A study of the electrochemical activity of some macrolide antibiotics on a gold electrode in a neutral electrolyte

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Abstract: The aim of the present study is to present the different reactivity of azithromycin and clarithromycin (pure and commercial) at a gold electrode in neutral electrolyte using cyclic linear sweep voltammetry under the same experimental conditions. A gold electrode was successfully used for the electrochemical qualitative and quantitative determination of azithromycin dihydrate and azithromycin from capsules (Hemomycin®) and for the separation of azithromycin from one of the excipients, lactose monohydrate. The good catalytically activity of the gold electrode was employed only for the qualitative electrochemical determination of pure clarithromycin by appearance of one cathodic and four anodic reactions, which enabled structural changes in this molecule during electrochemical reactions to be studied. Commercial clarithromycin, Clathrocyn® was qualitative determined by one reproducible anodic reaction. The activity of one of the excipients, Avicel, observed as a cathodic peak at different potential from the cathodic peak obtained with pure clarithromycin was used for the determination of its presence in Clathrocyn® tablets. FTIR Analysis showed the apparent changes in structure of pure clarithromycin, as well as in the molecule of clarithromycin in Clathrocyn® tablets. HPLC Analysis showed a significant decrease in the concentration of azithromycin, Hemomycin® clarithromycin and Clathrocyn® after the electrochemical reactions.

Keywords: azithromycin, clarithromycin, gold electrode, cyclic voltammetry.

INTRODUCTION

Macrolide antibiotics are active on both gram-positive and, to a lesser extent, on gram-negative microorganisms. They exert their antimicrobial action by binding to the bacterial 50S ribosomal subunit and inhibiting ribosomal assembly and protein synthesis. Erythromycin is a natural compound metabolized by a strain of Streptomyces erythreus. As a broad-spectrum antibiotic, it has proved invaluable for the treatment of bacterial infections in patients with β-lactam hypersen-
sitivity. From this parent macrolide, several derivatives have been synthesized. Beckmann rearrangement of the 9-oxime followed by reduction and methylation gives azithromycin, which shows good activity against gram-negative bacteria, including *Haemophilus influenzae*. If the 6-hydroxy group is methylated, clarithromycin is obtained, which has an improved pharmacokinetic profile compared to the parent molecule. Azithromycin has a methylated nitrogen atom at position number nine on the macrolide lactone ring, and clarithromycin has a methyloxy substitution at position number six of the macrolide ring (Fig. 1). These modifications affect the biological potency of the molecules, with azithromycin having a stronger affinity for ribosome and affecting equally the 50S ribosomal assembly and protein elongation.\(^1\)\(^,\)\(^2\) Clarithromycin also affects both processes but at a concentration 10 times higher than that of azithromycin, and erythromycin inhibits translation much more efficiently than the ribosomal assembly.\(^2\)

![Fig. 1. Chemical structures of azithromycin, erythromycin and clarithromycin.](image)

Azithromycin and clarithromycin present several clinical advantages over erythromycin, including better oral bioavailability, enhanced spectrum activity, higher tissue concentrations and improved tolerability.\(^3\) Clarithromycin is widely used for the eradication of *Helicobacter pylori*, which causes gastritis and gastric ulcers.\(^4\) Oral clarithromycin is used in combination with amoxicillin and lansoprazole or omeprazole (triple therapy) for the treatment of *Helicobacter pylori* infections and duodenal ulcers.\(^5\)\(^−\)\(^7\) Quantitative methods using high performance liquid chromatography (HPLC) procedures for the analysis of azithromycin and clarithromycin have been widely applied.\(^8\)\(^−\)\(^11\) Electrochemical methods for azithromycin determination are promising, being the cheaper and faster. Studies on the electrochemical oxidation and determination of azithromycin on glassy carbon and modified glassy carbon electrodes have been frequently published in the last few years. Hitherto, voltammetric determinations of azithromycin, including its determination in pharmaceutical dosage forms have been published.\(^12\)\(^,\)\(^13\) In
the above-mentioned publications, the electrode material was usually glassy carbon, graphite and carbon paste or multi wall carbon nanotubes.

The aim of the present study is to present the different reactivity of azithromycin and clarithromycin (pure and commercial) in order to develop an electrochemical method for their qualitative and quantitative determination, as well as to separate or determine them in the presence of some excipient compounds in capsule (tablets) form. The macrolide antibiotics were examined at a gold electrode in neutral electrolyte using cyclic linear sweep voltammetry under the same experimental conditions. HPLC and FTIR spectroscopy were used for the analysis of the bulk electrolyte after the electrochemical reactions.

