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The qualitative electrochemical determination of clarithromycin and spectroscopic detection of its structural changes at gold electrode

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Abstract

The aim of the present study was the qualitative determination of the pure clarithromycin using a gold electrode in neutral electrolyte by cyclic linear sweep voltammetry. It was shown that in the range of −1.2 V to 1.0 V vs. SCE in 0.05 M $NaHCO_3$, a gold electrode is successfully employed for the qualitative determination of clarithromycin by detection of the reproductive four anodic and one cathodic peaks. After the potentiostatic measurements at the potential values corresponded to current peaks, the bulk electrolyte was analyzed by FTIR spectroscopy to show the changes in molecular structure of clarithromycin. FTIR analysis of the bulk electrolyte after 4 h of holding the potential at −0.61 V vs. SCE (cathodic peak) showed the apparent changes in clarithromycin molecule structure: in the ester bond of the lactone and in ethers and acetal bonds.

Keywords: Clarithromycin; Qualitative determination; Gold electrode; Cyclic voltammetry; FTIR spectroscopy

1. Introduction

Erythromycin is a natural compound metabolized by a strain of *Streptomyces erythreus.* It has proved invaluable for the treatment of bacterial infections in patients with β-lactam hypersensitivity. From this parent macrolide, several derivatives have been synthesized. 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 methyloxyl substitution at position number six of the macrolide ring (Fig. 1). Azithromycin and clarithromycin present several clinical advantages over erythromycin, including enhanced spectrum activity, higher tissue concentrations, and improved tolerability [1–3]. Clarithromycin is widely used for the eradication of *Helicobacter pylori* that causes gastritis and gastric ulcers [4–7]. Quantitative methods using high performance liquid chromatography (HPLC) procedures for the analysis of azithromycin and clarithromycin have been widely applied [8–11]. Some information on clarithromycin voltammetry (Klaricid® tablets, obtained from Glaxowellcome, S. Korea) was presented in the literature related to electrochemical detection in HPLC procedures [12]. It is reported that amperometric detector with glassy carbon working electrode was used in a condition of hydrodynamic voltammetry after column-switching HPLC preparation of human plasma containing metabolized Klaricid® tablets. The data concerning the only electrochemical identification of not metabolized tablets, their content and its influence on the detection comparing to pure clarithromycin are not provided [12].

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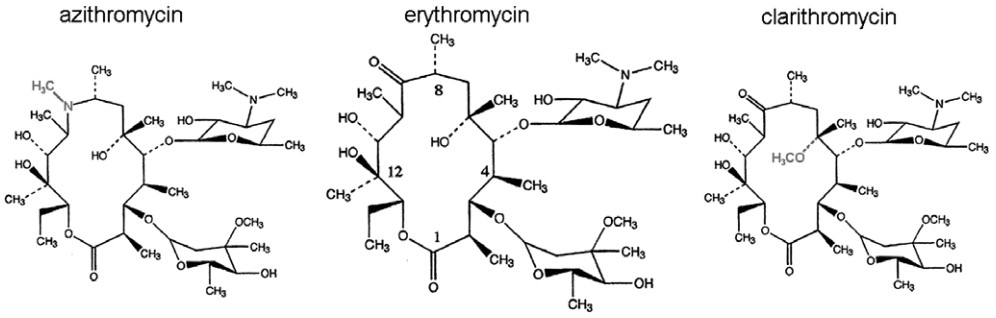

Fig. 1. Chemical structures of azithromycin, erythromycin and clarithromycin.

The electrochemical methods are cheaper and faster. Hitherto, voltammetric determinations of azithromycin [13,14] have been published. We previously reported the oxidative behavior and qualitative and quantitative determination of azithromycin at a gold electrode [15]. As in the case of azithromycin, gold electrode is selected as the best one for the examination of the next member of macrolide antibiotics family [15]. The aim of the present study was the qualitative determination of the pure clarithromycin using its reactivity at a gold electrode in neutral electrolyte by cyclic linear sweep voltammetry. After the potentiostatic measurements, the bulk electrolyte was analyzed by FTIR spectroscopy to detect the changes in the structure of clarithromycin molecule. It was also analyzed by HPLC.

