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Short Communication

Electrochemical Characterization of Oxaprozin on Bare Gold Electrode and Electrode Modified with Bovine Serum Albumin

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As the very first electrochemical investigation of oxaprozin, nonsteroidal anti-inflammatory drug, using cyclic voltammetry on gold electrode in 0.05 mol dm^{-3} $NaHCO_3$, the synthesized drug, its analytical standard and its content in Duraprox® tablets were characterized with one oxidation reaction and the three reduction reactions. All they exhibited the linear concentration dependency of anodic currents at 0.83V for the analytical standard and 0.85V for Duraprox® tablets in the range of concentrations 8.44 – 32.78×10^{-6} mol dm^{-3}. The strong adsorption of bovine serum albumin (BSA) on gold electrode in 0.1 mol dm^{-3} phosphate buffer solution (pH 7.4) is shown and concentration dependency of anodic currents of oxaprozin standard on BSA / Au is studied. Following the Langmuir adsorption thermodynamic equation, the binding constants of oxaprozin on BSA / Au electrode was calculated with the results 1.23×10^{5} dm^3 mol^{-1}.

Keywords: oxaprozin; gold electrode; bovine serum albumin; concentration dependency; Langmuir adsorption.

1. INTRODUCTION

Oxaprozin, 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanoic acid (Fig. 1), is one of the leading nonsteroidal anti-inflammatory drugs (NSAIDs) on the US market, which is used in the treatment of a number of inflammatory musculoskeletal diseases, including rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, tendinitis and bursitis [1,2]. It exhibits several advantages over other NSAIDs (e. g. aspirin, diclofenac, ibuprofen) including a low incidence of gastrointestinal side effects, a long half-life with long duration of action and good patient compliance with a once-daily oral regimen [3–5]. Several chromatographic methods have been presented in the literature for the determination of oxaprozin and its impurities in the bulk drug as well as in biological fluids [6,7]. A quantitative method

using electrophoresis has also been described [8]. So far the analytical utility of cyclic voltammetry in the determination of oxaprozin has not been reported.

Protein–drug binding has a determining role in the both pharmacokinetic and pharmacodynamic phases of drug action. Albumin is the most frequent serum protein which drug used for transport trough blood to different target sites and the most of drug used Tyr 411 for binding to serum albumin (Fig. 1). A variety of techniques have been developed to evaluate drug interactions with serum proteins, which are the most abundant soluble proteins in plasma. However, there is still a growing need for improved methods for this purpose [9]. The interactions of two derivatives of oxaprozin (Oxaprozin-E and Oxaprozin-P) with a model transport protein, bovine serum albumin (BSA), have been studied using spectroscopic methods including fluorescence and absorption spectroscopy [10,11]. Although relatively accurate binding parameters and structure information's can be provided in this way, their application is limited due to the poor sensitivity and selectivity and the requirement of large quantities of drugs and proteins [12,13]. On the other side, the electrochemical methods are regarded as faster, cheaper and superior to conventional chemical methods and have been successfully employed to investigate the binding of several drugs with proteins [14,15] as well as with DNA [3,8].