EXPERIMENTAL

Azithromycin dihydrate and clarithromycin, kindly provided by Hemofarm (Vršac, Serbia), were examined separately as pure substances, without further purification, in one set of electrochemical experiments. A comparative study was performed with capsulated azithromycin, marketed by Hemofarm as Hemomycin®, which except azithromycin contains the excipients: sodium lauryl sulfate, magnesium stearate, lactose monohydrate and corn starch. A comparative study was also performed with clarithromycin in tablet form, marketed by Hemofarm as Clathrocyn®, which in addition to pure clarithromycin contains the excipients: magnesium stearate, corn starch, Povidon 490, Avicel P401 and Prosolv SMC 90. The pure macrolide antibiotics and the content of the tablets (capsules) were added directly into the electrolytes in the concentrations given in detail in the Fig. captions. Before each measurement the electrolytes were purged with nitrogen for 20 minutes.

The NaHCO₃ used for the supporting electrolyte was of analytical grade (Merck). The solutions were prepared with 18 MΩ water. Standard equipment and a three electrode electrochemical cell were used for the cyclic voltammetry measurements, as previously described in detail.¹⁴⁻¹⁸ Polycrystalline gold (surface area 0.500 cm²), which served as the working electrode, was polished with diamond paste and cleaned with a mixture of 18 MΩ water and sulfuric acid. A platinum wire was used as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All the potentials are given vs. SCE. The electrode surface was controlled by cyclic voltammetry before each experiment. Prior to the control of the electrode surface and before the addition of the macrolide antibiotics, the electrolyte was purged with nitrogen. All the experiments were performed at room temperature.

The pH of the electrolyte was measured using a PHM 93 reference pH meter, Radiometer, Copenhagen.

The characteristics of the HPLC instrument are as follows: HPLC Instrument GBC, pump LC 1120, UV VIS detector LC 1205, manual injector RHEODYNE 7725i, column Asahipak ODP-50 (250 mm×4 mm), stationary phase L21 (USP-a rigid, spherical styrene–divinyl benzene copolymer, 5 µm), mobile phase 0.002 M diammonium hydrogen phosphate, propanol-2, acetonitrile (pH 9.5, flow rate 1.0 cm³ min⁻¹), wave length 215 nm.

The IR spectra were obtained using a FTIR BOMEM MB 100 Hartmann Braun FTIR spectrometer. The samples were analyzed in the form of KBr pellets after removal of the liquid under high vacuum at low temperature.

RESULTS AND DISCUSSION

A polycrystalline gold electrode was selected as the optimal working electrode for the examination of pharmaceutical compounds because it is completely defined with an always reproducible cyclic voltammogram and consequently, all
the electrochemical reactions at this electrode can be attributed only to the studied molecule. In order to avoid the influence of organic molecules (with direct oxidation/reduction ability or adsorption ability at a gold electrode) arising from a solvent or a part of a buffer solution in the electrolyte and to obtain only the reactions of the pharmaceutical compounds, 0.05 M NaHCO₃ was selected as the supporting electrolyte.

Cyclic voltammetry investigations of pure azithromycin showed that the oxidative peak of pure azithromycin at 0.6 V in 0.05 M NaHCO₃ at a scan rate of 50 mV s⁻¹ is a linear function of the concentration:

\[ i_{pa} \text{ (mA cm}^{-2}) = 0.023 (±0.0043) + 0.110 (±0.0099) c \text{ (mg cm}^{-3}) \text{, } r = 0.9921 \]  

Cyclic voltammograms presented in Fig. 2 show that for every investigated azithromycin dihydrate concentration only one very clear, wide and reproducible anodic peak was obtained in the concentration range 0.235–0.588 mg cm⁻³. It is to be mentioned that there is no cathodic activity of azithromycin.

![Fig. 2. Anodic part of the cyclic voltammogram of an Au electrode in 0.05 M NaHCO₃ (—) and with the addition of 0.235, 0.353, 0.471 and 0.588 mg cm⁻³ pure azithromycin dihydrate (full line); the lowest concentration is assigned by one arrow and the highest with four arrows. Sweep rate 50 mV s⁻¹.](image)

The anodic part of the cyclic voltammograms obtained with azithromycin in capsule form, Hemomycin®, is presented in Fig. 3, from which it is obvious that two peaks appeared. The peak at 0.6 V, which is the oxidative peak of pure azithromycin is already observed in Fig. 2, but second peak at 0.4 V is the oxidative peak of the one excipient, lactose monohydrate. Sodium lauryl sulfate, magnesium stearate and corn starch are electrochemically totally inactive under the applied experimental conditions. Using the anodic behavior of azithromycin in capsule, Hemomycin®, shown in Fig 3, an electrochemical method for the separation of azithromycin from lactose monohydrate at a gold electrode surface was developed. From the reproducible anodic peak at 0.4 V, lactose monohydrate is always recognized in the tested contents of capsule, which is important for the additional control of the contents of Hemomycin® capsules using a non time consuming and simple electrochemical method.
Each of the investigate concentrations of Hemonycin® gave a reproducible anodic oxidation peak and a linearity of the anodic peak current vs. concentration was obtained, as in the case of pure azithromycin. The linearity of the anodic peak current vs. concentration was obtained for lactose monohydrate in the investigated range of concentrations presented in Fig. 3, which opens the possibility for the development of a method for the quantitative detection of this compound.

