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Voltammetric studies of 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid as a novel prodrug of 5-aminosalicylic acid

Biljana Nigović [a,*], Zoran Mandić [b], Branimir Šimunić [b], Ines Fistrić [b]

[a] *Faculty of Pharmacy and Biochemistry, University of Zagreb, 10000 Zagreb, Croatia*
[b] *R&D, PLIVA, Pharm. Ind. Inc., Prilaz b Filipovica 25, 10000 Zagreb, Croatia*

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Abstract

The electrochemical properties of a colon-targeted prodrug of 5-aminosalicylic acid (5-ASA), 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid (SPSA), were investigated in aqueous solutions at glassy carbon electrodes using cyclic voltammetry and controlled potential electrolysis. The influence of the pH and experimental time domain on the reaction pathway has been studied. The electrochemical reduction of SPSA is identified as an ECE process always leading to the cleavage of azo bond. In an acidic media SPSA is reduced in a $4e^-/4H^+$ process yielding 5-ASA and sulfanilic acid. In neutral and weakly basic media SPSA is reduced in $2e^-/2H^+$ process resulting in the hydrazo intermediate that is stable enough to enable its reoxidation back to SPSA in the time scale of the cyclic voltammetry.
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1. Introduction

5-Aminosalicylic acid (5-ASA, mesalazine) is identified as an active component in the therapy of inflammatory bowel disease such as Crohn's disease and ulcerative colitis. The therapeutic action of 5-ASA is believed to be coupled to its ability to act as a free radical scavenger [1–4], acting locally on the inflamed colonic mucosa [5–7]. However, the clinical use of 5-ASA is limited, since orally administered 5-ASA is rapidly and completely absorbed from the upper gastrointestinal tract and therefore the local therapeutic effects of 5-ASA in the colon is hardly expected [8].

Presently, two azosalicylic acids, salicylazosulphapyridine (Sulfasalazine) and 3,3′-azobis-(6-hydroxybenzoic acid) disodium salt (Olsalazine) are employed in clinical praxis as 5-ASA precursors. The active part, 5-ASA, is produced in the colon

* Corresponding author. Tel.: +385-1-48-11559; fax: +385-1-48-56201.
E-mail address: biljana@nana.pharma.hr (B. Nigović).

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by bacterial reduction of azo bridge. However, both of these prodrugs show a considerably high rate of adverse reactions [9], what prevents their unlimited clinical administration. Most of the salicylazosulphapyridine side effects reported are ascribed to its sulphapyridine moiety, which is almost completely absorbed from the colon [10]. Several colon-specific prodrug approaches to the delivery of the active 5-ASA moiety to the colon without using sulphapyridine moiety have been reported [11,12]. Among them 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid (SPSA) has been designed [13]. It was found that orally administered SPSA 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 5-ASA. In addition, sulfanilic acid produced as a by-product after azo-bond cleavage has extremely low bioavailability, as expected from its extreme hydrophilicity [13].

Since biological cleavage of azo bond is reductive process much effort has been done to the investigation of mechanism and kinetics of the electrochemical reduction of azo compounds [14–17]. It has been found that the electrochemical reductions of unsubstituted aromatic azo compounds, or azo compounds with electron withdrawing substituents, occur in $2e^-$, $2H^+$ process to give hydrazo products. However, in the presence of strong electron donating substituents, such as hydroxyl and amino groups, reduction resulted in amino compounds as the final products. In spite of the fact that polarographic studies do not provide information about reverse reactions, very few attempts have been made to study the redox-reactions of azo compounds at solid electrodes [18,19].

In the present paper, the electrochemical behaviour of SPSA (Fig. 1) has been investigated at glassy carbon electrode. The objective was to investigate the mechanism of the electrochemical reduction of SPSA and to determine the possibility of reduction of SPSA under conditions close to those of biological system.

2. Experimental

2.1. Materials

SPSA was prepared according to the procedure described previously [13]. The identification of SPSA was performed by IR, NMR, MS and purity was determined by HPLC.

5-ASA and sulfanilic acid were obtained from Merck (Darmstadt, Germany). 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.

2.2. Cyclic voltammetry

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 -1000 to $+900$ mV employing scan rates between 10 mV/s and 1 V/s.

