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Electrochemical Oxidation of Sulfasalazine at a Glassy Carbon Electrode

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Abstract: Sulfasalazine (SSZ) is a pharmaceutical compound used for the treatment of rheumatoid arthritis. The electrochemical oxidation of SSZ at a glassy carbon electrode was studied by cyclic, differential pulse and square wave voltammetry in a wide pH range. For electrolytes with $pH < 11.0$, the oxidation is an irreversible, diffusion-control, pH-dependent process that involves the transfer of one electron and one proton from the hydroxyl group of the salicylic moiety. For $pH > 11.0$ the oxidation is pH-independent, and a $pK_a \approx 11$ was determined. The formation of a quinone-like oxidation product that undergoes two electrons and two protons reversible redox reaction was observed. Also, UV-vis spectra of SSZ were recorded as a function of supporting electrolytes pH. An electrochemical oxidation mechanism was proposed.

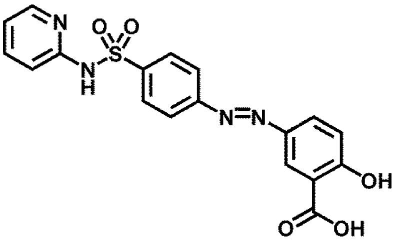
Keywords: Sulfasalazine · Oxidation mechanism · Glassy carbon electrode · Differential pulse voltammetry · UV-vis spectrophotometry

1 Introduction

Rheumatoid arthritis (RA) is an autoimmune disease which provokes disordered and systemic inflammation of the synovial joints, affecting many other tissues and organs, especially the lungs, pericardium and sclera [1–3]. The painful and disabling effects of the disease can lead to a loss of mobility and functioning. If not properly treated RA has a significant direct impact on economic activities [4].

The usual treatment of RA involves the use of non-steroid analgesics and disease modifying anti-rheumatic drugs which are capable of slowing the progression of the disease and allow the maintenance of personal economic activities. Methotrexate [1–5] is the first line of treatment when RA is first diagnosed but other drugs are administrated in later stages [6].

Sulfasalazine, 5-[4-(2-pyridylsulfamoyl) phenylazo] salicylic acid (SSZ), Scheme 1, is a pharmaceutical product that belongs to the anti-inflammatory class of azo-salicylic acid derivatives used for the treatment of RA [1–6]. SSZ is also recommended for the other types of inflammatory arthritis, such as psoriatic arthritis, and is used for treatment of Crohn's disease and ulcerative colitis. SSZ is poorly absorbed into the blood stream and serum levels below 50 $\mu g L^{-1}$ are considered safe to avoid side effects.



Scheme 1. Chemical structure of sulfasalazine.

SSZ is only soluble in alkaline media, and its absorption in vivo occurs only after the duodene in the small intestine, where the pH is 8.0 and 9.0. However, at this pHs the amount of SSZ solubilized is still low, which explains the reduced biodisponibility of the drug (around 15%) and the necessity of an increased SSZ daily dose (about 2–3 g/day) [2]. The drug is metabolized into 5-ASA and sulfapyridine which are immediately absorbed in vivo [5].

Monitoring SSZ levels is usually carried out every three months, but more frequently at the outset of the drug.

Analytical techniques for SSZ detection and quantification in the presence of other sulfonamides involve immunochemical methods [7], CE-MS detection [8], HPLC-MS [9] and micro-extraction-MS [10]. Electroanalytical techniques were used for the SSZ determinations at bismuth [11] and antimony [12] film modified glassy carbon electrodes with *LOD* of 1.2 μM and 0.58 μM, respectively. A molecular imprinted polymer modified carbon paste electrode allowed SSZ detection in down to 10^{-9} M [13].

Concerning the SSZ redox mechanism less information is available in the literature. Azo-salicylic compounds undergo electrochemical reduction at the azo nitrogen atom

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followed by the cleavage of the azo bond and releasing the 5-ASA [14–17]. On the other hand, salicylic derivatives undergo oxidation at the aromatic ring followed by nucleophilic addition of water producing benzoquinone derivatives in accordance to the degree and position of substituents in the aromatic ring [18–21].

The electrochemical behavior of pharmacological compounds can provide valuable information into their mechanism of action [22].

The aim of the present study is the investigation of SSZ anodic oxidation behavior, using cyclic, square wave and differential pulse voltammetry, at a glassy carbon electrode, in order to clarify the in vivo SSZ redox mechanism.