HPLC Analysis of the bulk of electrolyte confirmed the data obtained by analysis of the peak current values concerning the correlation with the investigate concentrations for azithromycin and Hemonycin®.

Azithromycin has a methylated nitrogen atom at position nine on the macrolide lactone ring and clarithromycin has a methyloxyl substitution at position six of the macrolide ring (Fig. 1), which suggests possible different electrochemical activities of this two compounds.

The anodic part of the cyclic voltammogram of pure clarithromycin is presented in Fig. 4, from which it can be seen that in the anodic direction, a small, wide and reproducible anodic peak appears at \(-0.58\) V.

Two reproducible anodic peaks were also observed at 0.10 V and at 0.33 V. In the region of AuO formation, a minor reproducible increase of the oxide peaks is seen. In this region the apparent activity of azithromycin (Fig. 2) can be used for its qualitative and quantitative determination. At \(-0.58\) V in the anodic direction, azithromycin is not active and at 0.10 V and at 0.33 V, just a small increase of anodic currents is obvious, but there are no peaks (Fig. 2).

In Fig. 5 it is shown that a reproducible cathodic peak was obtained with clarithromycin at \(-0.61\) V. However, with clarithromycin none of the observed peaks are proportional to the concentration of the antibiotic in the range of 0.110–\(-0.625\) mg cm\(^{-3}\), which is in accordance with the conclusion that four reproducible anodic peaks and one cathodic peak only qualitatively determine clarithromycin.

In order to get insight in the structural changes in the clarithromycin molecule during electrochemical reactions, electrochemical studies combined with the
analysis of the bulk electrolyte after the electrochemical reactions by FTIR spectroscopy and HPLC were performed.

![Anodic part of the cyclic voltammogram](image1)

**Fig. 4.** Anodic part of the cyclic voltammogram of an Au electrode in 0.05 M NaHCO$_3$ (---) and with the addition of 0.32 mg cm$^{-3}$ of pure clarithromycin, (full line). Sweep rate 50 mV s$^{-1}$.

![Cathodic part of the cyclic voltammogram](image2)

**Fig. 5.** Cathodic part of the cyclic voltammogram of an Au electrode in 0.05 M NaHCO$_3$ (---) and with the addition of 0.32 mg cm$^{-3}$ of pure clarithromycin, (full line). Sweep rate 50 mV s$^{-1}$.

The FTIR spectrum of pure clarithromycin and clarithromycin mixed with carbonates, both before the electrochemical experiment, served as reference for the further analysis. The observed changes in the molecule of clarithromycin were tracked with these data. The potential was held at selected values corresponding to all the observed current peaks (at $-0.58$ V, $0.10$ V, $0.33$ V and $-0.61$ V) for 4, 8 and 10 h and two samples of the electrolyte were analyzed by FTIR spectroscopy and HPLC. Significant changes in the molecular structure of clarithromycin were observed when the potential had been held at the potential of the cathodic peak observed in Fig. 5 ($-0.61$ V) for 4 h, 8 and 10 h.

The FTIR spectrum reveals two obvious changes after holding the potential: the disappearance of the 1730 cm$^{-1}$ peak corresponding to the carbonyl group vi-
bration of the lactone, and an intense reduction of the 1170 cm\(^{-1}\) peak, probably corresponding to the C–O vibration in the lactone, which implies changes in the ester bond of the lactone. The disappearance of the carbonyl band at 1690 cm\(^{-1}\) indicates a change in this group also. No absorptions were recorded in the 1000–1100 cm\(^{-1}\) range, which could be the result of changes in the ether and acetal bonds.\(^\text{15}\) When the potential was held at the cathodic peak potential observed in Fig. 5 (−0.61 V) for 8 and 10 h, the FTIR spectrum showed the same changes.

The FTIR analysis did not reveal clear changes in the molecule structure after 4 and 10 h of holding the potential at 0.10 V and at 0.33 V, except a minor reduction of the bands in the 1000–1100 cm\(^{-1}\) region.

