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Identification of 5-aminosalicylic acid, ciprofloxacin and azithromycin by abrasive stripping voltammetry

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Abstract

Solid microparticles of 5-aminosalicylic acid, ciprofloxacin, and azithromycin were mechanically immobilized on the surface of the paraffin impregnated graphite electrode and investigated by square-wave and cyclic voltammetry in order to develop a method for their qualitative determination. 5-Aminosalicylic acid is oxidized at 0.540 V in the quasireversible electrode reaction, which is followed by the chemical transformation of the product, while ciprofloxacin and azithromycin are oxidized at 1.2 V and 0.94 V, respectively, in totally irreversible electrode reactions. The detection of these drugs in commercial dosage formulations is reported.

Keywords: Abrasive stripping voltammetry; Microcrystals; 5-Aminosalicylic acid; Ciprofloxacin; Azithromycin

1. Introduction

Voltammetry of immobilized microcrystals is electrochemical method for qualitative analysis of minerals, alloys, and sparingly soluble organometallic and organic compounds [1–3]. The method is based on the mechanical transfer of insoluble solid microparticles on the surface of a carbon rod, which is immersed into an aqueous electrolyte and used as a working electrode in standard voltammetric experiments. Investigated materials can be either metallic or both ionic and electronic conductors. In the latter case, the change of charge on the metal ions in these compounds, which occurs in their electrochemical reactions, is accompanied by the exchange of charge compensating ions with the liquid electrolyte and by their diffusion through the particle body together with the propagation of electrons. So, the transfer of an electron across the electrode/particle interface and the transfer of an ion at the particle/solution interface occur simultaneously. This type of reactions was observed with some metal oxides and complexes [4,5], metal hexacyanometallates [6–8], and several organometallic compounds [9–12]. If the conduction of ions is hindered, the reaction will be localized at the particle/solution interface. Such surface reactions are characteristic for redox reactions which are coupled with the exchange of protons between some insoluble organic compounds and the dissolved acids [13–15]. Exhaustive bibliography of this research can be found on the following Internet address: www.iic.cas.cz/~grygar/AbrSV.html. Among others, the method can be applied for the direct identification of powders of pharmacologically important substances, such as famotidine, probucol, propylthiouracil, nicotinoylanilide, thionicotinoylanilide [2], cocaine [16], benzocaine, cinchocaine, lidocaine, procaine, and codeine [17]. In this communication the voltammetric properties of microparticles of three common drugs, 5-aminosalicylic acid, ciprofloxacin, and azithromycin are reported.

5-Aminosalicylic acid is an active component in the therapy of inflammatory bowel disease [18]. On carbon electrodes it can be oxidized to quinone-imine form [19,20]. This reaction was utilized for the determination of this compound in pharmaceutical formulations [21] and physiological samples [22].

Ciprofloxacin [23] and azithromycin [24] are broad-spectrum antibiotics. The first compound can be reduced at −1.4 V on mercury electrode [25] and oxidized at 0.95 V

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on carbon paste electrode [26], while the second substance can be oxidized at 0.75 V on carbon electrodes [27,28]. Electroanalytical methods for the determination of these drugs were developed [25–29].

2. Experimental

5-Aminosalicylic acid (Merck), ciprofloxacin, azithromycin dihydrate (both Pliva, Zagreb), $NaClO_4$, KNO_3, HCl, and 0.1 M buffer solutions pH 4.65 (sodium citrate–HCl), pH 7 (borax–KH_2PO_4), pH 9 and 10 (H_3BO_3–NaOH) (all Kemika, Zagreb, analytical grade) were used as received. Water was doubly distilled. Salofalk® delay-released tablets, each containing 500 mg of 5-aminosalicylic acid (Dr. Falk Pharma, Germany) and CIP 500® tablets, containing 500 mg of ciprofloxacin (Bayer, Germany) were finely powdered in an agate mortar. The contents of Sumamed® capsules, containing 250 mg of azithromycin (Pliva, Croatia), were completely removed from shells and analyzed directly. Excipients added to these dosage forms were calcium stearate, cellulose, polyvinyl-2-pyrrolidone, talc, titanium dioxide and iron oxide.