2 Experimental

2.1 Materials and Reagents

Sulfasalazine (SSZ) was obtained from Sigma Aldrich. A stock solution of 1 mM of SSZ was prepared in 1 mM NaOH and stored at 5 °C. Solutions of different concentrations of SSZ were prepared by dilution of the appropriate quantity in support electrolyte.

The 0.1 M ionic strength electrolyte solutions: pH 2.0 KCl/HCl, pH 3.4–5.4 acetate buffer, pH 6.1–8.0 phosphate buffer, pH 9.2–10.5 H_3BO_3/NaOH, and pH 12.0 NaOH/KCl were prepared [23] using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 10 μS/cm).

The pH measurements were carried out using a Crison micropH 2001 pH-meter with Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C) and microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Raining Instrument Co. Inc., Woburn, USA).

2.2 Instrumental

2.2.1 Voltammetric Parameters and Electrochemical Cells

Voltammetric experiments were carried out using an Autolab PGstat 10 with FRA module and USB interface running GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a three-electrode system in a 3 mL one compartment electrochemical cell (eDAQ, Poland). A glassy carbon electrode (GCE, $d = 1.0$ mm) was the working electrode, a Pt wire the counter electrode and an Ag/AgCl (3 M KCl) reference electrode.

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms, step potential of 2 mV and scan rate 5 mV s^{-1}. For square wave (SW) voltammetry were used: amplitude pulse of 50 mV, frequency of 25 Hz and a potential increment of 2 mV corresponding to an effective scan rate of 50 m s^{-1}. The experimental conditions for CV were scan rate 100 m s^{-1}.

The GCE was polished using diamond spray (particle size 1 μm) (Kemet, UK), before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following the mechanical treatment, the GCE was placed in supporting electrolyte and voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

2.2.2 Spectrophotometric Parameters

Absorption spectra were recorded using the UV-vis spectrophotometer SPECORD S100 from Carl Zeiss Technology with Win-Aspect software. The experimental conditions for absorption spectra were: integration time 25 ms and accumulation 1000 points. A quartz cuvette of 1.0 mm of optical path was used in the measurements.

2.3 Acquisition and Presentation of Data

All the voltammograms presented were baseline corrected using the moving average application with a step window of 2 mV included in GPES version 4.9 software. The mathematical treatment of the original voltammograms was used in the presentation of all experimental data for a better and clearer identification of the peaks. The values for peak current presented in all plots were determined from the original untreated voltammograms.

All spectra presented were shown without any further mathematical treatment and all values for λ_{max} and absorbance were obtained from the original spectra.

3 Results and Discussion

3.1 Voltammetry

3.1.1 Cyclic Voltammetry

Cyclic voltammograms were recorded in 50 μM SSZ in buffer solutions with pH between 2.0 and 12.0 at a scan rate of 100 m s^{-1}. One irreversible anodic peak 1_a was observed in all electrolytes, and for 0.1 M phosphate buffer pH 7.4 at $E_{p1a} = +0.79$ V (Figure 1A).

The peak current decreased in subsequent scans due to adsorption of the oxidation products at the electrode surface. For electrolytes with $\text{pH} < 2.0$ and $\text{pH} > 12$, no charge transfer reaction occurred.

The effect of scan rate on the oxidation peak 1_a was studied in solutions of 50 μM SSZ in 0.1 M phosphate buffer pH 8.0. CVs were recorded at different scan rates between 10 m s^{-1} and 400 m s^{-1}. Peak 1_a current increased with increasing scan rate following a linear relationship with $v^{1/2}$, in agreement with a diffusion controlled process.

3.1.2 Differential Pulse Voltammetry

The pH effect on the electrochemical oxidation of SSZ was studied by DP voltammetry in 50 μM SSZ in buffer supporting electrolyte $2.0 < \text{pH} < 12.0$ (Figure 2A).