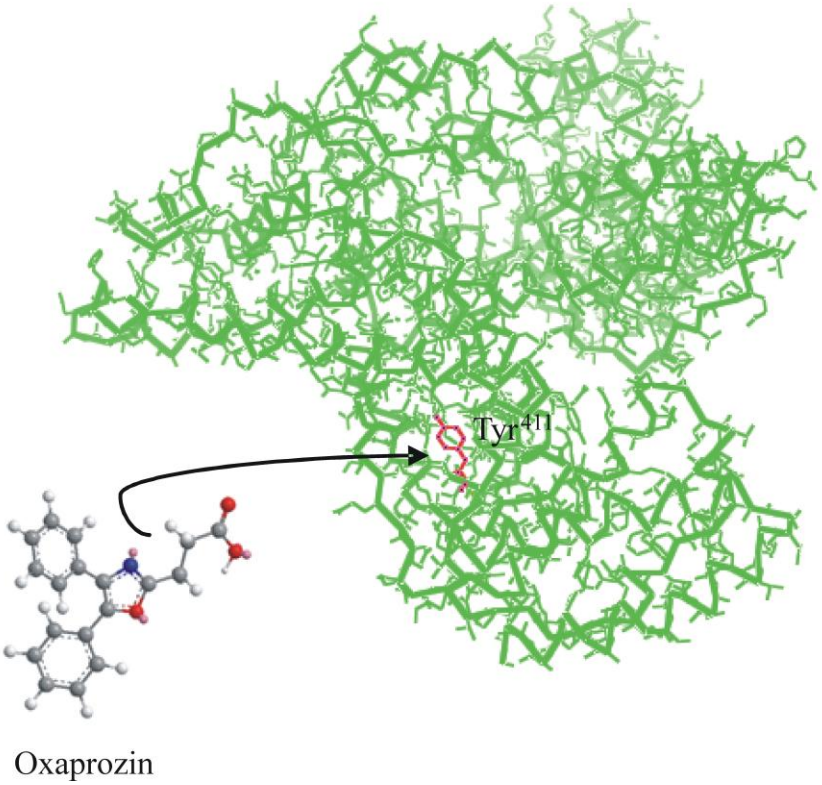


Figure 1. Interaction of oxaprozin with serum albumin. The 3D structure was obtained using Pymol vs. 0.99 and data obtained from Protein Data Bank (PDB). PDB code of serum albumin: 2BXM.

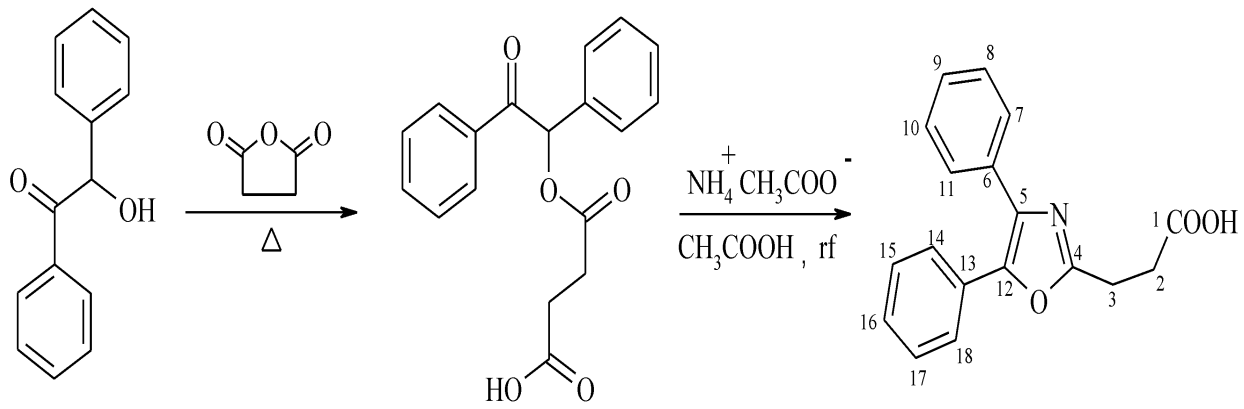
The aim of the present study is the characterization of oxaprozin by its reactivity on a bare gold electrode and Au / BSA electrode in neutral electrolytes using cyclic voltammetry. In this context, the electrochemical identification of the tablet content as well as the synthesized oxaprozin and its analytical standard are presented. The binding constant of oxaprozin on BSA / Au electrode was calculated.

2. EXPERIMENTAL

Oxaprozin standard, Sigma Aldrich and synthesized oxaprozin, were used as a pure substances without further purification, dissolved and added into the electrolytes (0.05 mol dm^{-3} $NaHCO_3$ and phosphate buffer, pH 7.4) in concentrations correctly assigned in the figure caption.

The commercial oxaprozin as a content of Duraprox® tablets (Tramedico, Netherlands) was added into 0.05 mol dm^{-3} $NaHCO_3$ following the procedure for the tablets solutions preparation described in [18,19] in the same concentration as standard. Bovine serum albumin, Sigma Aldrich, was used as a pure substance without further purification and modification. Phosphate buffer solution was prepared by standard procedure and pH was measured by Mettler Toledo (FiveGo) pH Meter. Monobasic sodium phosphate and dibasic sodium phosphate were p.a. purity. Water was purified by Milli-Q system

2.1 Synthesis of oxaprozin



Scheme 1. Synthesis of oxaprozin

Oxaprozin has been prepared according to the literature procedure [17] (Scheme 1) and characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR and FT-IR spectroscopy. *Oxaprozin*. Anal.Calcd. for $C_{18}H_{15}NO_3$ (%): C, 73.71; H, 5.15; N, 4.78. Found (%): C, 73.70; H, 5.36; N, 4.90. ^{1}H NMR (200 MHz, DMSO-d_6): 12.36 (*s*, 1H, O-H), 7.30–7.59 (*m*, 10H, 2Ph–H), 3.06 (*t*, 2H, J = 6.6Hz,

