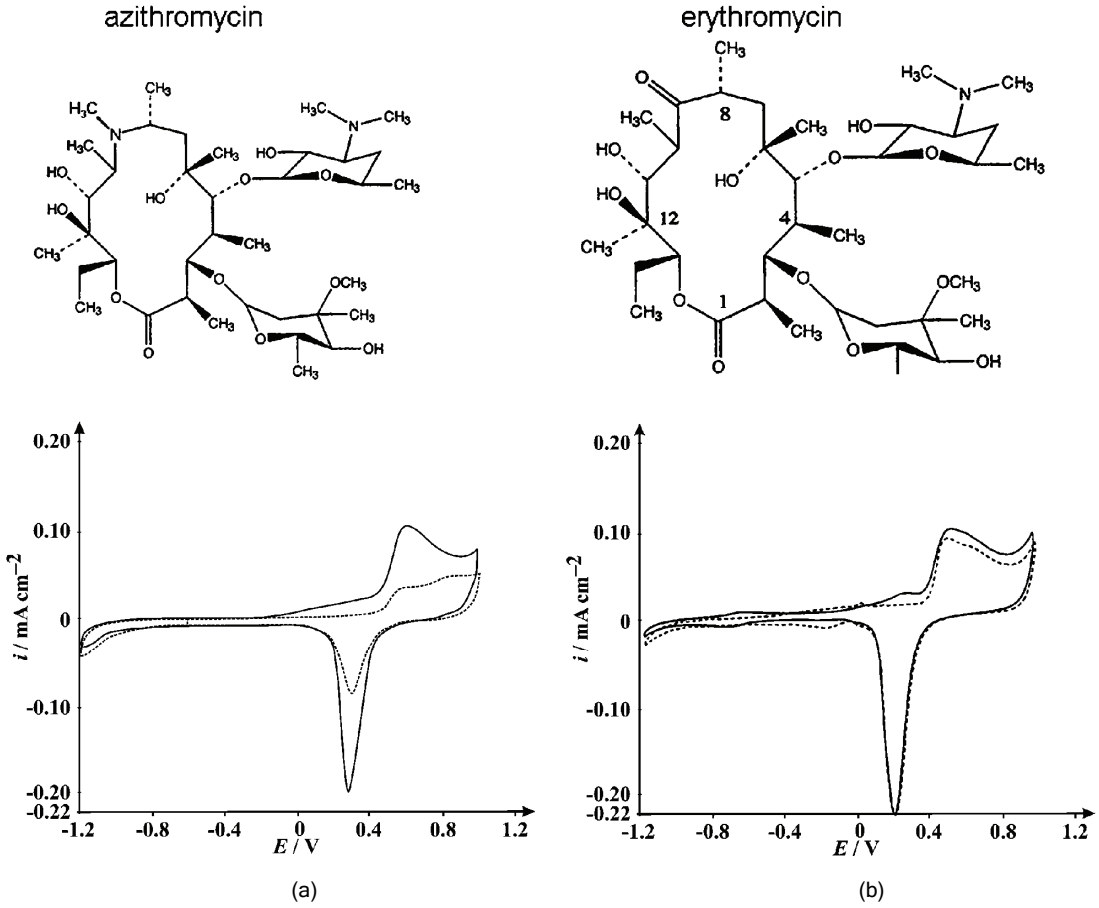

Figure 1. a) Chemical structure of azithromycin and cyclic voltammogram of gold electrode in 0.05 M $NaHCO_3$ (dashed line) and in the presence of 0.35 mg cm^{-3} of pure azithromycin dihydrate (full line), sweep rate 50 mV s^{-1}; b) chemical structure of erythromycin A and cyclic voltammogram of gold electrode in 0.05 M $NaHCO_3$ (dashed line) and in the presence of 0.40 mg cm^{-3} erythromycin succinate (full line), sweep rate 50 mV s^{-1}.

mycin and erythromycin A are presented in Fig. 2. Cyclic voltammetry shows that even starting from –0.6 V *vs.* SCE at a gold electrode in 0.05 M $NaHCO_3$, the apparent activity of both antibiotics is observed in a first forward sweep.

The aim of this work was to present and elucidate the catalytic role of the gold electrode surface in electrochemical activation of azithromycin dyhidrate, erythromycin A, roxithromycin and midecamycin. The behavior of azithromycin and erythromycin A was studied by cyclic voltammetry and at the potential of hydrogen evolution under potentiostatic conditions followed by pH measurements of the electrolyte and FTIR spectroscopy. Hydrogen evolution as the initial catalyst in the electrochemical activity of azithromycin was analyzed using the phenolphthalein reaction combined with UV-Vis spectroscopy. The catalytic role of the gold electrode in electrochemical behavior of roxithromycin and midecamycin was examined by cyclic voltammetry followed by the phenolphthalein reaction combined with UV-Vis spectroscopy.