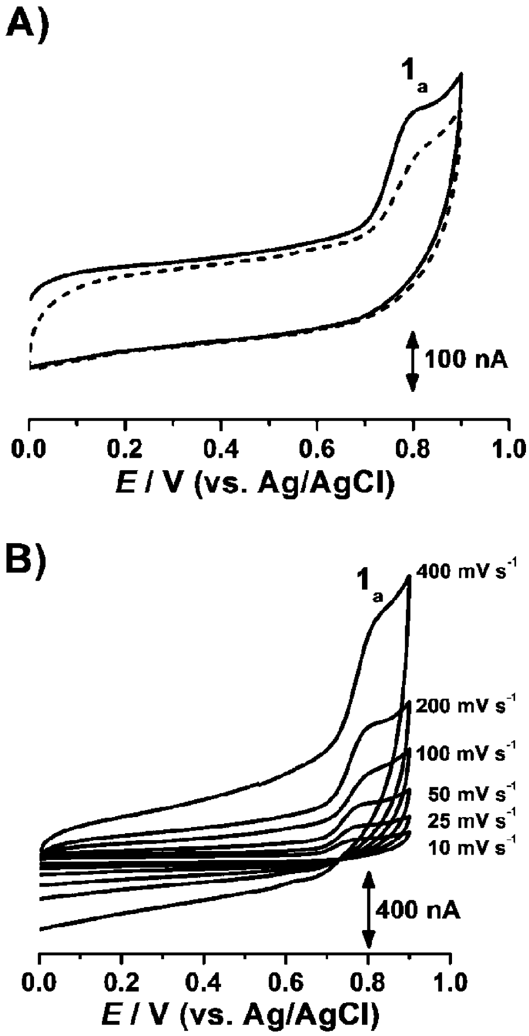


Fig. 1. CVs in 50 μM SSZ: (A) pH 7.4 (—) first and (- - -) second scans at $v = 100\ \mathrm{m\,s^{-1}}$ and (B) pH 8.0 at different scan rates.

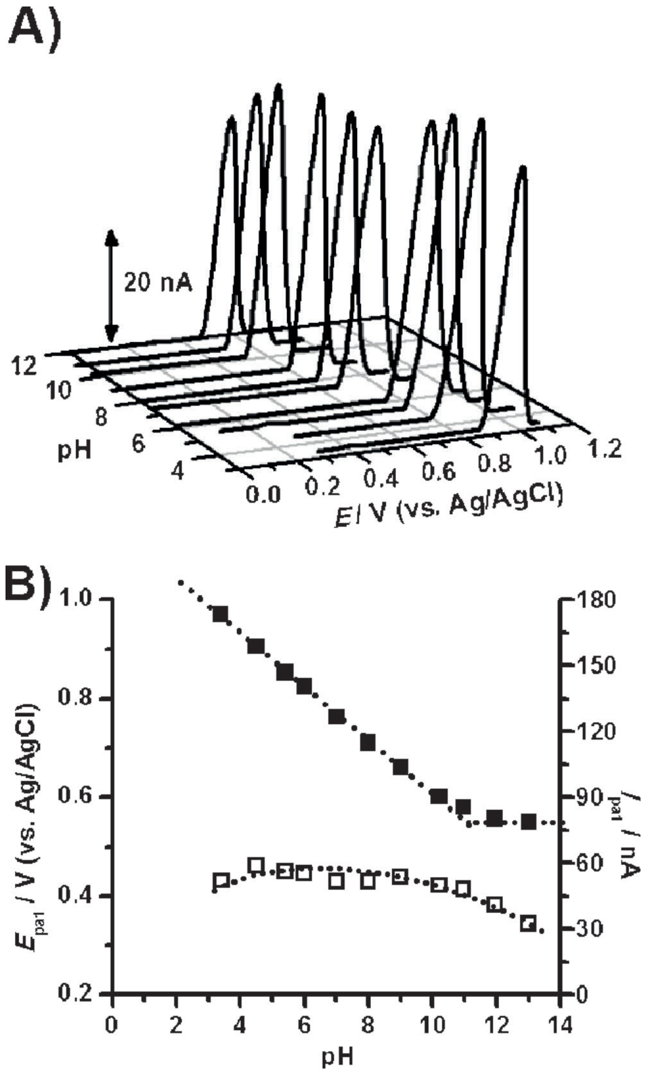


Fig. 2. (A) 3D plot of DP voltammograms baseline corrected in 50 μM SSZ; (B) Plot of peak 1_a (■) E_{p1a} and (□) I_{p1a} vs. pH.

For $pH < 11.0$, the oxidation of SSZ was pH-dependent and peak 1_a was shifted to a less positive potential with increasing pH following a linear relationship: E_{p1a} (V) $= 1.26 - 0.059$ pH (Figure 2B).

The slope of the dotted line, for E_{p1a} vs. pH (Figure 2B) indicated an equal number of protons and electrons involved in the electrochemical oxidation mechanism. The width at half-height of peak 1_a, $W_{1/2} = 98$ mV, is close to the theoretical value for the transfer of one electron. For electrolytes with $pH > 11.0$, the oxidation of SSZ was pH-independent (Figure 2B), in agreement with the chemical deprotonation of the molecule, and a $pK_a \approx 11$ was determined [30].