The working electrode was polished intensively with aluminium oxide on a polishing cloth and degreased in methanol prior to each electrochemical measurement. Stock solution (5.0 mM) of SPSA was prepared by dissolving in redistilled water. 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. The pH measurements were made with a Radiometer PHM 85 pH-Meter (Radiometer, Copenhagen, Denmark) using com-

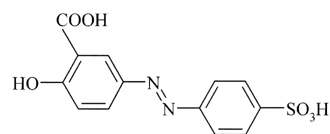

Fig. 1. Structural formula of 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid (SPSA).

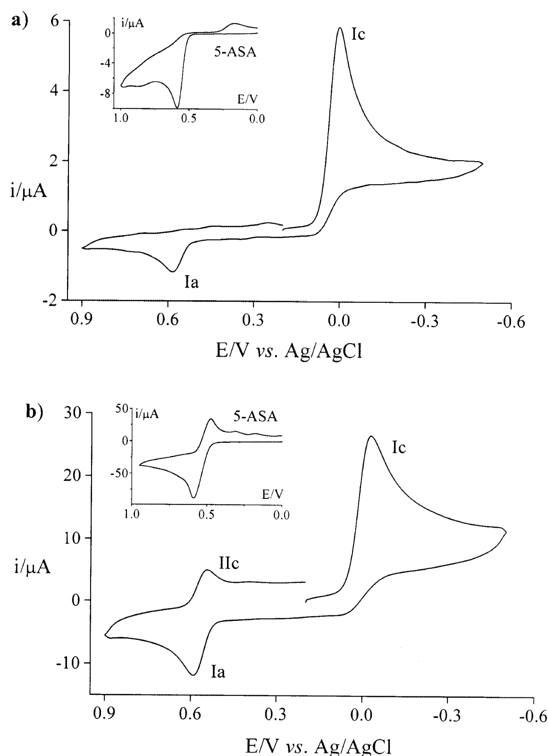

Fig. 2. Cyclic voltammograms of SPSA (0.5 mM) in Britton–Robinson buffer of pH 1.8 at a scan rates of (a) 20 mV/s and (b) 500 mV/s. Inset: Cyclic voltammograms of 5-ASA at the same conditions.

bined glass electrode (Radiometer GK 2322C). 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.

2.3. Controlled potential electrolysis

Controlled potential electrolysis was performed by using EG&G Princeton Applied Research Model 273A potentiostat. A large glassy carbon plate electrode ($A = 4$ cm^2) was employed as working electrode. A large carbon rod and Ag/AgCl served as a counter and a reference electrode respectively. SPSA (13.6 mg) was electrolysed in 20 ml of Britton–Robinson buffer in a conventional H-type electrolysis cell at the potentials of I_c current peak determined by cyclic voltammetry experiments. The progress of electrolysis was monitored by HPLC by taking aliquots of 100 μl at different time intervals. The solution was purged with nitrogen throughout the electrolysis.

2.4. HPLC analysis

HPLC analyses were carried out by using Hewlett Packard 1100 system (solvent delivery system: HP 1100; diode array detector HP 1100; column oven HP 1100; autosampler HP 1100 with Rheodyne injection valve with a 20 μl loop; column oven Hewlett Packard 1100. The analytical column was Waters Symmetry C 18 column, 3.5 μm, 100 × 4.6 mm ID.

The separation was obtained in 15 min using a flow rate of 1 ml/min in the gradient elution. Elution was carried out by binary gradient using phase A (0.05 M potassium phosphate buffer, pH 3.2) and phase B (methanol) according to the following program: from 0.1 to 40% B in 10 min. Diode array detection was used wavelengths set at 256 and 210 nm, and injection volumes were 10 μl.

3. Results and discussion

3.1. Cyclic voltammetry

Cyclic voltammograms of SPSA recorded at scan rates of 20 and 500 mV/s and pH 1.8 are shown in Fig. 2. In cyclic voltammograms one well-defined irreversible cathodic peak, Ic, was observed. The irreversibility of Ic current peak clearly shows that charge transfer is followed by a fast homogenous chemical reaction. The Ic current peak height is neither linear with scan rate, v, nor with $v^{1/2}$, suggesting a weak adsorption of SPSA at the surface of glassy carbon electrode. On the other hand, the value of current function, $i_p/v^{1/2}$, was found to decrease with increasing scan rate v (Fig. 3). The exponential nature of the current function *versus* the scan rate plot indicates the ECE nature of the electrode reaction in which

the chemical step is interposed between two electron transfers with charge transfer being at lower potential than the first.