EXPERIMENTAL

Azithromycin dihydrate and erythromycin ethyl succinate, kindly provided by Hemofarm Stada (Vršac, Serbia) and "Zorka Pharma" a.d. (Šabac, Serbia), were used as pure substances. Midecamicyin, kindly provided by Krka (Novo Mesto, Slovenia) and roxithromycin, by the Medicines and Medical Devices Agency of Serbia (Belgrade, Serbia) were also used as pure substances. All the antibiotics were added directly into electrolyte to obtain the following concentrations: azithromycin dihydrate (0.35 and 0.60 mg cm^{-3}), erythromycin succinate (0.40 mg cm^{-3}), roxithromycin (0.35 mg cm^{-3}) and midecamycin (0.40 mg cm^{-3}).

All reagents used were of analytical grade (Merck): $NaHCO_3$ and a 1 % ethanol solution of phenolphthalein. The solutions were prepared with 18 MΩ water and 0.05 M $NaHCO_3$ was used as the electrolyte. Standard equipment was employed for the potentiostatic measurements and the three electrode electrochemical cell was previously described in detail [1-5].

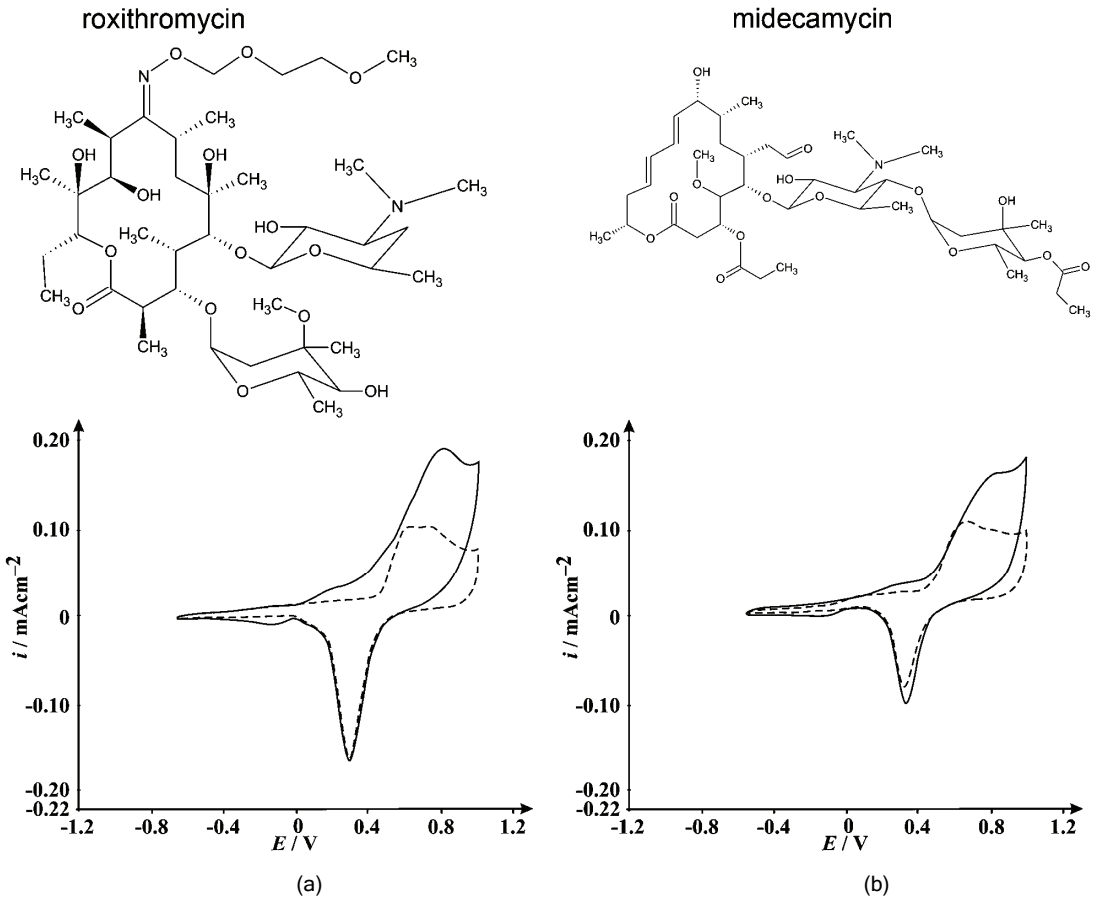

Figure 2. a) Chemical structure of roxithromycin and cyclic voltammogram of gold electrode in 0.05 M $NaHCO_3$ (dashed line) and in the presence of 0.35 mg cm^{-3} of pure roxithromycin (full line), sweep rate 50 mV s^{-1}; b) chemical structure of midecamycin and cyclic voltammogram of gold electrode in 0.05 M $NaHCO_3$ (dashed line) and in the presence of 0.40 mg cm^{-3} midecamycin (full line), sweep rate 50 mV s^{-1}.

The polycrystalline gold (surface area 0.500 cm^2), which served as the working electrode, was polished with diamond paste, cleaned with a mixture of 18 MΩ water and sulfuric acid and further cleaned with 18 MΩ water in an ultrasonic bath. A platinum wire served as the counter electrode, saturated calomel electrode as the reference electrode and all the potentials are given *vs.* SCE. The electrolyte was deoxygenated by purging with nitrogen. All the experiments were performed at room temperature. Before the potentiostatic measurements, the cyclic voltammetry response on the added concentration of azithromycin and of erythromycin ethyl succinate and HPLC analysis of the bulk of electrolyte were performed in the same manner as already described [1,5]. The characteristics of the employed HPLC instrument are as follows: HPLC Agilent 1100 binary pump 1100, electrochemical detector 1100, autosampler 1100 series.

In the experiments with phenolphthalein, 0.1 cm^3 of an alcoholic solution were added into 110 cm^3 of 0.05 M $NaHCO_3$ before the potentiostatic measurements. The pH of electrolyte was measured using a pH meter, Hanna Instruments 9321.

The UV-Vis spectra were obtained using a Shimadzu 1700 UV-Vis spectrophotometer. The IR spectra were recorded using a FTIR BOMEM MB 100 Hartmann Braun FTIR spectrometer. The analyses were performed as described earlier [2].

RESULTS AND DISCUSSION

