Electrochemical behavior of chloramphenicol on carbon electrodes in a microelectrochemical cell
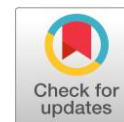
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Abstract

Express determination of antibiotics is an extremely important task today. Portable electrochemical microdevices are a viable alternative to traditional methods of analysis. The development of such devices requires the study of redox processes in detail. This article is devoted to the comparative study of the electrochemical behavior of chloramphenicol in water solvents in standard laboratory and portable microelectrochemical cells. It was found that the electrochemical reduction of chloramphenicol proceeds via a 3-electron mechanism to the formation of a dimer. In the transition from the macrocell to the microcell, a decrease in the electrochemical reduction current and a shift of the peak potential to the cathode region are observed, which is apparently associated mainly with the type of the electrode material. The best characteristics of the direct electrochemical response were obtained in the differential pulse voltammetry mode. Under the selected operating parameters, the peak current of the electrochemical reduction of chloramphenicol is linearly dependent on the concentration of the antibiotic in the range of $2 \cdot 10^{-3} - 1 \cdot 10^{-5}$ M with a detection limit of $3 \cdot 10^{-5}$ M. Obtained characteristics are sufficient for the quality control of pharmaceuticals and can be improved through the use of organic and hybrid modifiers of the working electrode surface.

1. Introduction

Chloramphenicol (CAP) is a broad-spectrum antibiotic utilized in veterinary and medicine due to its high efficacy and low cost. However, CAP entering the aquatic environment does not decompose and accumulates therein. Thus, it contaminats sediments and water systems and induces unhealthy effects, such as aplastic anemia (blood disorder), agranulocytosis, dosage independent suspected carcinogenity in humans, and also contributes to the development of bacteria resistance [1, 2]. Therefore, detection of chloramphenicol in food and environmental objects is an important task [3].

To determine the trace amounts of chloramphenicol in laboratory practice, chromatographic [4], spectroscopic [5], enzyme immunoassay [6], and other methods of analysis are used [7]. However, all these approaches require expensive equipment, reagents, and a rather lengthy analysis procedure. Of greatest interest is the electrochemical determination of chloramphenicol [8], mainly due to the ultra-

sensitivity of the determination, the possibility of miniaturization and the transition to portable test systems. Electrochemical analytical microdevices have great prospects, as they are suitable for the mass production, representing inexpensive, disposable sensor systems. In the designs of such devices, as a rule, three-electrode systems made by screen printing are used [9]. For example, Pakapongpan et al. developed an electrochemical sensor using a magnetic screen-printed electrode (SPE), which demonstrated a detection limit of 10 nM for the detection of CAP [10]. Li et al. invented a laser-enabled flexible electrochemical sensor on finger, which can be used for rapid real-time in-site electrochemical identification of CAP in meat (LOD 10 μM) [11]. The advantages of such electrochemical systems with printed electrodes are low cost and the possibility of one-time use, which simplifies the analysis procedure and improves analytical performance by eliminating the stage of electrode surface regeneration.

Transition from standard electrochemical cells to portable microdevices requires additional studies of the

electrode reactions because of the significant differences in geometry of a working electrode and electrochemical cell, parameters of diffusion and charge transfer, etc. [12, 13]. It can also influence the analytical signal parameters [14–17].

The aim of this article is the comparative study of the electrochemical behavior of CAP in standard laboratory and portable microelectrochemical cells, and the estimation of the possibility of quantitative electrochemical determination of CAP using the direct electrochemical response.

2. Experimental

2.1. Reagents and Chemicals

All solutions were prepared with ultrapure water from a Branstead Pacific TII Water Purification System. Britton–Robinson buffer solutions (BRB) (pH 2–10) were prepared from 0.04 M orthophosphoric acid, 0.04M acetic acid and 0.04 M boric acid; the pH values were adjusted with sodium hydroxide. The pH was measured with a FiveEasy pH Meter (Mettler Toledo). All chemicals were purchased from Sigma Aldrich: orthophosphoric acid, acetic acid, boric acid, sodium hydroxide, lithium perchlorate, acetonitrile, potassium chloride, ferrocyanide ($K_4Fe[CN]_6$), potassium ferricyanide ($K_3Fe[CN]_6$).