The reduction of SPSA at the potentials of Ic current peak gives rise to the formation of a new electroactive species that can be oxidised in the reverse scan at a potential of ca. + 0.58 V (Ia). When the scan direction was changed toward negative potentials again, new reduction peak (IIc) appeared at scan rates higher then 100 mV/s, forming quasi-reversible pair with Ia.

The oxidation of a new electroactive species formed after reduction of azo compound resulted in the electrochemical behaviour characteristic for 5-ASA (Fig. 2 inset). At slow scan rates an irreversible oxidation wave is observed and a single reduction wave is seen at much more negative potential than would be expected for reversible reduction. This indicates that the initial product of the oxidation undergoes a chemical reaction to yield a second product that can be reduced at more negative potentials. As the scan rate is increased the oxidation becomes quasi-reversible. This is in a good agreement with previous findings which indicated a mechanism involving the initial two-electron, two proton oxidation of 5-ASA to the quinone imine, followed by hydrolysis to produce the corresponding quinones [20,21].

The evidence of 5-ASA 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

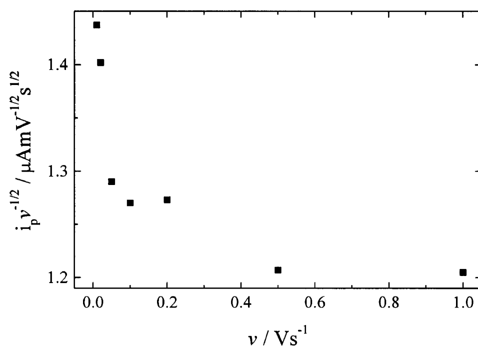

Fig. 3. Observed dependence of $i_p/v^{1/2}$ for Ic current peak on scan rate v for SPSA at pH 1.8.

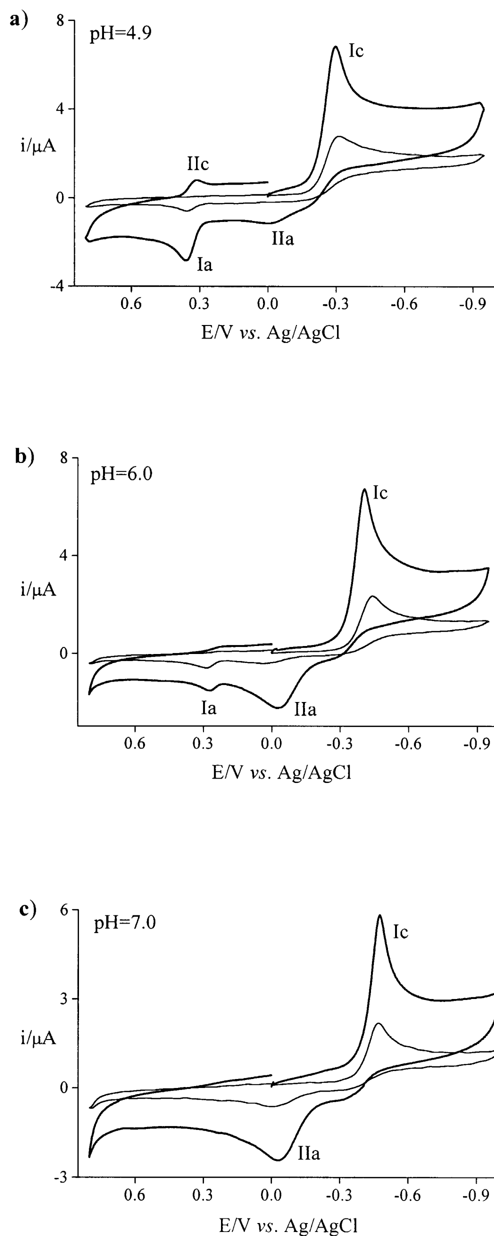

Fig. 4. Cyclic voltammograms of SPSA (0.5 mM) recorded in Britton–Robinson buffer at (a) pH 4.9, (b) pH 6.0 and (c) pH 7.0 with a scan rates of 10 mV/s (—) and 50 mV/s (—).

investigated and therefore it could not be detected by the electrochemical methods.