An apparent suppression of the hydrogen evolution at a gold electrode surface in 0.05 M $NaHCO_3$ at the beginning (at -1.2 V) and during the electrochemical reactions in the case of azithromycin and erithromicin A [1,5] is presented by cyclic voltammograms in Fig. 1. Potentiostatic measurements at -1.2 V could aid in the better understanding of the observed effect. The first experiment was performed by potential holding at -1.2 V at clean gold electrode during four hours. The next experiment was performed in the same way but in the presence of 0.6 mg

cm^{-3} azithromycin. In addition, the same potentiostatic measurements were performed in the presence of 0.6 mg cm^{-3} of erythromycin A. The cyclic voltammograms, in the first stage of the clean gold electrode and then in the presence of azithromycin and erythromycin A are displayed in Fig. 1. The pH value of the electrolyte was measured before and at the end of each experiment. During four hours at the potential holding at -1.2 V the pH changed from 8.40 to 8.96 and from 8.40 to 9.18, in 0.05 M $NaHCO_3$ solution and azithromycin solution, respectively. This indicates the increased hydrogen acceptance by azithromycin, *i.e.*, by the dominant proton acceptor, the tertiary nitrogen of the macrolide ring. It is known that protonation of amine moieties leads to water soluble ammonium salts [8,9]. In order to confirm the hydrogen acceptance by azithromycin using another method, experiments in the absence and in the presence of an antibiotic were performed in the same manner as described above but with the addition of phenolphthalein [10]. Before and after the potentiostatic measurements for four hours, the pink-colored electrolytes in an electrochemical cell were photographed and analyzed by UV-Vis spectroscopy. The results are presented in Fig. 3. The pink color of the electrolyte before the electrochemical treatment was more intensive in the presence of azithromycin then without it (Fig. 3a). The affinity to the hydrogen consumption by azithromycin from 0.05 M $NaHCO_3$ without any electrochemical reaction is obvious. It is also obvious (Fig. 3b) that the pink color of the electrolyte after the the evolving of hydrogen at -1.2 V at gold electrode during four hours was apparently more intensive in the presence of azithromycin. The difference can be attributed only to the consumption of the hydrogen evolved at gold electrode by azithromycin, as indicated by cyclic voltammetry (Fig. 1) [1]. The UV-Vis spectra of the bulk electrolyte before and after holding the potential during four hours clearly show that the value of the absorbance was much higher after the electrochemical treatment then at the beginning of the potential holding in the presence of the antibiotic (Fig. 3b). These data are in accordance with a detected increase in the alkality of the electrolyte.