$CH_2\underline{CH_2}CO_2H$), 2.79 (*t*, 2H, J = 7Hz, $\underline{CH_2}CH_2CO_2H$); ^{13}C NMR (50 MHz, DMSO-d_6): 173.5 (C1), 162.71 (C4), 144.9 (C5), 134.6 (C12), 132.3 (C13), 129.2 (C15,C17), 129.1 (C6), 128.9 (C8,C10), 128.7 (C16), 128.4 (C10), 127.7 (C14,C18), 126.6 (C7,C11), 30.5 (C2), 23.2 (C3). FT-IR (KBr disc, cm^{-1}): 2930 mw, 2611 w, 1720 vs, 1568 mw, 1502 mw, 1443 mw, 1362 w, 1321 vw, 1275 mw, 1218 w, 1060 w, 965 w, 922 w, 757 s, 705 vw, 695 vs, 586 w, 524 w.

2.2. Apparatus and preparation of electrode surfaces

Standard equipment was used for the cyclic voltammetry measurements and the three electrode electrochemical cell was described in detail previously [18,19]. Polycrystalline gold **(**bare gold) served as the working electrode; a gold wire was used as the counter electrode and a saturated calomel electrode as the reference electrode. All the potentials are given *vs.* SCE. Prior to the addition of oxaprozin, the electrolytes were deoxygenated by purging with nitrogen. All the experiments were performed at room temperature.

BSA modified gold electrode was prepared by transferring a droplet of 20 μdm^3 of BSA solution onto the surface of gold electrode and by air-drying overnight. The electrode was then soaked in sterile water for at least 4 h before being rinsed with water to remove any unadsorbed BSA [20].

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 deionised water and sulfuric acid and further cleaned with 18 MΩ cm deionised water in an ultrasonic bath.

3. RESULTS AND DISCUSSION

A polycrystalline gold electrode has already been selected as the optimal working electrode for the examination of the pharmaceutical compounds because it is defined with a completely reproducible cyclic voltammograms and, consequently, all the electrochemical reactions at this electrode can be attributed only to the studied molecule [18,19]. As described in the experimental part, gold electrode has been prepared only by mechanical polishing, followed by cleaning. This influenced the surface roughness and after repeated “rough” mechanical polishing the changes can be seen only on the nanoscale. The cyclic voltammograms have always been the same and reproducible under the described experimental conditions. For carbon based electrodes, the situation is quite different. Their structure changes during mechanical polishing in a way which leads non-reproducible voltammograms, which is obvious from the different values of the double layer current. Different pretreatments for preparing and activating the surface of glassy carbon electrodes for electrochemical measurements have been widely discussed [18].

As is presented in Fig. 2A, the cyclic voltammogram shows that on gold electrode in the presence of oxaprozin standard, the apparent reaction of the oxidation occurred in the area of the oxide formation with big anodic current plateau between +0.6V and +0.9V. In the reverse sweep, the reaction of the oxide reduction was decreased in the presence of oxaprozin which can be attributed to the

reduction of products formed in described anodic reaction. In the cathodic direction in reverse sweep, the two additional reduction reactions occurred: the first with the cathodic peak at –0.2 V and second one, with cathodic peak at –0.55 V. By cyclic voltammetry on gold electrode, the synthesized oxaprozin as well as its analytical standard was characterized with one oxidation reaction and the three reduction reactions. The synthesized oxaprozin and analytical standard exhibited the identical electrochemical behavior and the concentration dependency of anodic currents at 0.83V in the range of concentrations 8.44 – 32.78×10^{-6} mol dm^{-3}. This linearity is presented in the left corner of Fig. 2A. In Fig. 2B is shown the voltammetric characterization of the content of oxaprozin tablets (Duraprox®). Cyclic voltammograms of oxaprozin standard and as a content of Duraprox® tablets show an identical electrochemical activity of examinated drugs and clearly indicate that the present exscipients in tablets have no any influence on oxaprozin activity. Excipients were examinated separately and did not exhibit any electrochemical activity under the experimental conditions presented in Figure 2B and in [18,19]. The concentration dependency of anodic currents of content of oxaprozin tablets (Duraprox®) at 0.85V in the range of concentrations 8.44 – 32.78×10^{-6} mol dm^{-3} is linear. This linearity is shown in the left corner of Figure 2B.