The shape and characteristics of cyclic voltammograms depend strongly on the pH of the

Table 1
The potentials and heights of Ic current peak of SPSA at different pH values using a scan rate of 50 mV/s

pH	*E* (V)	*i* (μA)
1.8	−0.01	9.1
3.7	−0.16	9.0
4.9	−0.30	7.1
6.0	−0.41	6.7
7.0	−0.48	5.8
9.0	−0.69	4.3

Table 2
Effect of scan rate on the heights of Ia and IIa current peaks for 0.5 mM of SPSA at pH 6.0

Scan rate (mV/s)	Ia (μA)	IIa (μA)	Ia/IIa
10	0.29	0.21	1.38
20	0.19	0.41	0.46
50	0.26	1.39	0.19
100	0.06	1.91	0.03
200	Shoulder	2.81	–

medium (Fig. 4). All the potentials shift toward more negative direction with an increase of pH (Table 1). The following relations can represent the effect of pH on the potential of the Ic peak over the pH range 1.8–9.0:

$$E_p = 185\text{–}95 \text{ pH mV } vs. \text{ Ag/AgCl} \quad |r| = 0.9991$$

Since a reduction of the azo compounds should require the uptake of one proton per electron to yield a hydrazo compound [14], or an amine, a slope of −59 mV/pH is expected for the reduction potential as a function of the pH. The experimental value of the slope of the E_p *versus* pH plot was found to be −95 mV/pH (Fig. 5). There are several possible reasons for this deviation from the expected value of −59 mV/pH including, for example, pH dependent adsorption and the possibility that the protonation of the reaction products changes in the investigated pH range. The $-NH_3^+$ group of 5-ASA has a pK_a value of 5.78 and sulfanilic acid has pK_{a2} value 3.25 [22]. A slope of −90 mV/pH would hence be expected at pH values lower than the pK_a values of the products.

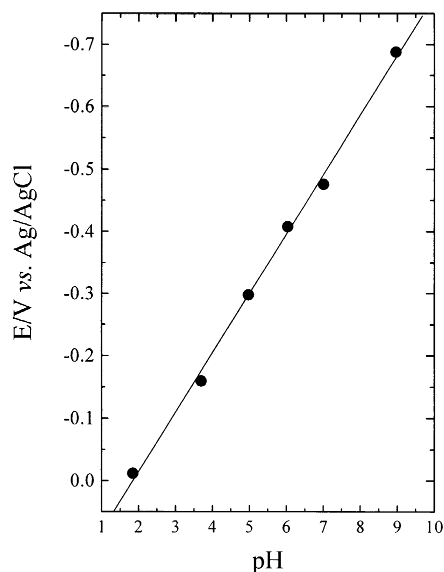

Fig. 5. Dependence of potentials on pH for the reduction peak Ic of SPSA at a scan rate of 50 mV/s.

The height of Ic reduction peak was found to decrease with increasing pH (Table 1). At pH 9.0 the peak current was almost half the current obtained at pH 1.8. At the intermediate pH values (pH 4.9) another anodic peak (IIa) appears on the cyclic voltammograms at scan rates higher then 50 mV/s at potentials less positive then the potentials ascribed to the oxidation of 5-ASA (Fig. 4). With a further increase of pH, peak IIa becomes more and more pronounced, being the only one observed at the pH ≥ 7.0. Peaks Ia and IIa seem to be mutually related since peak IIa increases on the expense of peak Ia. In addition, the ratio of Ia/IIa current peak heights depends on the scan rate (Table 2) suggesting that the species that are oxidized at the potentials of IIa current peak, are slowly transformed by chemical reaction into 5-ASA. The chemical transformation is acid catalysed process and could be observed only in the pH region where the rate of transformation is comparable with the time-scale of the experiment.