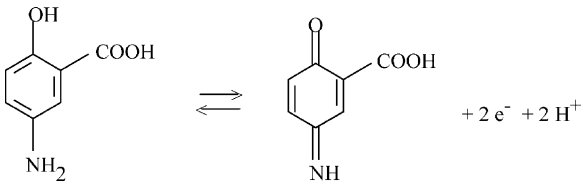
Voltammetric measurements were performed by using a multimode polarograph Autolab 30 (EcoChemie, Utrecht). The working electrode was a spectral-grade paraffin-impregnated graphite rod (diameter 5 mm, length 50 mm). Its clean and carefully polished circular surface was contaminated with microparticles of solid organic compounds by pressing it into a small pile of substance powder on a highly glazed ceramic tile and moving it with a circular motion for about 10 s. After each measurement, the graphite rod was rinsed with distilled water, polished on wet polishing cloth, rinsed again, dried with fine-grade tissue and carefully polished on a dry white paper sheet. The Pt wire was an auxiliary electrode and Ag/AgCl/3 M KCl (Metrohm) was a reference electrode (E = 0.208 V versus SHE).

Liquid electrolytes were degassed with high purity nitrogen for 20 min prior to the measurements. A nitrogen blanket was maintained thereafter. The cell was kept at 20 °C in a thermostat.

The working electrode was immersed in the electrolyte only during the voltammetric measurements. Less than 1 mm of the graphite rod was immersed in the electrolyte, with the objective of restricting its contact with the solution to the working surface itself as much as possible. By this procedure the residual current was diminished and the signal-to-noise ratio was increased.

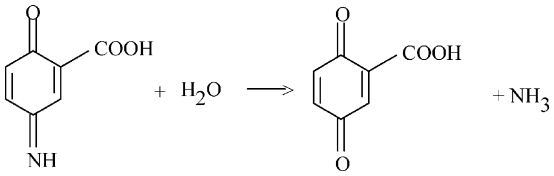
3. Results and discussion

Fig. 1 shows cyclic voltammograms of microcrystals of 5-aminosalicylic acid immobilized on a paraffin impregnated graphite electrode and immersed into an aqueous perchlorate solution at pH 1. If the scan rate is 1 V/s, the voltammogram consists of an oxidation peak at 0.540 V versus Ag/AgCl/3 M KCl and two reduction peaks at 0.471 V and 0.061 V, respectively (see Fig. 1B). If the scan rate is lower than 0.5 V/s, the first reduction peak disappears while the second one increases (see Fig. 1A). The relationships between the peak potentials of the oxidation and the second reduction processes and the logarithm of the scan rate are linear, with the slopes 22.5 mV/d.u. and −31 mV/d.u., respectively. This is an indication that both processes are controlled by the kinetics of electrode reactions and characterized by the products βn_{ox} = 1.3 and αn_{red} = 0.97, respectively. Here β and α are average transfer coefficients, while n_{ox} and n_{red} are numbers of electrons transferred in the oxidation and the reduction, respectively. These results are in the agreement with the responses of the dissolved 5-aminosalicylic acid molecules [19–21]. They can be explained by an EC mechanism in which the product of the oxidation of 5-aminosalicylic acid undergoes chemical transformation into the form which is stable at potentials higher than 0.2 V. According to literature data [19,20], 5-aminosalicylic acid is oxidized to quinone-imine in a quasireversible electrode reaction:



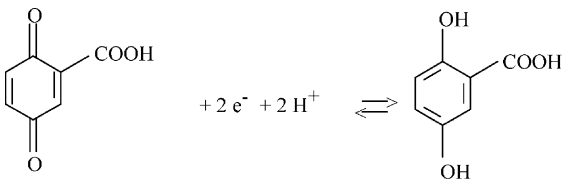
(1)

So, the peaks at 0.540 V and 0.471 V correspond to the oxidation of 5-aminosalicylic acid and the reduction of quinone-imine, respectively. However, in water quinone-imine is irreversibly transformed into quinone:



(2)

This reaction occurs during the period in which the electrode potential is scanned from 0.540 V to 0.650 V and back to 0.470 V. If the scan rate is 1 V/s, the duration of this period is 0.29 s, but if the scan rate is 0.1 V/s, the period lasts 2.9 s. So, at high scan rates quinone-imine is only partly transformed and the response of its reduction can be registered, but at low scan rates quinone-imine is almost completely transformed and the response of its reduction disappears. The peak at 0.091 V (see Fig. 1A) can be ascribed to the reduction of quinone to gentisic acid:



(3)