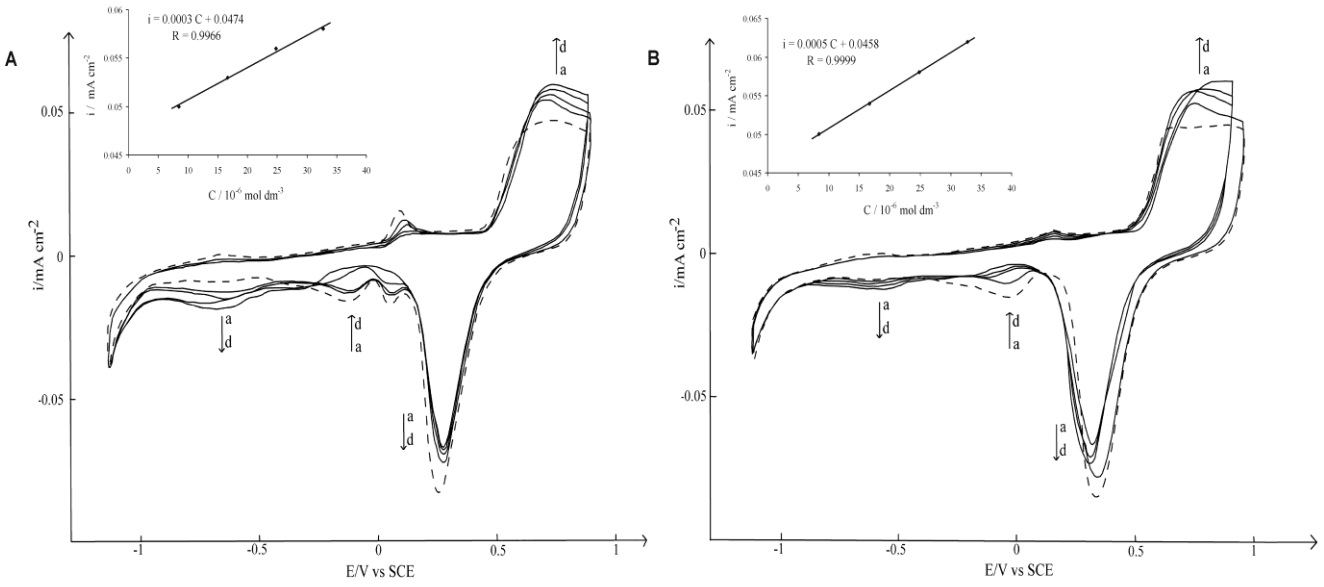


Figure 2. The cyclic voltammograms on bare gold electrode (-----) as well as in the presence of oxaprozin standard (A) and oxaprozin tablets (Duraprox®) (B) (full lines) in the range of concentrations: a = 8.44 mol dm^{-3}; b = 16.71 mol dm^{-3}; c = 24.83 mol dm^{-3}; d = 32.78 mol dm^{-3} in 0.05 mol dm^{-3} $NaHCO_3$, sweep rate 50 mV s^{-1} (only the first sweep is recorded).

NSAIDs of the arylpropionic acid type, which possess a carboxylate at the end of an extended hydrophobic molecule, bind to site II on albumin [21]. It was suggested that a tyrosine residue on albumin (Tyr411, site II) act as a nucleophile in the covalent interaction of oxaprozin [2] (Fig. 1). This gives the possibility for the modification of gold electrode with BSA and big probability for an interaction of oxaprozin with BSA.

In Fig. 3 is presented the cyclic voltammogram of bare gold electrode (.....) and modified with BSA (-----) as well as in the presence of different concentrations of oxaprozin standard (full lines) in 0.1 mol dm^{-3} phosphate buffer solution (pH 7.4). It is clear that gold modified by BSA exhibited two times lower currents in the whole region of the applied potential which shows the strong adsorption of BSA on gold. As is shown in Fig. 3, the oxidative peak current increased gradually with the concentrations 8.44 – 24.83 mol dm^{-3} and then reached its saturation value at the concentration of oxaprozin more than 32.78×10^{-6} mol dm^{-3}, which indicated that the binding of oxaprozin with BSA had reached its equilibrium.