Therefore, taking into account negligible effect of the pH on diffusion coefficient of SPSA, it was concluded that the mechanism of electrode process occurring in an acidic medium is different from the process occurring at pH ≥ 7.0 and that charge consumed in the reduction process at

pH ≥ 7 is half the charge consumed in acidic media.

3.2. Constant potential electrolysis

Constant potential electrolysis has been carried out at glassy carbon plate electrode at pH 3 and pH 8. The potentials of the electrolysis were set at the potentials of Ic current peak determined by cyclic voltammetry experiments. The progress of the reduction was monitored by HPLC.

After 4 h of electrolysis, the concentration of SPSA dropped almost to zero and at the same time the concentrations of both 5-ASA and sulfanilic acid reached the value of the SPSA concentration at the start of the electrolysis (Fig. 6). The charge consumed in electrolysis in both cases amounted to $z = 4 \pm 0.2$ F/mol. This means that electroreductive cleavage of azo bond proceeds smoothly without any side reactions, which might complicate the reaction.

There seems to be a discrepancy between results obtained by cyclic voltammetry and the results obtained by constant potential electrolysis. Namely, since Ic peak height in mild basic media is half the value of Ic peak in acidic media, one would conclude that charge consumed at pH 8 should amount to 2 F/mol. However, considering the time scales of the two experiments it is obvious that chemical reaction taking place between two charge transfer processes is too slow to be registered by cyclic voltammetry.

3.3. Mechanism

From the results presented it is obvious that the electrochemical reduction of SPSA is ECE process leading to the cleavage of azo bond and resulting in 5-ASA and sulfanilic acid. The first stage of the reaction is the uptake of $2e^-$ and $2H^+$ giving corresponding hydrazo intermediate (first E process, reaction 1). Hydrazo intermediate is not electroactive in the potential range investigated, but N–N bond is cleaved by acid catalyzed process (C process, reaction 2). When pH < 5 hydrazo intermediate is readily protonated enabling a fast scission of N–N bond leading to sulfanilic acid and 5-ASA quinoneimine. As the reduction potential of 5-ASA quinoneimine is more positive then the reduction potential of SPSA, it is further reduced by $2e^-/2H^+$ process to 5-ASA as soon as it is formed (second E process, reaction 3).

Therefore, in an acidic media only one step of the reduction of SPSA is observed. The SPSA is reduced at the potentials of Ic current peak in a $4e^-/4H^+$ process yielding 5-ASA and sulfanilic acid. In neutral and weakly basic media SPSA is reduced in $2e^-/2H^+$ process resulting in the hy-

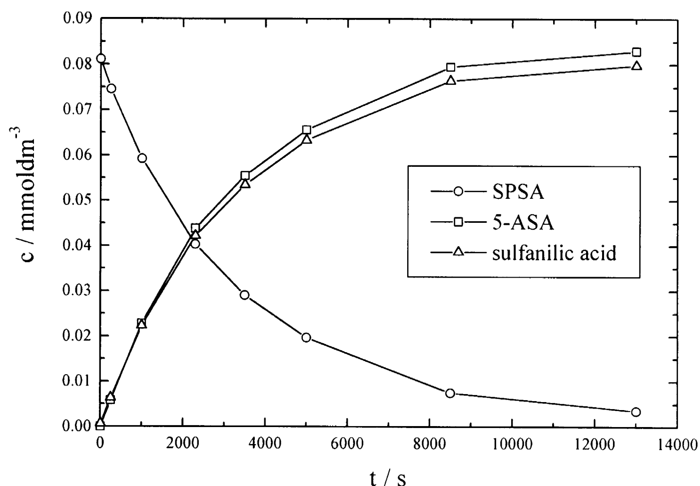

Fig. 6. Electrochemical reduction of SPSA in Britton–Robinson buffer, pH 3. Concentration profiles of reactant and products *vs.* time.