2.2. Electrochemical research

Cyclic voltammograms (CV), differential pulse voltammograms (DPV), square wave voltammograms (SWV) and chronoammograms (CA) were recorded on a potentiostat/galvanostat µAutolab Type III. The three-electrode standard cell (Figure 1a) consisted of a glassy carbon working electrode (GCE), an Ag/AgCl/KCl reference electrode, and a carbon rod as an auxiliary electrode. A Micrux AIO-platform with a three-electrode system made by screen printing was used as a microelectrochemical cell (Figure 1b).

3. Results and Discussion

The study of the electrochemical behavior of chloramphenicol in water solutions was carried out both in the standard three-electrode cell and in the microcell.

Cyclic voltammograms (Figure 2a), registered on the glassy carbon electrode, show a pronounced cathodic peak at a potential of –0.7 V and an anodic peak at a potential of 0.3 V, which increases with further cycling. Also, in the second cycle, a peak at a potential of –0.3 V appears, and the current of the cathodic peak at a potential of –0.7 V decreases. It probably indicates a two-stage reduction process. On the CV registered in microcell, the electrochemical behavior of CAP is similar (Figure 2b). The shift of the cathodic peak potential in the negative region can be associated with the type of the electrode material and the differences in effective surfaces (Figure 2c).

Studies on the effect of pH on the electroreduction (Figure 3) in the Britton–Robinson solution were carried out to estimate the proton influence. With an increase in pH, a shift of the peak to the cathodic region is observed. Thus, we can assume a proton-dependent reduction mechanism. In an aqueous medium $\Delta E/\Delta pH = -43$ mV, which indicates an equal number of protons and electrons involved in the reaction. The maximal peak current is observed at pH = 5 (Figure 3b).

Figure 4 shows the dependences of the recovery peak current on the square root of the scan rate ($v^{1/2}$) in the potential scan rate range from 1 to 2000 mV/s. The peak current has a linear relationship with the square root of the scan rate over the pH range under study. Thus, the electrochemical reduction of chloramphenicol is a diffusion-controlled process.

The effective number of electrons, involved in CAP electroreduction, was estimated as 3, using the Randles-Sevcik theory (Equation 1) [18]:

$$I_p = (2.69 \times 10^5)\, n^{3/2} A D^{1/2} c\, v^{1/2}, \qquad (1)$$

where I_p is the peak current, A; n is the number of transferred electrons; A is the area of the electrode, cm²; D is the diffusion coefficient, cm²/s; c is the concentration, M; and v is the potential scan rate, V/s.

It is known that the diffusion coefficient for the nitroaromatic compounds in aqueous media is ~10^{-5} cm² [19], the diameter of GCE is 2 mm, the concentration of CAP is $2 \cdot 10^{-3}$ M (in BRB pH = 5), the scan rate is 2 V/s.
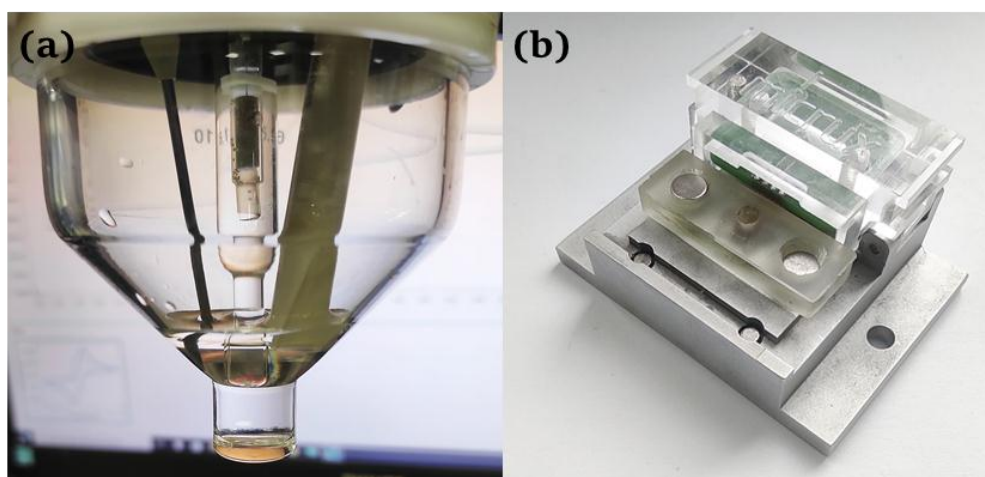