2. Experimental

Clarithromycin, provided by pharmaceutical company, Hemofarm, was used as a pure substance. It was added directly into the electrolyte, which was purged with nitrogen for 20 min before each measurement, in the concentrations in the range of 0.235–0.588 mg cm^{-3}. $NaHCO_3$ used for the supporting electrolyte were 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 [15–19].

Polycrystalline gold (surface area 0.500 cm^2), which served as the working electrode, was prepared by polishing with diamond paste, and cleaning with a mixture of 18 MΩ water and sulfuric acid. Platinum wire was used as the counter electrode and a saturated calomel electrode as the reference electrode. Prior to the control of the electrode surface, which was performed by cyclic voltammetry before each experiment, the electrolyte was purged with nitrogen. All the experiments were performed at temperature of 20 °C, and all the potential values are given vs. saturated calomel electrode.

The pH of the electrolyte before and after addition of clarithromycin was measured using 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 × 4 mm), stationary phase L21 (USP-a rigid, spherical styrene–divinyl copolymer, 5 μm), mobile phase 0.002 M diammonium hydrogen phosphate, propanol-2, acetonitrile (pH 9.5, flow rate 1.0 ml/min), 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.

3. Results and discussion

A polycrystalline gold electrode was already selected as the optimal working electrode for the examination of different organic molecules including azithromycin [15,18,19]. As in a case of azithromycin, for clarithromycin, our choice was 0.05 M $NaHCO_3$ as the supporting electrolyte [15]. The solubility of clarithromycin in water is very poor, and it is slightly soluble in methanol [20]. The

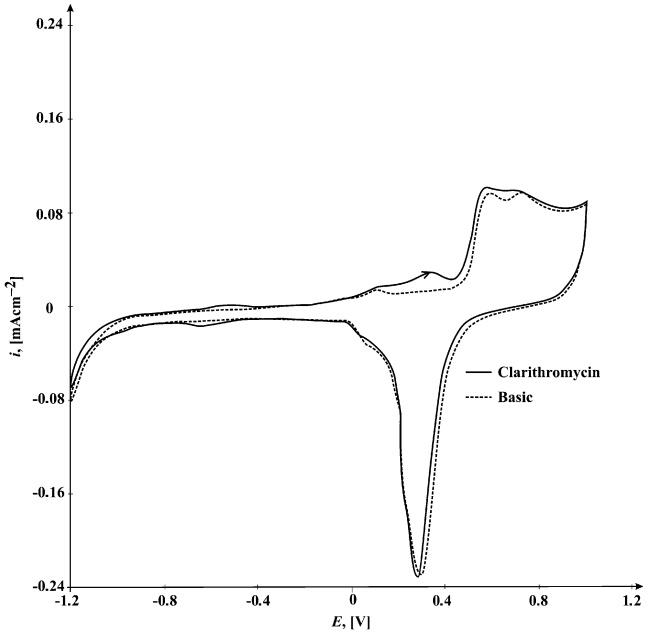

Fig. 2. Cyclic voltammogram of the Au electrode in 0.05 M $NaHCO_3$ (——) and with the addition of 0.4 mg cm^{-3} of pure clarithromycin, third sweep, (full line), sweep rate: 50 mV/s.

methanol is avoided as the solvent (in a mixture with water) because of its activity on the oxides of gold and silver electrodes in different electrolytes [17–19].