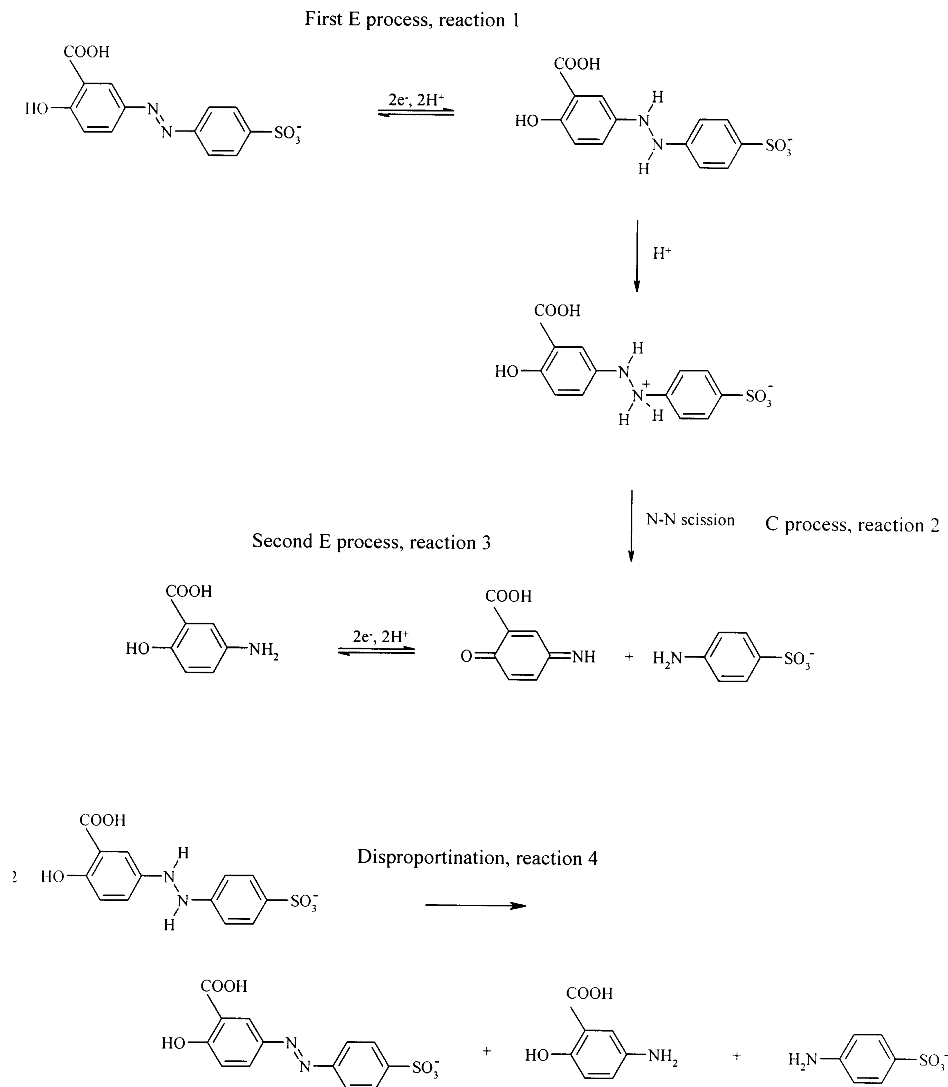

Fig. 7. Overall mechanism of electroreduction of SPSA at GC electrode.

drazo intermediate that is stable enough to enable its reoxidation back to SPSA (peak IIa) in the time scale of the cyclic voltammetry experiment.

It is possible that after the chemical cleavage of N–N bonds a disproportionation reaction of the hydrazo intermediate takes place (reaction 4) together with the second E step. However, how much disproportionation reaction participate in the overall mechanism is not possible to conclude with the given experimental techniques. To distinguish between ECE and disproportionation mechanism, optical techniques or double potential step chronoamperometry could be used (Fig. 7).

4. Conclusions

SPSA is electrochemicaly reducible at glassy carbon electrode. The reduction follows ECE pathway with the cleavage of azo bond representing chemical step. The electron-donating OH group in *para* position to azo bridge enables acid

catalysed cleavage of azo bond, although SO_3 group render the hydrazo intermediate more stable comparing to the hydrazo derivatives with two electron donating groups in *para* positions [19].

Electrochemical characteristics of SPSA are comparable to those of Olsalazine and Sulfasalazine [19] what makes SPSA a suitable candidate for possible medical applications. In addition, slow chemical cleavage of hydrazo bond might improve the pharmacological activity of 5-ASA prodrug when taken orally, since it could enable the hydrazo intermediate to pass intact through the upper gastrointestinal tract.

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