The peak 1_a current varied with pH (Figures 2A and B). In acid media a smaller peak was observed, in agreement with the acid-base equilibrium of the carboxyl group ($pK_a = 2.98$) in the SSZ molecule salicylic moiety which became negatively charged for $pH > 3.0$ [24]. For $3 < pH < 10.0$ peak 1_a current was constant and for electrolytes with $pH > 10.0$, the deprotonation of the hydroxyl group was responsible for the decrease of peak 1_a current.

Successive DP voltammograms were recorded in the same solution without cleaning the GCE surface. The first voltammogram recorded in 50 M SSZ at pH 8.0 showed peak 1_a and in the second voltammogram, a new small peak 2_a, at $E_{p2a} = +0.10$ V, corresponding to the oxidation of a SSZ oxidation product appeared (Figure 3). Peak 1_a current decreased and shifted to more positive potentials with increasing number of scans.

Peak 2_a was pH-dependent (Figure 4A), and E_{p2a} shifted to a less positive potential with increasing pH (Figure 4B). The variation of E_{p2a} with pH, $E_{p2a} = 0.624 - 0.059$ pH, indicates that the oxidation process involves the same number of protons and electrons.

The width at half-height of the peak 2_a, $W_{1/2} \approx 61$ mV, is close to the theoretical value for the transfer of two electrons [25]. Thus, the oxidation of SSZ redox product involved two electrons and two protons.

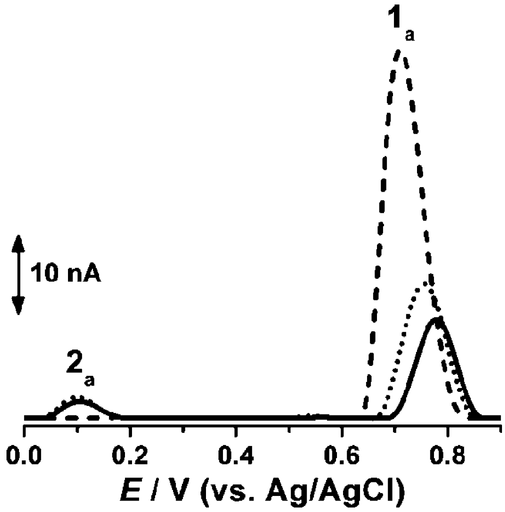


Fig. 3. DP voltammograms baseline corrected in 50 µM SSZ in pH 8.0; (- - -) first, (•••••) second and (—) third scan.

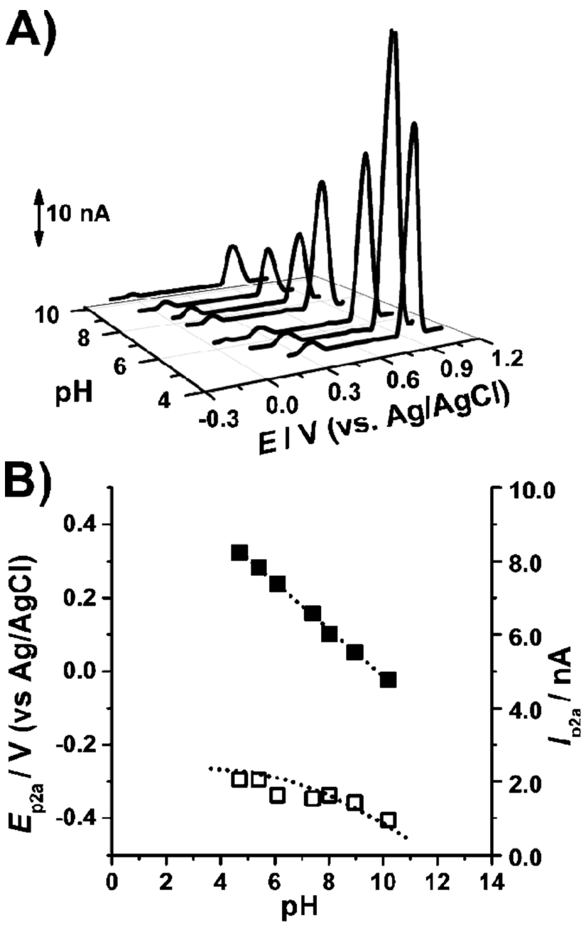


Fig. 4. A) 3D plot of DP voltammograms baseline corrected in 50 µM SSZ; B) Plot of peak 2_a (■) E_{p2a} and (□) I_{p2a} vs. pH.