In the first stage, after addition of the antibiotic into the electrolyte, the potential was cycled between −0.6 V and 1.0 V. The electrochemical activation of clarithromycin is not observed as was the case with azithromycin [15]. It was necessary to reach the negative potential value of −1.2 V vs. SCE with the hydrogen evolution occurrence at the gold electrode. The electrochemical activation of clarithromycin and the hydrogen evolution suppression is obvious from Fig. 2 and the same effects were already observed with azithromycin [15]. In Fig. 2 is also presented that starting from −1.2 V vs. SCE in anodic direction, the cyclic voltammogram first shows one small, wide and reproducible anodic peak with a current maximum at −0.58 V vs. SCE. The two reproducible anodic peaks were also observed at +0.10 V vs. SCE and at +0.33 V vs. SCE. In the region of AuO formation, a minor increase of the oxide peaks of the gold electrode was observed. The reproducible cathodic peak is present in the reverse direction with a current maximum at −0.61 V vs. SCE.

It is shown for clarithromycin that observed peaks are not proportional to the concentration of antibiotic in the range of 0.235–0.588 mg cm^{-3}. In this range of concentrations the four reproducible anodic and one cathodic peaks always qualitatively determine clarithromycin. In order to investigate the structural changes in clarithromycin molecule, electrochemical studies combined with the analysis of the bulk electrolyte after the electrochemical reactions by FTIR spectroscopy and HPLC were performed.

FTIR spectrum of pure clarithromycin and clarithromycin mixed with carbonates, both before the electrochemical experiment, served as reference for the further analysis (Fig. 3). The observed changes in the molecule of clarithromycin were tracked with these data. The potential was held