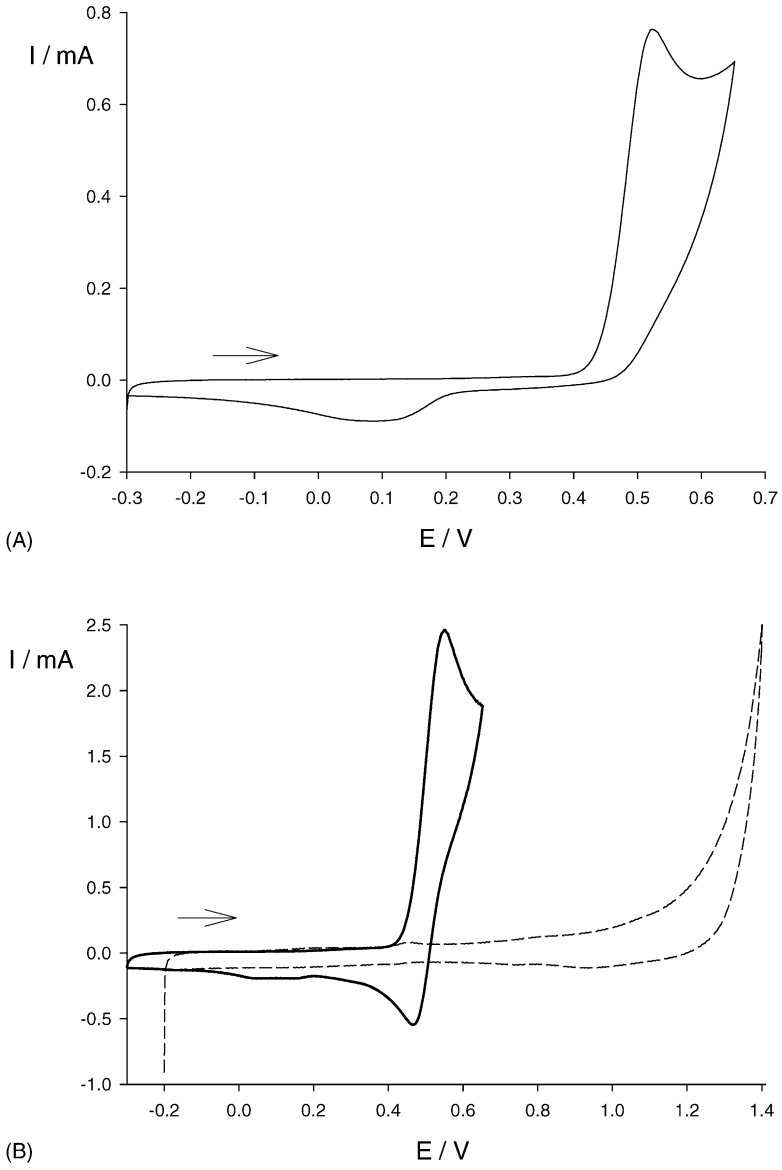


Fig. 1. Cyclic voltammetry of 5-aminosalicylic acid microparticles in 1 M $NaClO_4$ + 0.1 M HCl. Starting potential is −0.3 V and scan rates are 0.1 V/s (A) and 1 V/s (B). The solid particles are mechanically transferred to the surface of the paraffin impregnated graphite electrode and immersed into a liquid electrolyte. A dashed line in (B) is the response of bare electrode.

This assignation is based on the comparison with cyclic voltammograms of microcrystals of quinhydrone [2].

Fig. 2 shows three consecutive cyclic voltammograms of microcrystals of 5-aminosalicylic acid in the acidic solution (pH 1). A new oxidation peak at 0.250 V, corresponding to the oxidation of gentisic acid to quinone appears in the second and the third cycle. All three peaks are diminished from cycle to cycle, but the decreasing of the oxidation peak of 5-aminosalicylic acid is the most severe. This can be explained by assuming that the transformation of quinone-imine into quinone occurs on the surface of the particle and that both quinone and gentisic acid remain bound to the particle surface blocking the oxidation of 5-aminosalicylic acid in the second and the third cycles. However, the diminution of the pair of peaks corresponding to the quinone/gentisic acid redox couple indicates that the smallest, the most reactive particles are consumed during the voltammetric experiment. So, a slow dissolution of gentisic acid must be also considered.