Figure 1 The standard three-electrode cell (a); 'Micrux' AIO-platform for screen-printed electrodes (SPE) (b).
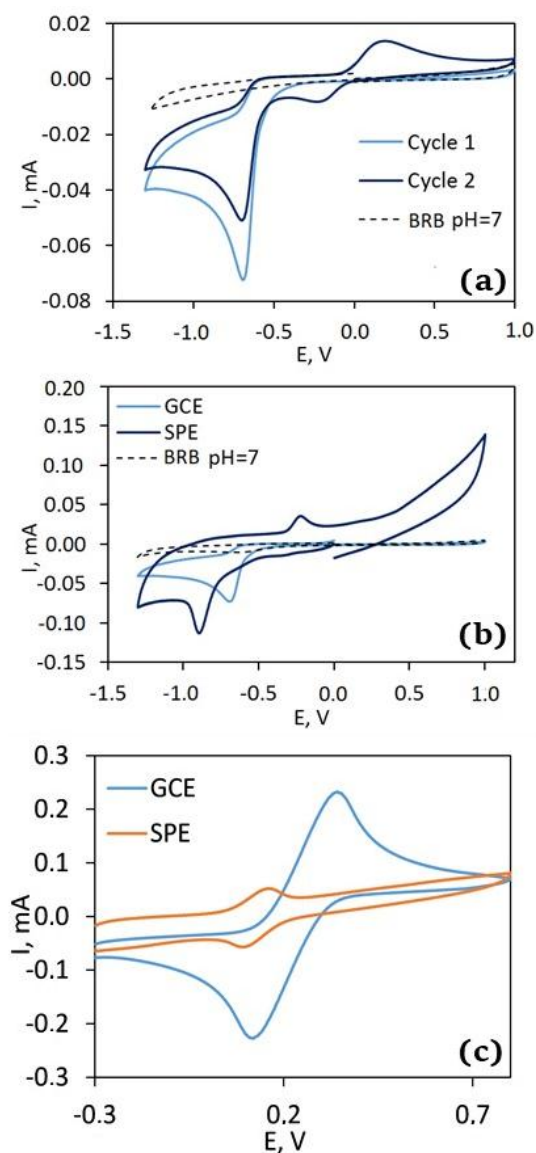
Figure 2 CV registered in presence of 2·10⁻³ M CAP in BRB pH = 7 on GCE (a), on GCE and SPE (b), CV registered in presence of redox indicator 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ on GCE and SPE, 0.1 KCl, scan rate 0.1 V/s (c).
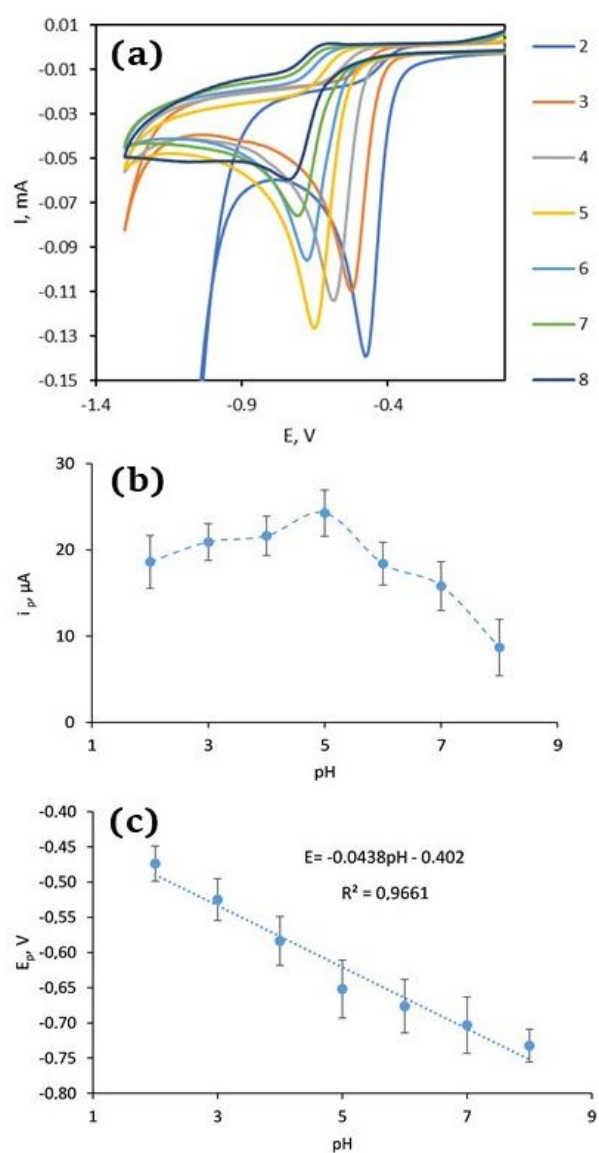
Figure 3 CVs registered in presence of 2·10⁻³ M CAP on GCE, BRB pH = 2–10, scan rate 0.1 V/s (a); plot $I = f(pH)$ (b) and plot $E = f(pH)$ (c).