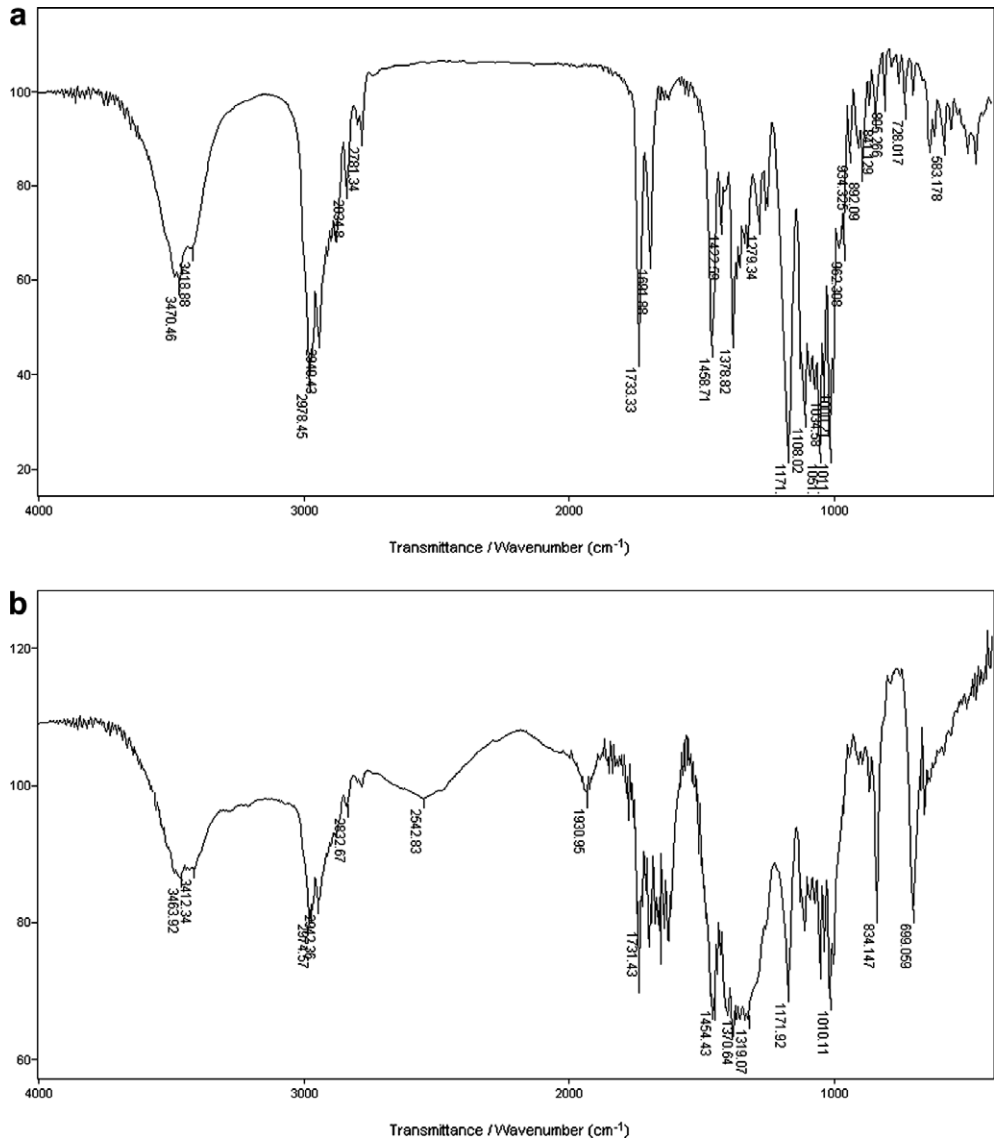

Fig. 3. Infrared spectra of pure clarithromycin (a) and pure clarithromycin mixed with carbonate ($NaHCO_3$) (b).

at selected values corresponded to all observed current peaks for 4 h. The first sweep after holding the potential was recorded by cyclic voltammetry and two samples of the electrolyte were analyzed by FTIR and HPLC. The potential was held for 4 h at −0.58 V, at +0.10 V, at +0.33 V and at −0.61 V vs. SCE.

The significant changes in clarithromycin molecular structure were observed when potential was held for 4 h at −0.61 V, at the cathodic peak, observed in Fig. 2. The first sweep after 4 h of holding the potential is presented in Fig. 4a and shows that, in addition to the current increase previously described in Fig. 2 (around 0.70 V vs. SCE) the anodic current rises by the end of the anodic scan. A current increase at the potentials corresponding to OH^- adsorption/desorption was observed, not only during the first sweep, but also in the three subsequent sweeps at least.

The FTIR spectrum reveals two obvious changes after potential holding (Fig. 4b): the disappearance of the 1730 cm^{-1} peak corresponding to the carbonyl group vibration 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.

The FTIR analysis did not reveal clear changes in the molecule after 4 h of holding the potential at +0.10 V vs.