Square-wave voltammogram of 5-aminosalicylic acid microcrystals is shown in Fig. 3. In this technique the high-amplitude, high-frequency square-wave is combined with the staircase waveform. Relative to the scan direction, forward and backward pulses can be distinguished. The currents are measured at the end of each pulse and the difference between the currents measured on two successive pulses is recorded as a nett response. Additionally, the currents of the forward and backward series of pulses are reported as a function of the corresponding potential of the staircase waveform [30]. In Fig. 3 these compo-

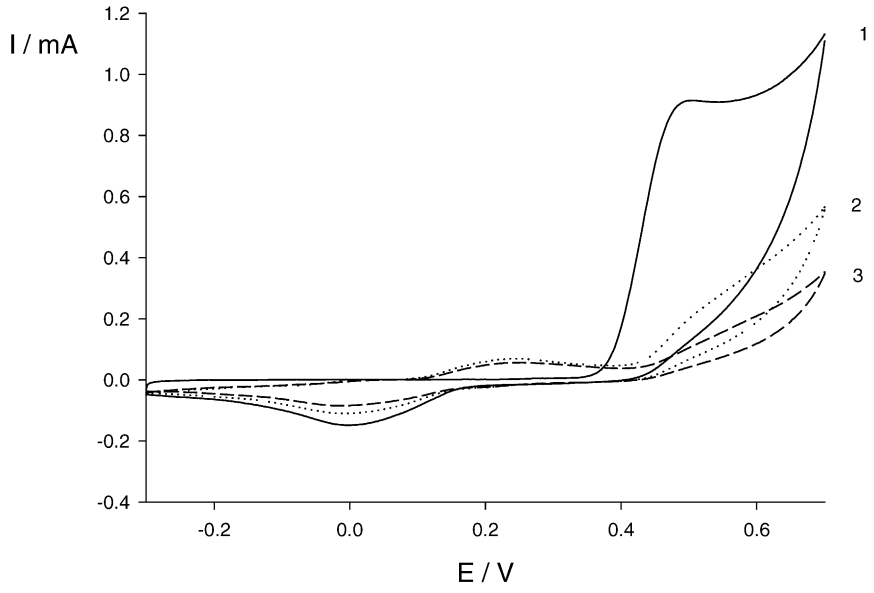


Fig. 2. Multiple cyclic voltammetry of 5-aminosalicylic acid microparticles in 1 M $NaClO_4$ + 0.1 M HCl. A scan rate is 0.05 V/s. The first (–), the second (. . .) and the third (- - -) cycles are shown.

nents correspond to the oxidation of 5-aminosalicylic acid and the reduction of quinone-imine, respectively. As the frequency is 100 Hz, the duration of each pulse is 5 ms. This is 58 times shorter than the duration of the period between the oxidation and reduction in cyclic voltammetry if the scan rate is 1 V/s. So, the chemical transformation of quinone-imine during the anodic pulse is negligible and the cathodic component of the response is well developed. The peak potential of the nett response is 0.504 V, which is equal to the median potential of cyclic voltammogram shown in Fig. 1B. If pH > 6, the peak potential of nett response in square-wave voltammetry and the median potential in cyclic voltammetry do not depend on pH: E_P = 0.370 V (if the frequency is 100 Hz) and $\bar{E}$ = 0.395 V (if the scan rate is 1 V/s). At lower pH values the relationships between these potentials and pH are linear, with slopes $\Delta E_P/\Delta pH$ = −27 mV and $\Delta\bar{E}/\Delta pH$ = −20 mV. This is in agreement with the results of previous measurements [21].

Fig. 4 shows square-wave voltammogram of the powdered Salofalk® tablets. A well-developed response of 5-aminosalicylic acid and no interference from excipients can be observed. This shows that abrasive stripping

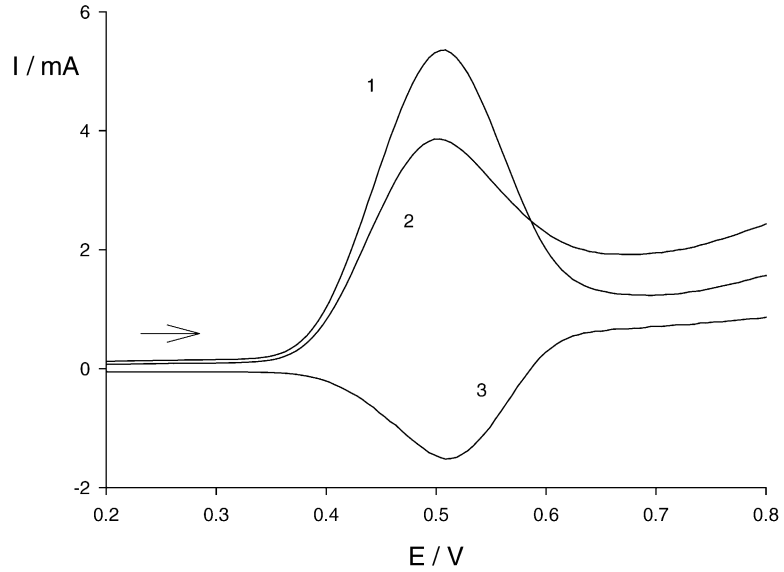


Fig. 3. Square-wave voltammetry of 5-aminosalicylic acid microparticles in 1 M $NaClO_4$ + 0.1 M HCl. Nett response (1) and its forward (2) and backward (3) components. Starting potential 0 V, frequency 100 Hz, amplitude 75 mV and potential increment 2 mV.