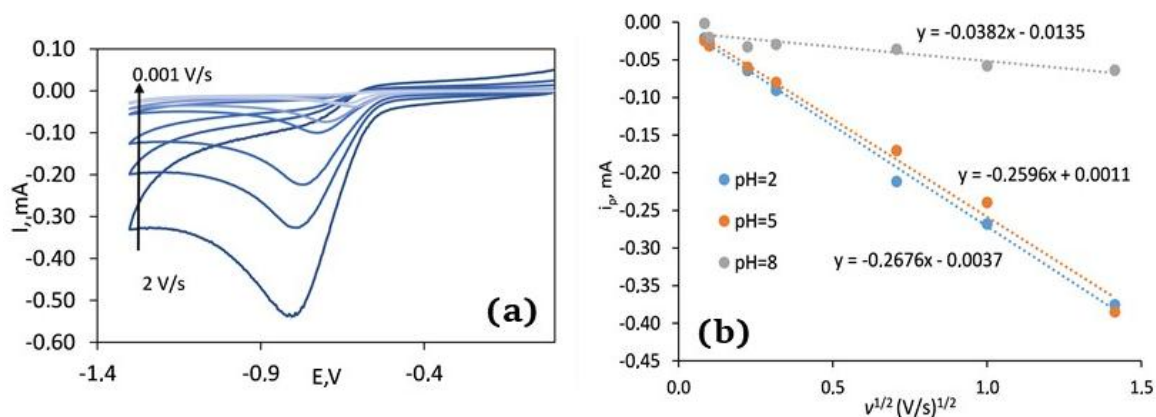**Figure 4** CVs registered in presence of 2·10⁻³ M CAP on GCE, BRB pH = 5 (a); plot peak recovery current vs scan rate (b).

Based on the obtained results, we can assume that the electrochemical behavior of chloramphenicol in microcell accompanied with a pronounced cathodic peak corresponds to the 3-electron and 3-proton electroreduction of the nitro group with the formation of the corresponding dimer (Scheme 1).
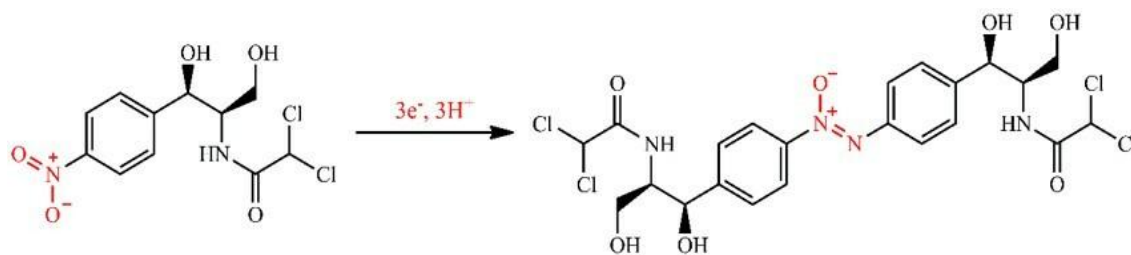
Scheme 1 Proposed mechanism of CAP electrochemical reduction in microcell.

To achieve the best analytical characteristics of the CAP determination, we optimized the voltammetric mode both in standard and micro-electrochemical cells using CV, DPV and SWV. Figure 5 shows that during the changing registration mode from the LV (–0.65 V) to the SWV (–0.54 V) and DPV (–0.56 V), the cathodic peak potential shifts to the anodic region and the peak current increases significantly. The best characteristics were obtained using the DPV mode (Figure 5). Similar tendency was also observed in the Micrux cell.

When registering the DPV of CAP in a microvolume of the sample (the volume of the sample was 50 µL), the cathodic peak current significantly decreased, which is probably associated with a complication of charge transfer and a less developed surface of the screen-printed electrode (Figure 6).

Thus, the process of electrochemical reduction of CAP, registered in DPV mode can be used as a direct analytical signal in the portable electrochemical cells for express determination of the antibiotic in microvolumes of samples.