The experiments with erythromycin A, starting from the pH measurements after four hours of potential holding at -1.2 V, showed that the pH of the 0.05 M $NaHCO_3$ changed from 8.40 to 8.77. This change was smaller than that observed with azithromycin. This can be attributed to the lower ability of the carbonyl group of erythromycin for proton acceptance compared to the tertiary nitrogen of azithromycin as a part of a similar nucleus (macrolide ring) [9]. This indicates a lower consumption of hydrogen species by erythromycin A.

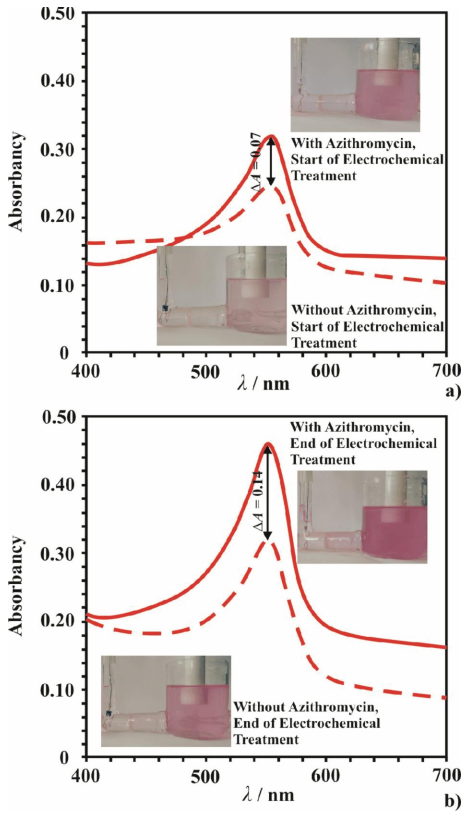

Fig. 3. UV-Vis spectra and photographs of the bulk electrolyte (0.05 M $NaHCO_3$) in a electrochemical cell in the absence and in the presence of 0.60 mg cm^{-3} of azithromycin dehydrate a) before and b) after four hours of holding the potential at -1.2 V vs. *SCE.*

The bulk electrolytes containing azithromycin and erythromycin A were analyzed by FTIR spectroscopy. FTIR spectra of pure azithromycin, erythromycin and azithromycin and erythromycin mixed with carbonates before the electrochemical experiment served as references for further analysis. The azithromycin FTIR spectrum reveals obvious changes after potential holding for 4 h at -1.2 V: an intense reduction of the 1721 cm^{-1} peak corresponding to the carbonyl group vibration of the lactone, and the disappearance of the 1180 cm^{-1} peak, probably corresponding to the C-O vibration in the lactone, which implies changes in the ester bond of the lactone. No absorptions were recorded in the 1000-1100 cm^{-1} range, which could be the result of changes in the ether and acetal bonds. Concerning erythromycin, similar changes were observed: the disappearance of the 1736 cm^{-1} and the 1168 cm^{-1} peak and no absorption in the 1000-1100 cm^{-1} range. FTIR spectra of azithromycin and erythromycin suggest apparent changes in the lac-

tone of macrolide ring, *i.e.*, changes in the ester bond of the lactone, and in the ether and acetal bonds.

HPLC analysis of the bulk electrolyte after four hours of the potential holding at –1.2 V showed that the concentration of azithromycin dihydrate and erythromycin A slightly decreased in agreement with the changes observed by FTIR spectroscopy and according to previous publications [1-5] and literature data [11-14].

All the experimental data suggest that the role of the evolution of hydrogen at the gold electrode, as a catalyst for the electroactivation of azithromycin and erythromycin at the gold electrode in 0.05 M $NaHCO_3$, is in protonation of the macrolide ring and in improved ionization. Many publications report the successful electrochemical oxidation and determination of macrolide antibiotics in aqueous electrolytes and buffers containing organic molecules [15-18] in order to attain their ionization. The herein presented results in pure 0.05 M $NaHCO_3$ give the possibility of employing the effect of hydrogen evolution for improvement of the ionization of azithromycin dihydrate and erythromycin A in cases when organic molecules should be obligatorily avoided.