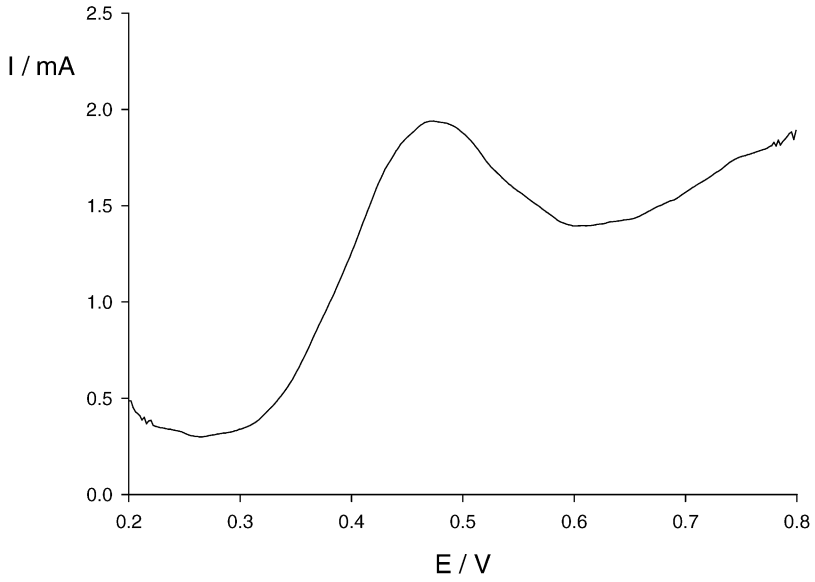


Fig. 4. Square-wave voltammetric detection of 5-aminosalicylic acid in Salofalk® tablets. A liquid electrolyte is 1 M $NaClO_4$ + 0.1 M HCl, frequency is 100 Hz and amplitude is 75 mV.

square-wave voltammetry can be used for direct electrochemical characterization of solid 5-aminosalicylic acid and its determination in pharmaceutical formulations.

3.1. Ciprofloxacin

Figs. 5 and 6 show cyclic voltammograms (three successive cycles) and square-wave voltammogram of ciprofloxacin microcrystals mechanically immobilized on the electrode surface. Both responses indicate totally irreversible oxidation, with the peak potentials 1.2 V and 1.32 ± 0.02 V, respectively. Within an interval $1 < pH < 10$, the peak potentials are independent of pH in both techniques. According to the literature data [26,29], it is not known which part of ciprofloxacin molecule is oxidized in the electrode reaction but it can be assumed that it is one of three amino groups that is oxidized to the cation radical [31–37]. This reaction is followed by the fast and totally irreversible transformation of the molecule either by the dimerization [31,33], or by the reaction with water [34–37]. The response does not change significantly in the second and the third cycles. This indicates that the oxidation products are dissolved and the surface of particles is renewed in each cycle. Square-wave voltammetric response of ciprofloxacin

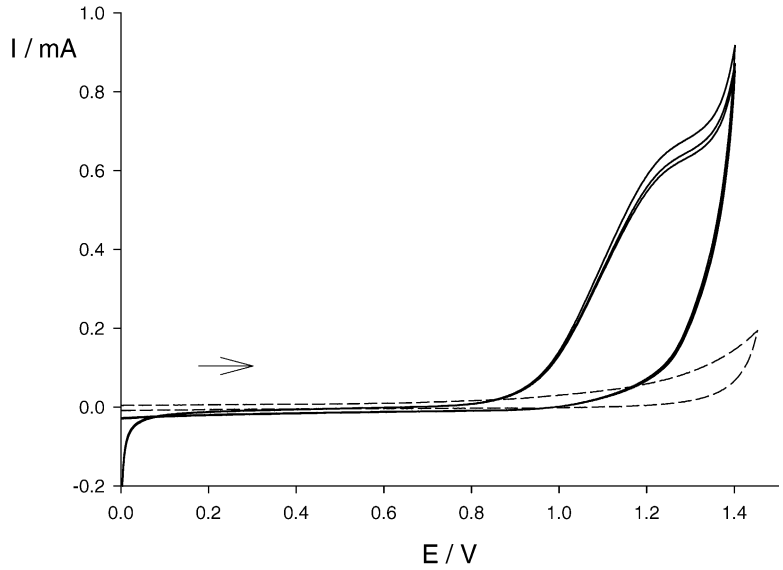


Fig. 5. Cyclic voltammetry of ciprofloxacin microparticles in 1 M KNO_3 + 0.01 M HCl. The first three successive cycles are shown. Scan rate is 0.01 V/s. A dashed line is the response of bare electrode.

