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Comparison of the electrochemical properties of some colon-specific prodrugs of mesalazine

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The electrochemical behavior of 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid, 2-hydroxy-5-[(3-sulfophenyl)azo]benzoic acid and 2-hydroxy-5-[(2-sulfophenyl)-azo]benzoic acid was investigated by cyclic voltammetry using a glassy carbon electrode. The influence of the pH and position of the substituents on the reaction pathway has been studied. The results obtained are compared to the electrochemical properties of olsalazine. The reduction of these compounds is identified as an ECE process always leading to the cleavage of the azo bond. The electron-donating hydroxyl group in *para* position with respect to the azo bridge weakens the N-N bond, whereas the presence of the sulfo group strengthens this bond and renders the hydrazo intermediate more stable comparing to the hydrazo derivatives with two electron-donating groups in *para* positions.

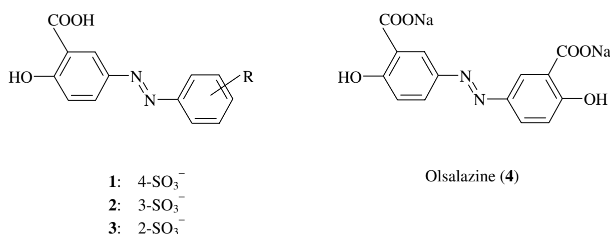
1. Introduction

Mesalazine (5-aminosalicylic acid) is an intiinflammatory drug primarily employed in the treatment of chronic inflammatory bowel diseases [1–3]. Mesalazine containing pharmaceutical preparations are designed to release therapeutic quantities of the drug in the terminal ileum and colon [4]. However, approximately 20% to 30% are absorbed after oral ingestion [5]. Two azo compounds, olsalazine (3,3′-azobis-(6-hydroxybenzoic acid) disodium salt) and sulfasalazine (salicylazosulphapyridine) have been used clinically as mesalazine precursors. The active part, mesalazine, is produced in the colon by bacterial reduction of the azo bridge [6]. The clinical use of sulfasalazine has been limited by the side effects that are ascribed to its sulphapyridine moiety [7]. Several colon-specific prodrug approaches to the delivery of the active mesalazine moiety to the colon have been reported [8, 9]. Among them 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid (**1**) has been designed [10]. It was found that orally administered **1** was hardly absorbed from the gastrointestinal tract and was not cleaved by digestive enzymes in the small-intestinal tract. As a result, much larger amounts of it could be transferred to the colon, where the azo bond was easily cleaved by intestinal microflora to yield mesalazine. In addition, sulfanilic acid produced as a by-product after azo bond cleavage has extremely low bioavailability, as expected from its extreme hydrophilicity [10].

Since biological cleavage of the azo bond is a reductive process, increased knowledge about the redox chemistry of azo compounds could facilitate the understanding of the pharmaceutical properties of such drugs.

In a previous paper [11] we have described the electrochemical behaviour of **1** at the glassy carbon electrode. It has been found that the electrochemical reduction of **1** follows an ECE mechanism always leading to the cleavage of the azo bond resulting in mesalazine and sulfanilic acid. The chemical step between the two charge transfers is an acid catalysed N-N bond cleavage or disproportionation reaction. These characteristics make **1** a suitable candidate for the medical applications.

In this work we extend our investigations to the study of other sulfo derivatives. The aim has been to compare the electrochemical properties of 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid (**1**), 2-hydroxy-5-[(3-sulfophenyl)-azo]benzoic acid (**2**) and 2-hydroxy-5-[(2-sulfophenyl)-azo]benzoic acid (**3**) and to explore the feasibility of application of these sulfo compounds in the treatment of inflammatory bowel diseases. The comparison of the electrochemical behaviour of **1**, **2** and **3** with the electrochemical behaviour of olsalazine, **4**, has been given and the influence of the position of the substituents on the stability of the hydrazo reduction intermediate is also discussed.