The SSZ oxidation product, peak 2_a oxidation current, did not change when DP voltammograms were registered in low (25 µM) or high (100 µM) SSZ concentrations. This indicates the formation of an adsorbed monolayer of the SSZ oxidation product on the glassy carbon surface.

3.1.3 Square Wave Voltammetry

SW voltammograms were recorded in 50 µM SSZ in electrolytes with different pH and the anodic peak 1_a occurred on the first scan.

In pH 8.0, peak 1_a, at $E_{p1a} = +0.75$ V, was observed (Figure 5A).

On the second SW voltammogram without cleaning the GCE surface, reversible peak $2_a/2_c$, at $E_{p2a} = +0.10$ V, appeared (Figure 5B).

Plotting the forward and the backward components of the total current of peak $2_a/2_c$, they occur at similar potentials and with similar currents, in agreement with the SSZ oxidation product, peak $2_a/2_b$, reversible charge transfer reaction.

3.2 UV-Vis Spectrophotometry

The UV-vis spectra of SSZ and salicylic acid (SA) were recorded at pH 7.4 (Figure 6A).

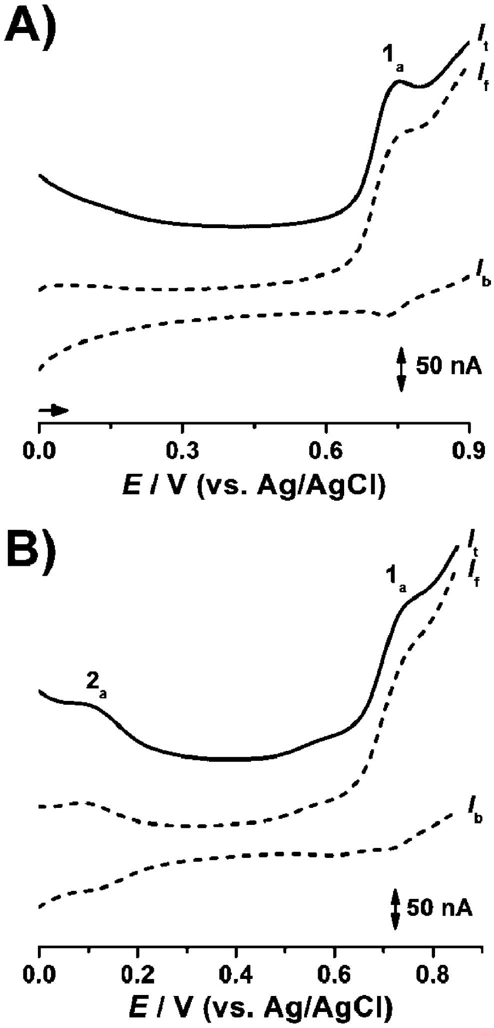


Fig. 5. SW voltammograms in 50 µM SSZ in pH 8.0: (A) first and (B) second scan; I_t - total, I_f - forward and I_b - backward current.