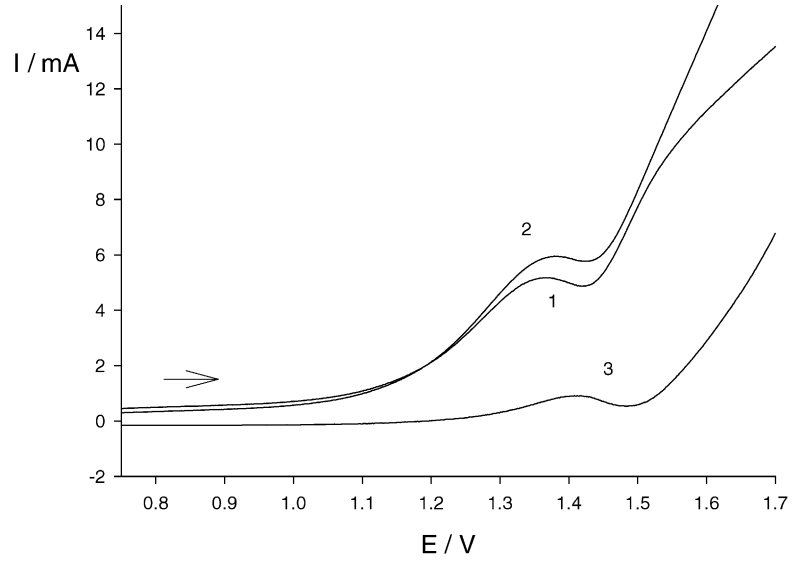


Fig. 6. Square-wave voltammetry of ciprofloxacin microparticles in 1 M KNO_3 buffered to pH 9. Nett response (1) and its forward (2) and backward (3) components. Frequency 10 Hz, amplitude 75 mV and potential increment 2 mV.

present in the powdered CIP 500® tablets is shown in Fig. 7.

3.2. Azithromycin

Oxidation of azithromycin microcrystals is totally irreversible electrode reaction, as can be seen in Fig. 8. At pH 4.65, the peak potential is 0.936 V, if the scan rate is 0.1 V/s. This is in agreement with the response of dissolved molecules [27]. In the acidic electrolyte (pH < 3) no response can be recorded. In the slightly acidic medium (4 < pH < 7) the potential of the oxidation peak is a linear function of pH, with the slope −52 mV. At pH 7, the peak potential is 0.814 V, for the same scan rate. In moderately basic solutions (pH 9 and 10) a new peak at 0.67 V appears. This is shown in Fig. 9. The potential of the main peak, at 0.81 V, is independent of pH. Similar changes of the response were observed in square-wave voltammetry. Both forward and backward components of the response are oxidation currents, analogously to Fig. 6, which are the characteristics of totally irreversible processes [30]. The peak potentials of the nett response vary from 0.918 V at pH 4.65, to 0.803 V at pH 7, 0.73 V at pH 9 and 0.72 V at pH 10. The last two responses consist of two small, poorly separated peaks. The origin of the first peak is not known, but the main peak is ascribed to the oxidation of dimethy-