Cyclic voltammogram investigation of clarithromycin in tablets, Clathrocyn\(^\text{®}\), indicated a different behavior from the pure antibiotic and that observed activities can be attributed to the excipient Prosolv, as an anodic peak at 0.33 V and to the excipient Avicel, as a cathodic peak at −0.90 V, which could be important for the electrochemical control of theirs contents in Clathrocyn\(^\text{®}\),\(^\text{16}\) although a concentration dependency of commercial clarithromycin and Avicel was not observed.

The anodic part of the cyclic voltammogram of Clathrocyn\(^\text{®}\) as presented in Fig. 6, shows only one reproducible and characteristic, sharp anodic peak in the potential region from 0.60 V to 0.80 V in the range of concentrations 0.110–0.725 mg cm\(^{-3}\).

![Anodic part of the cyclic voltammogram of an Au electrode in 0.05 M NaHCO\(_3\) (---) and with addition of 0.56 mg cm\(^{-3}\) of Clathrocyn\(^\text{®}\) (full line). Sweep rate: 50 mV s\(^{-1}\).
](image)

In order to investigate the structural changes in the clarithromycin molecule in Clathrocyn\(^\text{®}\) tablets caused by electrochemical processes, electrochemical studies combined with the analysis of the bulk electrolyte after the electrochemical reactions by Fourier transform infrared spectroscopy and high performance liquid chromatography were performed, similar to the investigation of pure clarithromycin.\(^\text{16}\)

The FTIR spectrum of the bulk electrolyte after cycling the potential, in the range presented in Fig. 6 for 8 h shows: the disappearance of the peak at 1730 cm\(^{-1}\).
(carbonyl group of lactone) and the very small peak at 1170 cm\(^{-1}\) (C(O)–O–C vibrations of the lactone), indicating the disappearance of the lactone structure. Also, the reduction of intensity of the vibration band at 1695 cm\(^{-1}\) indicates changes of the carbonyl group at position 9 of the clarithromycin molecule in Clathrocyn\textsuperscript{®} tablets. The same changes in the clarithromycin molecule in Clathrocyn\textsuperscript{®} tablets were observed under the experimental conditions previously published.\textsuperscript{16} As in the case of pure clarithromycin, this FTIR data combined with cyclic voltammetry can be helpful in the qualitative detection of the examined commercial clarithromycin.

HPLC Analysis of the bulk of electrolyte (the potential was held for 4 h at the 0.10 V and 0.70 V) showed a significant decrease (40 %) of the concentration of clarithromycin, both pure and commercial, as a consequence of the electrochemical reactions which had occurred.

Erythromycin was chosen as the macrolid antibiotic for the subject of the present studies because today it is the most safe antimicrobes drug and it was 162\textsuperscript{nd} of the top 300 prescribed drugs in the year 2005 (which includes different drugs, for example, drugs for neoplastic action (20) (FDA)),\textsuperscript{19} while azithromycin was 176\textsuperscript{th} in the year 2005.\textsuperscript{20} Cyclic voltammetry, HPLC and FTIR results with erythromycin suggest electrochemical activity similar to clarithromycin.\textsuperscript{20}

**CONCLUSIONS**

In a conclusion, it should be pointed out that clarithromycin investigated under the same experimental conditions as azithromycin showed quite different behavior, which is attributed to the fact that azithromycin has a methylated nitrogen atom at position number nine on the macrolide lactone ring while clarithromycin has a methyloxyl substitution at position number six of the macrolide ring. It was shown that a gold electrode can be successfully employed for the qualitative and quantitative electrochemical determination of azithromycin dihydrate and azithromycin from capsules (Hemomycin\textsuperscript{®}) and for the separation of azithromycin from lactose monohydrate. Lactose monohydrate can also be quantitatively determined.

The good catalytic activity of a gold electrode can be employed only for the qualitative electrochemical determination of pure clarithromycin by the appearance of the one cathodic and four anodic reactions, which enables structural changes in this molecule during electrochemical reactions to be studied. FTIR analysis showed significant structural changes in the clarithromycin molecule, i.e., changes in the ester bond of the lactone and changes in the ether and acetal bonds. Clarithromycin in tablets, Clathrocyn\textsuperscript{®}, is defined by one reproducible anodic peak and the activity of the excipient Avicel was used for the determination of its presence in Clathrocyn\textsuperscript{®} tablets. FTIR analysis showed changes in the structure of the molecule indicating the disappearance of the lactone moiety and changes of carbonyl group at position 9. HPLC Analysis of the bulk elec-
trolyte showed a significant decrease in the concentration of all the examined macrolide antibiotics, as a consequence of the electrochemical reactions which had occurred.

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REFERENCES