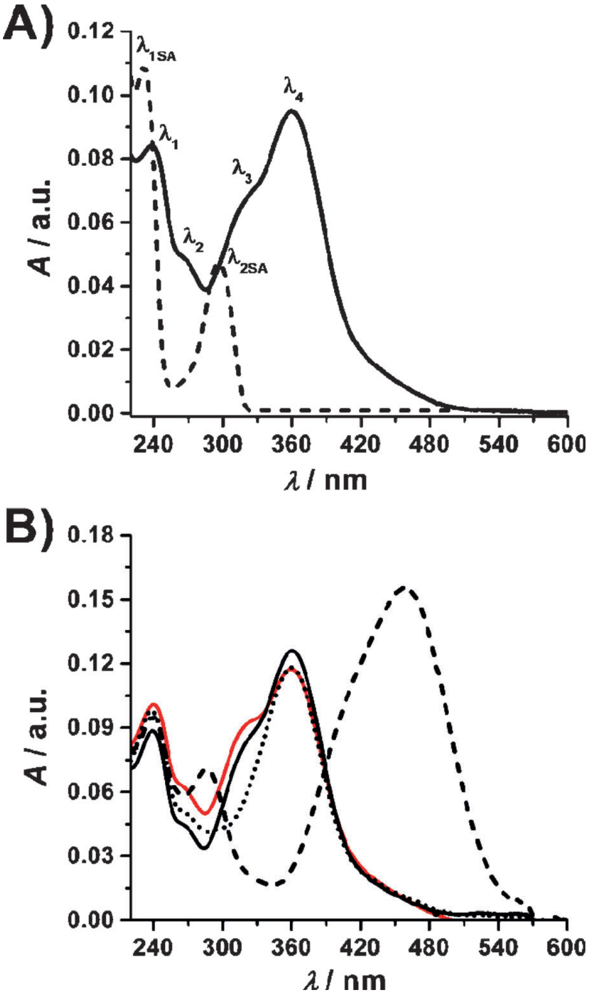


Fig. 6. UV-vis spectra of 50 μM: (A) (—) SSZ and (- - -) salicylic acid in pH 7.4 and (B) SSZ in pH (red line) 3.4, (—) 7.0, (•••••) 10.0, (- - -) 13.0.

The SA spectrum presented two absorption bands at $\lambda_{1SA}=230$ nm and at $\lambda_{2SA}=297$ nm, which are due to the π→π* transition in the carboxyl and aromatic ring, respectively.

The SSZ spectrum showed four absorption bands all due to the π→π* transitions. The adsorption bands at $\lambda_1=239$ nm and $\lambda_4=360$ nm correspond to primary and secondary transitions in the benzene ring of the salicylic moiety. The adsorption band at $\lambda_3=320$ nm was due to a transition in the *p*-substituted sulfonamide benzene ring. The adsorption band $\lambda_2=270$ nm corresponds to the secondary band transition in the pyridine moiety and is shifted due to the presence of nitrogen as heteroatom and the electron releasing substituent N from sulfonamide [26].

The azo group and the phenolic group are responsible for the major batochromic effect observed on SSZ salicylic moiety bands (Figure 6A).

When SSZ is excited, both groups have *n* (non-bonding) electrons which can be transferred to the π system in the aromatic ring resulting in a charge transfer excited state. This effect is magnified due to the *para* positioning of the substituents [26].

The pH effect on SSZ UV-vis spectra was also investigated, Figure 6B. The adsorption band $\lambda_3=320$ nm decreased with increasing pH and disappeared for pH > 8.0. In strong alkaline solutions, pH > 12.0, the SSZ spectra changes drastically and the adsorption band at $\lambda_4=360$ nm shifts to 460 nm due to degradation and the formation of the phenoxide group, in agreement with the voltammetric results. The presence of more electrons promotes delocalization due to charge transfer excited state effect and allows the π→π* transition to occur more frequently requiring less energy [26]. At the same time, the change of the solution color, from brilliant yellow to deep orange, was visually observed.

3.3 Electroanalytical Determination

The SSZ electroanalytical determination was performed in 0.1 M phosphate buffer pH 7.0, in a concentration range from 10 to 50 μM (Figure 7), following the equation I_{pa} (nA) = 0.948 + 0.966 [SSZ]. The detection limit $LOD=3.43$ μM and quantification limit $LOQ=11.39$ μM were calculated from the calibration curve using the criteria of 3 *m*/*s* and 10 *m*/*s* respectively. These values are comparable to the bismuth film modified glassy carbon electrode [11], but are less sensitive than using the antimony film [12] and molecular imprinted polymer modified carbon electrodes [13].

The GCE can be applied to determine SSZ as a fast and routine analysis in pharmacological compositions, in which the drug is usually available in 500 mg tablets. The electrode needs to be polished after each measurement to remove the oxidation products adsorbed at the GCE surface.

Most sensors developed for SSZ quantification are based in the reduction reaction of the azo group and it is necessary to purge the solution with N_2.