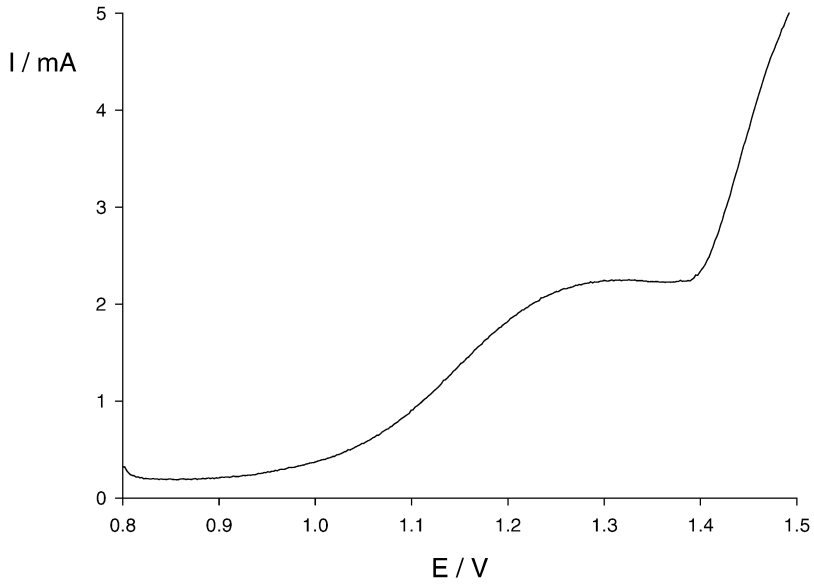


Fig. 7. Square-wave voltammetric detection of ciprofloxacin in CIP 500® tablets. A liquid electrolyte is 1 M KNO_3 buffered to pH 9, frequency is 10 Hz and amplitude is 75 mV.

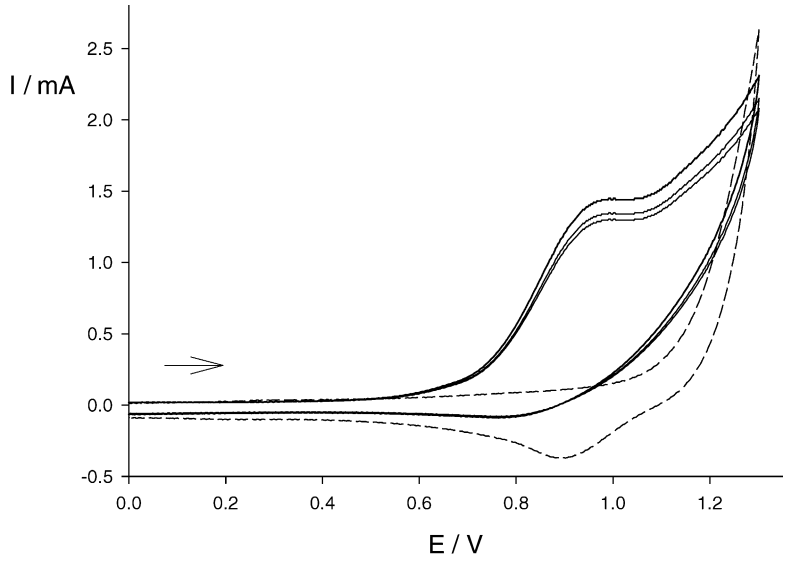


Fig. 8. Cyclic voltammetry of azithromycin microparticles in 1 M $NaClO_4$ buffered to pH 4.65. The first three consecutive cycles are shown. Scan rate is 0.1 V/s. A dashed line is the response of bare electrode.

lamino group on the desosamine sugar part of the molecule [27]. The product of this electrode reaction is cation radical, which undergoes irreversible transformation by the reaction with water [31–37]. This attribution is based on the fact that erythromycin, which is structurally analogous but has no nitrogen atom in macrocyclic lactone ring, exhibits similar voltammetric response [38]. The final products are probably dissolved because the response does not vanish in the second and third voltammetric cycles. Square-wave voltammetric detection of azithromycin in the Sumamed® capsules is shown in Fig. 10.

Regarding the reactivity of insoluble microparticles, it is explained by the theory of three-phase boundary at which the electrode, the liquid electrolyte and the immobilized microcrystal meet [2,39]. The reaction starts from this line and expands over the surface of the particle by a series of electron jumps, which are accompanied by the exchange of ions or protons with the electrolyte. This type of conductivity is a consequence of the gradient of the electrochemical potential on the surface of the particle. The reaction is localized on the particle/electrolyte interface because proton donors cannot penetrate into the body of the particle. This mechanism was observed in voltammetry of azobenzene microcrystals [39]. In some cases the mass transfer is achieved by the surface diffusion of partly hydrated molecules of the solid [17]. The electrode reaction can be either destructive, i.e. leading to a dissolution of the particle, or not, depending on the solubility of the products. It seems that electrochemical oxidations of ciprofloxacin and azithromycin are destructive, while in the