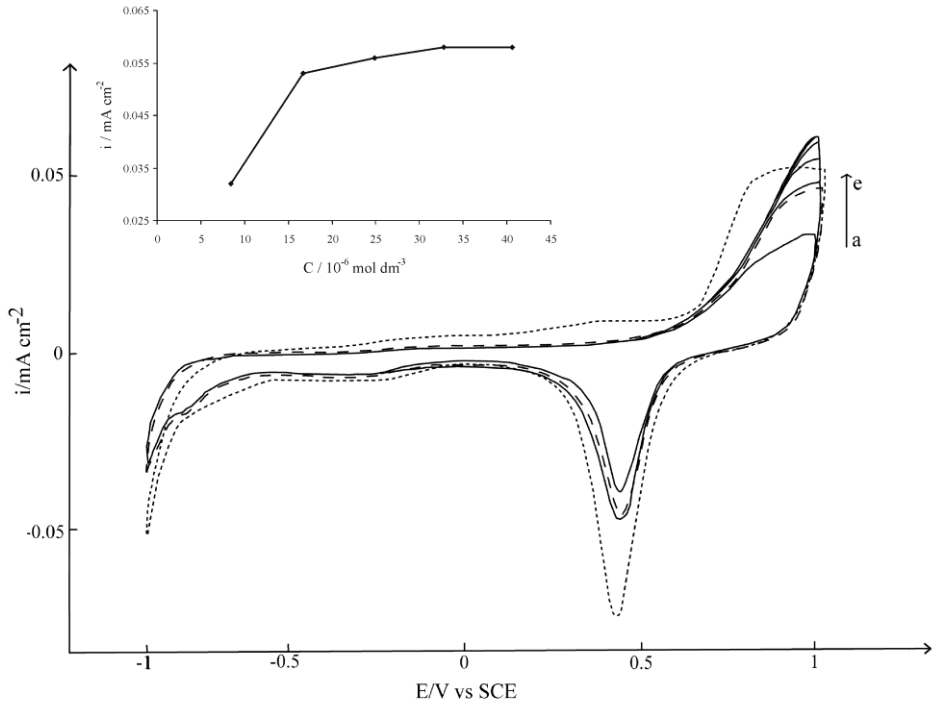


Figure 3. The cyclic voltammogram of bare gold electrode (.....) and modified with BSA (-----) as well as in the presence of oxaprozin standard (full lines) in the range of concentrations: a = 8.44 mol dm^{-3}; b = 16.71 mol dm^{-3}; c = 24.83 mol dm^{-3}; d = 32.78 mol dm^{-3} in 0.1 mol dm^{-3} phosphate buffer solution (pH 7.4), sweep rate 50 mV $s^{-}1$ (only the first sweep is recorded).

The binding of oxaprozin with BSA modified gold electrodes seems to obey a Langmuir isotherm, since adsorption sites (site II on albumin, Tyr411) are probably not close to each other, so the free energy of adsorption should not depend on coverage. This means that interaction between adsorbents should minimal. Due to this, the adsorption constant of oxaprozin at BSA modified gold electrode surfaces was calculated according to the following Langmuir adsorption thermodynamic equation [22]:

$$\frac{C}{i} = \frac{1}{K \cdot i_{max}} + \frac{C}{i_{max}}$$

where i represents anodic peak current, i_{max} the maximum of peak current, C the concentration of oxaprozin and K the binding constant of oxaprozin on the BSA modified gold electrode. From the equation it could be seen that the relationship of C/i and C should be a straight line. From the slope and intercept the value of i_{max} and K can be obtained.

A plot of C/i *versus* C on BSA / Au electrode was constructed and from the corresponding linear regression equation (C/i = 13.94·C + 113.59; R = 0.9892) the binding constant of oxaprozin on BSA / Au electrode was calculated to be 1.23×10^5 dm^3 mol^{-1}. The value of the binding constant of oxaprozin on BSA / Au electrode indicated that oxaprozin has higher binding affinity for BSA than its derivatives (oxaprozin-P and oxaprozin-E) [10,11].

4. CONCLUSION

As the very first electrochemical investigation of oxaprozin, nonsteroidal anti-inflammatory drug, using cyclic voltammetry on gold electrode in 0.05 mol dm^{-3} $NaHCO_3$, the synthesized drug, its analytical standard and its content in Duraprox® tablets were characterized with one oxidation reaction and the three reduction reactions. All they exhibited the linear concentration dependency of anodic currents at 0.83V for the analytical standard and 0.85V for Duraprox® tablets in the range of concentrations $8.44 - 32.78\times10^{-6}$ mol dm^{-3}.

Since the binding of oxaprozin with BSA modified gold electrodes seems to obey a Langmuir isotherm, the adsorption constant of oxaprozin at BSA modified gold electrode surfaces was calculated according to the following Langmuir adsorption thermodynamic equation with the results 1.23×10^5 dm^3 mol^{-1}.

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