2. Investigations, results and discussion

The cyclic voltammograms of **1**, **2** and **3** in acidic media show one well-defined irreversible cathodic peak, Ic (Fig.1). The irreversibility of the Ic current peak clearly shows that charge transfer is followed by a fast homogenous chemical reaction. Moreover, the value of current function, $i_p/\nu^{1/2}$, was found to decrease with increasing scan rate ν, indicating the ECE nature of the electrode

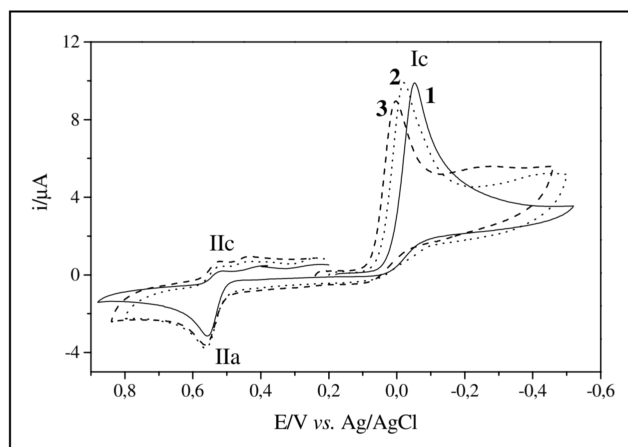


Fig. 1: Cyclic voltammograms of 5×10^{-4} M compounds **1**, **2** and **3** in Britton-Robinson buffer at pH 2.5 with a scan rates of 50 mVs^{-1}

reaction in which the chemical step is interposed between two electron transfers. The reduction of these azo compounds gives rise to the formation of a new electroactive species (IIa) that can be oxidised in the reverse scan at potentials which are characteristic for mesalazine [12]. When the scan direction was changed toward negative potentials again, new reduction peak (IIc) appeared at scan rates higher then $100\,mVs^{-1}$, forming a quasi-reversible pair with IIa. The evidence of mesalazine in a reaction medium confirms the proposed mechanism of azo bond cleavage upon electrochemical reduction. Another possible reduction product, the sulfanilic acid, is electrochemically inactive in the potential range investigated and therefore it could not be detected by electrochemical methods.

The shape and characteristics of cyclic voltammograms depend strongly on the pH of the medium for all species investigated. The potential of the Ic current peak shifts for all species toward a more negative direction with an increase of pH. The dependence of potentials on pH suggests that the reduction of the azo compounds is accompanied with an uptake of one proton per each electron. At the same time, Ic current peak height decreases with an increase of pH, and at pH > 7 being half the height of the current peak in acidic media.

In neutral and weakly basic media another anodic peak (Ia) appears on the cyclic voltammograms at scan rates higher then $50\,mVs^{-1}$ and at the potentials less positive then the potentials ascribed to the oxidation of mesalazine (Fig. 2). With a further increase of pH, peak Ia becomes more and more pronounced, being the only one observed at the pH $\geq$ 7.0. Peaks Ia and IIa seem to be mutually related since peak Ia increases on the expense of peak IIa. In addition, the ratio of IIa/Ia current peak heights depends on the scan rate suggesting that the species that are oxidized at the potentials of Ia current peak are slowly transformed by chemical reaction into mesalazine.

From the results presented it is obvious that the electrochemical reduction of these azo compounds is an ECE process leading to the cleavage of the azo bond and resulting in mesalazine and sulfanilic acid (Scheme). The first stage of the reaction is the uptake of $2e^-$ and $2H^+$ giving the corresponding hydrazo intermediate (first E process). The rate of the hydrolysis of the hydrazo bond is an acid catalyzed process and depends on the pH and the position of the sulfo substituent (C process). When pH < 5 the hydrazo intermediate is readily protonated enabling a fast splitting of the N-N bond leading to sulfanilic acid and 5-aminosalicylic acid quinoneimine. As the reduction potential of 5-aminosalicylic quinoneimine is more positive then the reduction potential of azo compounds, it is further reduced by a $2e^-/2H^+$ process to mesalazine as soon as it is formed (second E process). At pH > 7 the hydrazo intermediate is stable enough to be reoxidized back into the starting compound in the time scale of the cyclic voltammetry experiment.