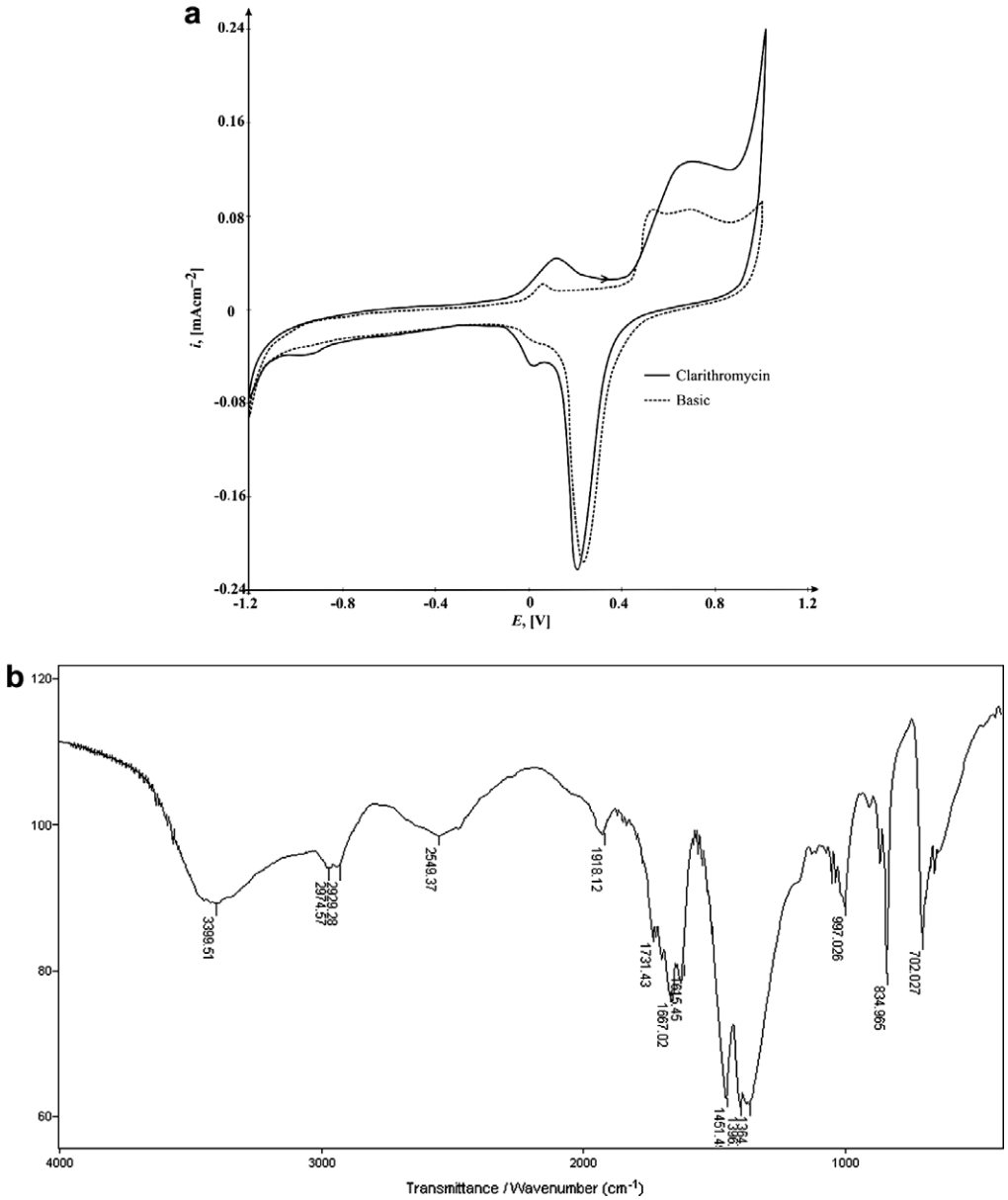

Fig. 4. (a) Cyclic voltammogram of the first sweep of the Au electrode in the presence of 0.4 mg cm^{-3} pure clarithromycin after the potential had been held for 4 h at −0.61 V vs. SCE in 0.05 M $NaHCO_3$ (full line). The voltammogram of the pure gold electrode is presented by the dashed line, sweep rate: 50 mV/s. (b) Infrared spectrum of 0.4 mg cm^{-3} pure clarithromycin in 0.05 M $NaHCO_3$ solution after 4 h electroreduction at −0.61 V vs. SCE under the conditions described in (a).

SCE and at +0.33 V vs. SCE, except a minor reduction of the bands in the 1000–1100 cm^{-1} region.

HPLC analysis of the bulk electrolyte, showed a significant decrease in the concentration of clarithromycin after the potential was held at selected values for 4 h.

The qualitative determination of commercial clarithromycin (provided by Hemofarm and tested in tablets) at gold electrode, followed by FTIR analysis, is already successfully performed and will be published separately [21].

4. Conclusion

A gold electrode can be successfully employed for the qualitative electrochemical determination of pure clarithromycin in 0.05 M $NaHCO_3$. The good catalytical surface of gold electrode for the activation and consequently for the one cathodic and four anodic reactions of pure clarithromycin enables structural changes in this molecule during electrochemical reactions to be studied.

FTIR analysis of the bulk electrolyte after 4 h of holding the potential at −0.61 V vs. SCE (cathodic peak) showed significant structural changes: changes in the ester bond of the lactone and changes in ethers and acetal bonds.

HPLC analysis of the bulk electrolyte, after 4 h of the applied electrochemical conditions, showed a significant decrease in the concentration of clarithromycin.

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