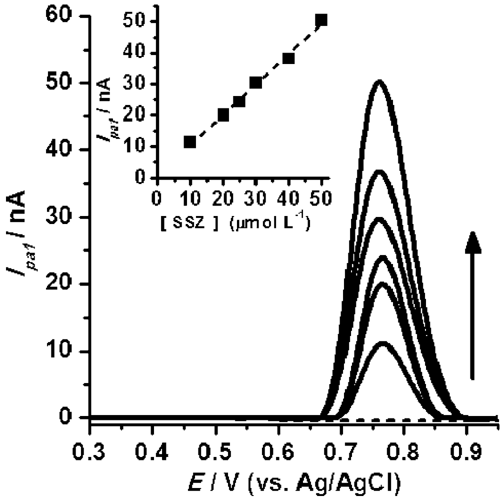
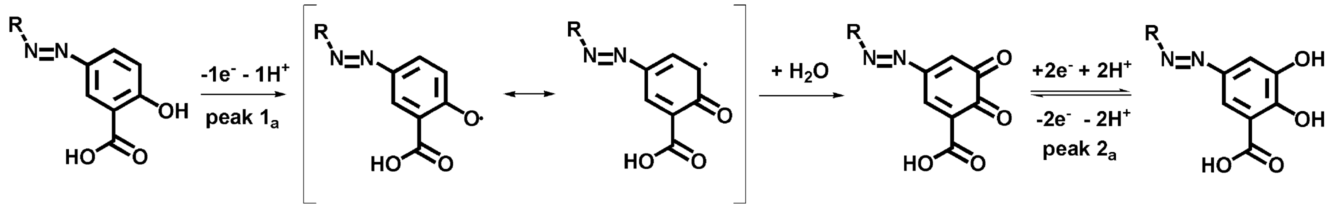


Fig. 7. DP voltammograms baseline corrected in pH 7.0, for 10, 20, 25, 30, 40 and 50 μM SSZ. Inset: Calibration curve.



Scheme 2. Proposed oxidation mechanism of sulfasalazine.

The advantage of the GCE is enabling the SSZ electroanalytical determination to be based on the oxidation reaction with no need for N_2 purging.

3.4 Oxidation Mechanism

The SSZ oxidation at peak 1_a is an irreversible, pH-dependent process for $pH < 11.0$, and involves the transfer of one electron and one proton leading to the formation of an oxidation product that undergoes a two electrons and two protons reversible redox reaction (Scheme 2).

The SSZ oxidation occurs at the hydroxyl group of the salicylic moiety producing a thermodynamically unstable phenoxyl radical [27] in different isomeric states. The stabilisation of the radical occurs through nucleophilic addition of water to the ortho- position of the aromatic ring since the *para*- and *meta*-positions are either occupied or unfavourable for any kind of chemical reaction. This results in a catechol derivative which at the peak 1_a potential is immediately oxidised and is reduced after reversing potential [27] (Figure 5B and Scheme 2).

The catechol derivative undergoes a reversible two electrons and two protons oxidation, peak $2_a/2_c$, in a mechanism similarly to phenol, p-substituted phenols oxidation, and salicylic acid [27–29].

For $pH > 11.0$, SSZ undergoes chemical deprotonation with $pK_a \sim 11.0$ [30]. SSZ is only soluble in alkaline media [30], and its absorption in vivo occurs where the pH is 8.0 and 9.0 [31].

4 Conclusions

The electrochemical oxidation of sulfasalazine, a pharmaceutical compound used for the treatment of rheumatoid arthritis was studied at a glassy carbon electrode by cyclic, differential pulse and square wave voltammetry in a wide pH range.

In acid, neutral and mild alkaline electrolytes, the oxidation of sulfasalazine is an irreversible, diffusion-control and pH-dependent process. It involves the transfer of one electron and one proton from the hydroxyl group of the salicylic moiety followed by nucleophilic addition of water. The formation of a quinone-like oxidation product that undergoes two electrons and two protons reversible redox reaction was observed. For strong alkaline electrolytes, the oxidation is pH-independent and $pK_a \approx 11$ of SSZ was determined.

Also, sulfasalazine UV-vis spectrophotometric behavior was studied as function of pH and compared to salicylic acid. The spectra of SSZ showed a major batochromic effect on all maximum absorptions which is characteristic to the formation of charge transfer excited state promoted by the azo group and phenolic group in *para*-positioning. An electrochemical oxidation mechanism was proposed.

The SSZ electroanalytical determination in 0.1 M phosphate buffer pH 7.0, in a range from 10 to 50 µM, was performed. The detection limit $LOD = 3.43$ µM and the quantification limit $LOQ = 11.39$ µM were obtained.