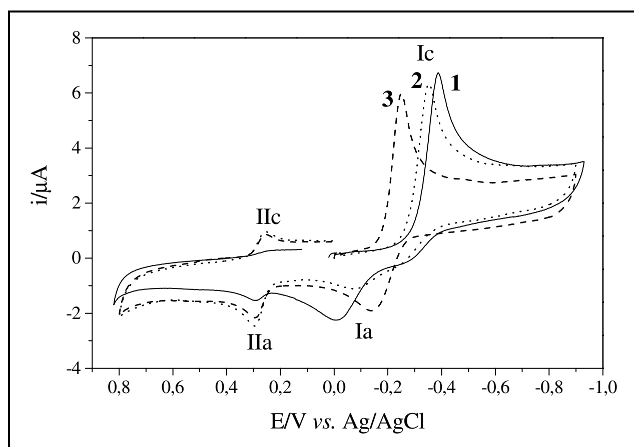
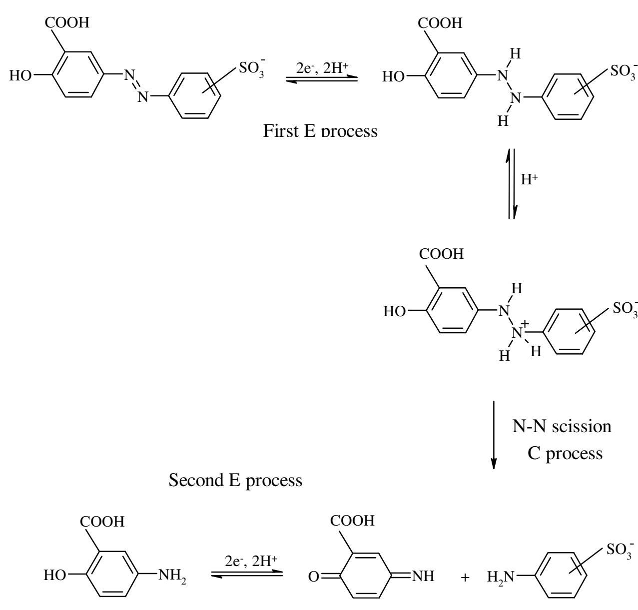


Fig. 2: Cyclic voltammograms of 5×10^{-4} M compounds **1**, **2** and **3** in Britton-Robinson buffer at pH 6.0 with a scan rates of $50\,mVs^{-1}$

Scheme



The potentials of the Ic current peak, as well as the rate of hydrazo bond cleavage depend on the position of the sulfo group relative to the azo bridge (Table). The reason for the more negative reduction potential for compound **1** is the fact that this compound has the electron withdrawing sulfo group in *para* position to the azo bond. On the other hand, the sulfo group in *meta* position strongly weakens the N-N bond and causes the fast scission of the hydrazo intermediate of compound **2**. A slow proton catalysed cleavage of the hydrazo bond of compound **3** can be explained by the influence of the *ortho*-sulfo substituent and possible steric hindrance.

The comparison of cyclic voltammograms of commercially available drug olsalazine (**4**) with the cyclic voltammogram of the *para*-sulfo derivative is given in Fig. 3. The reduction potential of olsalazine is more negative than the reduction potentials of sulfo derivatives. This behaviour could be expected taking into account that the hydroxyl group in *para* position relative to the azo bridge has a strong electron donating effect compared to the electron withdrawing effect of a sulfo group. However, the cleavage of N—N bond is for olsalazine so fast that the corresponding hydrazo intermediate could not be detected over the whole pH region investigated.