Under the chosen operating parameters, a calibration plot $I = f(C)$ was obtained in model solutions of CAP (Figure 7). The regression is linear ($R^2 = 0.9984$) in the wide concentration range of the CAP. The detection limit, estimated according with the 3-sigma criterion, was $3 \cdot 10^{-5}$ M.

The obtained characteristics are sufficient for the quality control of pharmaceuticals and can be improved using organic and hybrid modifiers of the working electrode surface.

4. Conclusions

In this work, the electrochemical behavior of chloramphenicol in aqueous solvents was studied in the standard three-electrode cell and the portable electrochemical cell with the screen-printed three-electrode system. A similar RedOx behavior of CAP was obtained, accompanied with the pronounced irreversible reduction peak corresponding to the 3-electron and 3-proton electrochemical conversion into the corresponding dimer. The best characteristics of the response were obtained in the differential pulse voltammetry mode. In the transition from the macrocell to the microcell, a decrease in the electroreduction current and a shift of the peak potential to the cathodic region are observed, which is apparently associated with the type of the working electrode. Under the experimentally optimized operating parameters, the current of the CAP electroreduction peak linearly depends on the antibiotic concentration in the range of $2 \cdot 10^{-3}$–$1 \cdot 10^{-5}$ M with a detection limit of $3 \cdot 10^{-5}$ M and
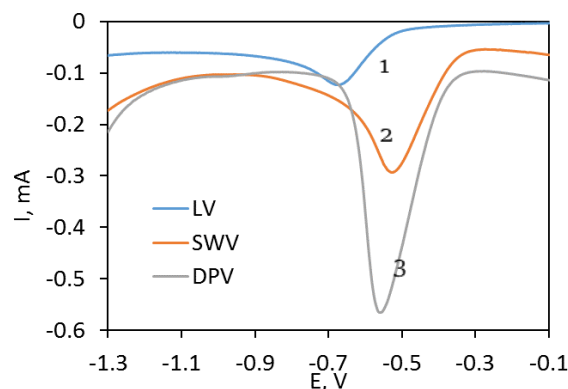

Figure 5 LV, scan rate 0.1 V/s (1); SWV, modulation amplitude 0.2 V, frequency 25 Hz (2); DPV, modulation amplitude 0.2 V, modulation time 0.005 s (3). $2 \cdot 10^{-3}$ M chloramphenicol, glassy carbon electrode, BRB pH = 5.
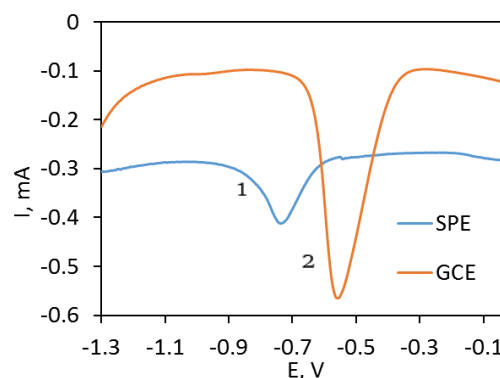

Figure 6 DPV, $2 \cdot 10^{-3}$ M chloramphenicol, SPE (1), GCE (2). BRB pH = 5, modulation amplitude 0.2 V, time modulation 0.005 s.
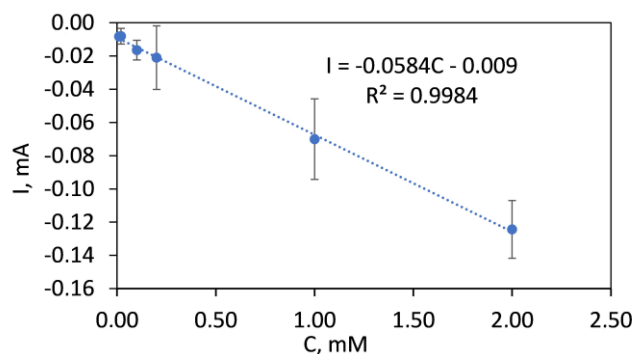

Figure 7 Calibration plot $I = f(C_{CAP})$ obtained in the Micrux cell on the SPE, DPV modulation amplitude 0.2 V, modulation time 0.005 s, BRB pH = 5.

can be used as an analytical signal of an electrochemical sensor. An increase in the detection sensitivity can be achieved using organic and hybrid modifiers of the working electrode surface.

Supplementary materials

No supplementary materials are available.

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Author contributions

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Funding acquisition: T.S.S., M.V.M.
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Conflict of interest

The authors declare no conflict of interest.

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