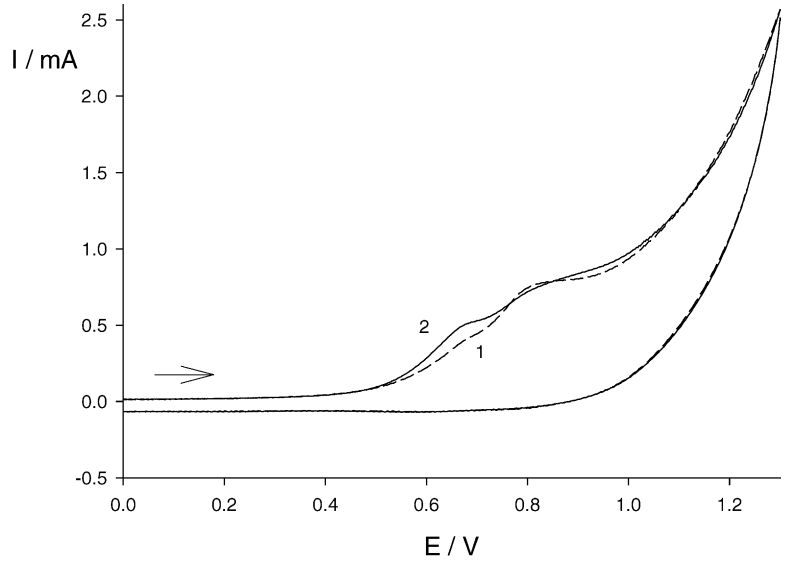


Fig. 9. Cyclic voltammetry of azithromycin microparticles in 1 M $NaClO_4$ buffered to pH 9 (1) and 10 (2). Scan rate is 0.1 V/s.

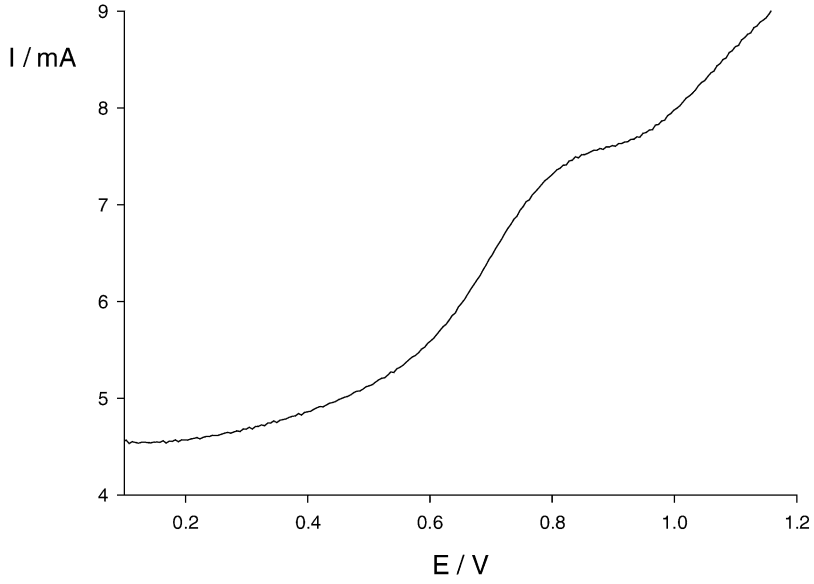


Fig. 10. Square-wave voltammetric detection of azithromycin in Sumamed® capsules. A liquid electrolyte is 1 M $NaClO_4$ buffered to pH 7, frequency is 100 Hz and amplitude is 75 mV.

oxidation of 5-aminosalicylic acid poorly soluble products are formed.

The described results show that a minute amount of powder of insoluble electroactive compound can be directly characterized. The responses of microparticles of 5-aminosalicylic acid are not significantly different from the responses of the dissolved compound. So, the electrode reactions of both immobilized microparticle and dissolved molecule can be explained by the same mechanism. By the analogy, it can be assumed that the responses of the other two drugs, electrochemical properties of which are less known, can be also explained by the mechanisms which apply to their solutions.

The measurements of powdered tablets and the content of capsules show that the matrix is electroinactive in the potential range in which the investigated drugs are oxidized. So, the matrix does not influence the responses of the drugs.

The limit of detection for a certain solid compound depends on the electrode reaction of its microparticles. The experiments with azobenzene showed that the response of 1 μA corresponded to about 5 nmol/cm^2 of solid azobenzene [17]. For cocaine microparticles the detection limit was 0.3 μg/cm^2 [16], and for some pesticides this limit was only 1 ng/cm^2 [14]. However, for the investigated drugs the detection limits coincide with the amounts present in the inspected pharmaceutical dosage formulations. The proposed method can be used for a quick detection of the active component in the formulation, before the quantitative analysis is performed.

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