Table: The potentials of Ic current peak of azo compounds in Britton-Robinson buffer at pH 7 using scan rate of $50\,mVs^{-1}$

Compound	E(V)
1	−0.32
2	−0.44
3	−0.48
4	−0.53

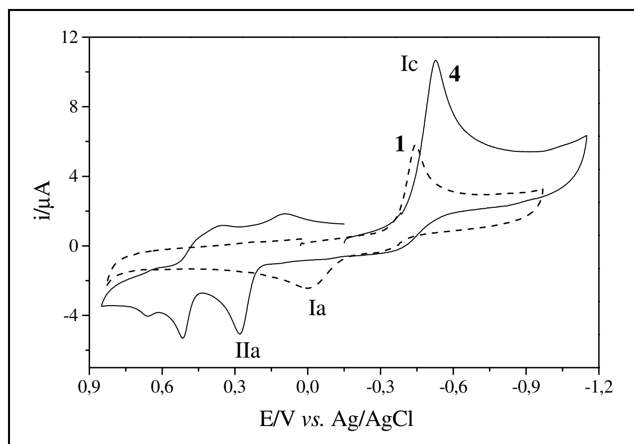


Fig. 3: Cyclic voltammograms of 5×10^{-4} M olsalazine (**4**) and compound **1** in Britton-Robinson buffer at pH 7.0 with a scan rates of 50 mVs^{-1}

Contrary to the sulfo derivatives, olsalazine yields two oxidation processes in the reverse scan. The electroactive species that can be oxidised at a potential of ca. +0.28 V is attributed to mesalazine and the anodic peak seen at +0.52 is due to the oxidation of the azo compound resulting in the formation of a benzoquinone diimine structure.
The electrochemical characteristics of sulfo derivatives are comparable to the electrochemical characteristics of olsalazine what makes sulfo derivatives suitable candidates for medical application. In addition, slow chemical cleavage of the hydrazo bond might improve the pharmacological activity of a mesalazine prodrug when taken orally, since it could enable the hydrazo intermediate to pass intact through the upper gastrointestinal tract. Accordingly, 2-hydroxy-5-[(2-sulfophenyl)azo] benzoic acid, **3**, might be a best clinical candidate due to the slowest rate of hydrazo bond cleavage what could enable its heighest efficiency to pass upper gastrointestinal tract.

3. Experimental

3.1. Reagents

Compounds **1**, **2** and **3** were prepared according to a literature procedure [10]. These compounds were identified by IR, NMR, MS and purity was determined by HPLC.
Mesalazine and sulfanilic acid were obtained from Merck (Darmstadt, Germany). Olsalazine were obtained from Aldrich (Milwaukee, USA). All other chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany) except sodium hydroxide that was purchased from Kemika (Zagreb, Croatia). Britton-Robinson buffers (pH 1.8–9.0) were prepared from boric, acetic and phosphoric acids and adjusted to the desired pH values with sodium hydroxide.
Stock solutions (5.0 mM) were prepared by dissolving the azo compounds in redistilled water. Since olsalazine has a low solubility in water, a drop of 2 M NaOH was added to facilitate the dissolution. Sample solutions (0.5 mM) were prepared from the stock solution by dilution of 1 ml of the stock solution to 10.0 ml with Britton-Robinson buffer of the desired pH.

3.2. Apparatus

The cyclic voltammetry experiments were carried out with an EG & G Princeton Applied Research Model 273A potentiostat controlled by the Model 270/250 Research Electrochemistry Software v. 4.30. Cyclic voltammetric studies were carried out using a glassy carbon working electrode ($A = 0.03\ cm^2$), a platinum auxiliary electrode, and an Ag/AgCl reference electrode. The potentials were scanned from −1200 to +900 mV employing scan rates between 10 mVs^{-1} and 1 Vs^{-1}. The glassy carbon electrode was polished intensively with aluminium oxide on a smooth polishing cloth and degreased in methanol prior to each electrochemical measurement. All the solutions examined by electrochemical techniques were first deaerated for at least 10 min with argon, after which a continuous stream of argon was passed over the solutions during the measurements.
The pH measurements were made with a Radiometer PHM 85 pH-Meter (Radiometer, Copenhagen, Denmark) using combined glass electrode (Radiometer PHC 2406L).

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