The cyclic voltammograms, in the first stage of the clean gold electrode and then in the presence of roxithromycin and midecamycin, characterizing their electrochemical activity are displayed in Fig. 2. From Fig. 2a, it is obvious that starting from –0.6 V, the apparent electrochemical activity of roxithromycin is obtained in the first cycle. Under identical experimental conditions, in contrast to azithromycin and erythromycin A, it is sufficient to begin sweeping from the potential in the area of a double layer region at the gold electrode in order to catalyze the roxithromycin activity. The observed characteristic behavior is attributed to the fact that the lactone ring erithronolide A is modified by the replacement of the 9 keto group by an etheroxime side chain, in molecule of roxithromycin [9] as is presented in Fig. 2. From Fig. 2b, is also clear that starting from –0.6 V, the apparent electrochemical activity of midecamycin was obtained in the first cycle. The independence of the beginning of its electrochemical activity on hydrogen evolution at a gold electrode could be attributed to the existence of the aldehyde group in the sixth position of the macrolide nucleus as is presented in Fig. 2. The catalytic role of the gold electrode is the same as in the case of roxithromycin.

The experiment with phenolphtalein shows that the pink color of the electrolyte remains the same in the presence of roxithromycin and midecamycin *i.e.* is the same as it was observed at the start of the electrochemical treatment without the antibiotic (photos at the bottom in Fig. 3a). The UV-Vis spectra confirmed the opinion showing the exactly same value of the absorbance. pH measurements show that pH value of the electrolyte remains unchanged in the presence of roxithromycin and midecamycin. It clearly indicates that there is no affinity to the hydrogen consumption by roxithromycin and midecamycin from 0.05 M $NaHCO_3$ without and with electrochemical reaction.

CONCLUSION

The catalytic role of the gold electrode differs in the case of azithromicin and erythromycin A from roxithromycin and midecamycin under the identical experimental conditions.

By cyclic voltammetry it was shown that the beginning of the hydrogen evolution is necessary in the electrochemical activation of azithromycin dihydrate and erythromycin A. They also exhibited the affinity to the hydrogen consumption from electrolyte. This was confirmed by potentiostatic measurements followed by pH measurements, phenolphtalein reaction followed by UV-Vis spectroscopy and FTIR spectroscopy. Contrary to azithromycin dihydrate and erythromycin A, it is not necessary to reach the potential of the hydrogen evolution for the beginning of roxithromycin and midecamycin electroactivity. Cyclic voltammetry shows that their activity in the first forward sweep began starting from the area of a double layer region. Besides the absence of the necessities for the hydrogen evolution for promoting their electrochemical activity, roxithromycin and midecamycin exhibited no affinity to the hydrogen consumption from electrolyte. This was confirmed by pH measurements and phenolphtalein reaction followed by UV-Vis spectroscopy.

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MILKA L. AVRAMOV IVIĆ[1]
SLOBODAN D. PETROVIĆ[2,3]
PREDRAG M. ŽIVKOVIĆ[2]
DUŠAN Ž. MIJIN[2]
KATICA M. DRLJEVIĆ[4]

[1]IHTM – Centar za elektrohemiju, Univerzitet u Beogradu, Beograd
[2]Tehnološko-metalurški fakultet, Univerzitet u Beogradu, Beograd
[3]Hemofarm, Stada, Farmaceutska i hemijska industrija, Vršac,
[4]Agencija za lekove i medicinska sredstva Srbije, Beograd

NAUČNI RAD

ISPITIVANJE KATALITIČKE ULOGE ELEKTRODE OD ZLATA U ELEKTROHEMIJSKOJ AKTIVACIJI ČETIRI MAKROLIDNA ANTIBIOTIKA U NATRIJUM-BIKARBONATNOM ELEKTROLITU

Metodom ciklične voltametrije je pokazano da je izdvajanje vodonika na elektrodi od zlata neophodno za elektrohemijsku aktivaciju azitromicin-dihidrata i eritromicina A. Četiri sata držanja potencijala na vrednosti 1,2 V vs. *SCE je rezultovalo u promeni pH elektrolita sa 8,40 na 8,96; sa 8,40 na 8,77 u prisustvu eritromicina A, i sa 8,40 na 9,18 u prisustvu azitromicina, ukazujući na reakciju čestica izdvojenog vodonika sa antibioticima. Efekat je potvrđen upotrebom fenolftalein indikatora i analizom boje elektrolita UV-Vis spektroskopijom, a molekula antibiotika FTIR spektroskopijom. Pod istim eksperimentalnim uslovima na elektrodi od zlata, u suprotnosti sa ponašanjem azitromicin-dihidrata i eritromicina A, elektroaktivnost roksitromicina i midekamicina je uočena počevši od oblasti formiranja dvojnog sloja, tj. bez ikakvog učešća čestica izdvojenog vodonika.*

Ključne reči: makrolidni antibiotici; katalitička uloga; elektroda od zlata; UV-Vis; spektroskopija.