Acknowledgements

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References

[1] I. M. M. Laurindo, A. C. Ximenes, F. A. C. Lima, G. R. C. Pinheiro, L. R. Batistella, M. B. Bertolo, P. Alencar, R. M. Xavier, R. D. N. Giorgi, R. M. Ciconelli, S. C. Radominski, *Rev. Bras. Reumatol.* **2004**, *44*, 435.
[2] V. Majithia, S. A. Geraci, *Am. J. Med.* **2007**, *120*, 936.
[3] M. C. Boissier, L. Semerano, S. Challal, N. Saidenberg-Kermanac'h, G. Falgarone, *J. Autoimmun.* **2012**, *39*, 222.
[4] C. G. Helmick, D. T. Felson, R. C. Lawrence, S. Gabriel, R. Hirsch, C. K. Kwoh, M. H. Liang, H. M. Kremers, M. D. Mayes, P. A. Merkel; S. R. Pillemer, J. D. Reveille, J. H. Stone, *Arthritis Rheum.* **2008**, *58*, 15.
[5] D. L. Scott, F. Wolfe, T. W. Huizinga, *Lancet* **2010**, *376*, 1094.
[6] K. E. Donahue, G. Gartlehner, D. E. Jonas, L. J. Lux, P. Thieda, B. L. Jonas, *Ann. Intern. Med.* **2008**, *148*, 124.
[7] N. Pastor-Navarro, E. Gallego-Iglesias, A. Maquieira, R. Puchades, *Anal. Chim. Acta* **2007**, *583*, 377.
[8] G. Font, A. Juan-Garcia, Y. Pico, *J. Chromatogr. A* **2007**, *1159*, 233.
[9] A. Krivohlavek, Z. Smit, M. Bastinac, I. Zuntar, F. Plavsic-Plavsic, *J. Sep. Sci.* **2005**, *28*, 1434.

[10] V. K. Balakrishnan, K. A. Terry, J. Toito, *J. Chromatogr. A* **2006**, *1131*, 1.
[11] B. Nigovic, B. Simunic, S. Hocevar, *Electrochim Acta* **2009**, *54*, 5678.
[12] B. Nigovic, S. Hocevar, *Electrochim Acta* **2011**, *58*, 523.
[13] S. Sadeghi, A. Motaharian, A. Z. Moghaddam, *Sens. Actuators B, Chem.* **2012**, *168*, 336–344.
[14] Z. Mandic, B. Nigovic, B. Šimunic, *Electrochim. Acta* **2004**, *49*, 607.
[15] A. Eriksson, L. Nyholm, *Electrochim. Acta* **2001**, *496*, 1113.
[16] A. Eriksson, L. Nyholm, *Electrochim. Acta* **1999**, *44*, 4029.
[17] B. Nigovic, S. Komorsky-Lovric, B. Simunic, *Electroanalysis* **2005**, *17*, 10.
[18] A. Eriksson, L. Nyholm, *Electroanalysis* **1998**, *10*, 198
[19] A. A. J. Torriero, J. M. Luco, L. Sereno, J. Raba, *Talanta* **2004**, *62*, 247.
[20] A. Simić, D. Manojlović, D. Šegan, M. Todorović, *Molecules* **2007**, *12*, 2327.
[21] V. Supalkova, J. Petrek, L. Havel, S. Krizkova, J. Petrlova, V. Adam, D. Potesil, P. Babula, M. Beklova, A. Horna, R. Kizek, *Sensors* **2006**, *6*, 1483.
[22] A. D. R. Pontinha, S. M. A. Jorge, V. C. Diculescu, M. Vivan, A. M. Oliveira-Brett, *Electroanalysis* **2012**, *24*, 917.
[23] D. D. Perrin, B. Dempsey, *Buffers for pH and Metal Ion Control*, Wiley, Chichester **1974**.
[24] D. R. Lide, *CRC Handbook of Chemistry and Physics*, 86th ed., CRC Press, Taylor & Francis, Boca Raton **2005**.
[25] C. M. A. Brett, A. M. Oliveira-Brett, *Electrochemistry – Principles, Methods, and Applications*, Oxford University Press, Oxford **1993**.
[26] D. M. Pavia, G. L. Lampman, G. S. Kritz, *Introduction to Spectroscopy*, 3rd ed., Thomson Learning, London **2001**, p. 355–383.
[27] T. A. Enache, A. M. Oliveira-Brett, *J. Electroanal. Chem.* **2011**, *655*, 9–16.
[28] T. A. Enache, O. F. -Filho, A. M. Oliveira-Brett, *Comb. Chem. High Through. Scr.* **2010**, *13*, 569–577.
[29] W. Zhang, B. Xu, Y.-X. Hong, Y.-X. Yu, J.-S. Ye, J.-Q. Zhang, *J. Solid State Electrochem.* **2010**, *14*, 1713–1718
[30] A. Avdeef, *Absorption and Drug Development – Solubility, Permeability, and Charge State*, Wiley, Chichester **2003**
[31] A. C. Guyton, J. E. Hall, *Textbook of Medical Physiology*, Elsevier Saunders, 11th ed., **2006**.

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