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Short communication

Determination of 5-aminosalicylic acid in pharmaceutical formulation by differential pulse voltammetry

Biljana Nigović [a,*], Branimir Šimunić [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 oxidative behaviour of 5-aminosalicylic acid (5-ASA) has been investigated by differential pulse voltammetry using a glassy carbon electrode in different buffer systems. Linear sweep voltammetry was used to study the influence of pH on the peak current and peak potential. The solution conditions and instrumental parameters were optimized to obtain a good sensitivity. The Britton–Robinson buffer of pH 1.81 was selected as a suitable analytical medium in which 5-ASA exhibited a sensitive diffusion controlled oxidative peak at 0.564 V (vs. Ag/AgCl). The peak current varied linearly with drug concentration in the range between 1×10^{-4} and 2×10^{-6} M. The proposed voltammetric method has been applied to the determination of the drug in commercial delayed-release tablet forms. A mean recovery of 101.23% with a relative standard deviation of 1.35% was obtained.

Keywords: 5-Aminosalicylic acid; Mesalazine; Differential pulse voltammetry; Electrochemical oxidation; Delayed-release tablets

1. Introduction

5-Aminosalicylic acid (5-ASA, mesalazine) is an antiinflammatory drug primarily intended for the therapy of acute bowel disease such as Crohn's disease and ulcerative colitis [1,2]. Its action mechanisms are far from being completely elucidated. 5-ASA has been shown to be a potential scavenger of oxygen free radicals that play a significant role in the pathogenesis of inflammatory bowel disease [3,4]. It can reduce leukotriene production and inhibit the cellular release of interleukin-1 [5]. All these properties seem to be important in reducing the acute inflammatory response. Orally administered 5-ASA is rapidly and completely absorbed from the upper gastrointestinal tract and therefore an enteric-coated formulations have been designed for drug release in the terminal ileum and colon [6]. After absorption, 5-ASA is metabolized mainly to its *N*-acetyl derivative.

Prolonged treatments as well as the need for clinical and pharmacological studies require fast and sensitive analytical techniques of the drug presence determination in several biological samples. Up to now, most common procedures for the

* Corresponding author. Fax: +385-1-4856-201.
E-mail address: biljana@pharma.hr (B. Nigović).

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determination of 5-ASA in pharmaceutical dosage forms [7,8] and biological fluids [9–16] were based on chromatographic techniques. High-performance liquid chromatographic methods with UV [9], fluorescence [11,14,16] and electrochemical [13,15] detection were primarily used for the analysis of 5-ASA in biological samples. Spectrophotometry [17] and colorimetry [18] were also used for the compound quantitation. Electrochemical methods have been recently introduced in the analysis of this drug [19]. To the best of our knowledge, there are no available literature data on electroanalytical investigations based on differential pulse voltammetry for the determination of this compound. Chromatographic methods need sophisticated equipment or require lengthy extraction and clean-up procedures. The voltammetric techniques offer another possibility for the estimation of this compound. Hence the development of electrochemical determination assumes importance.

The aim of the present study has been to examine the voltammetric behaviour of 5-ASA, based on the oxidation on the surface of glassy carbon electrode, by using a differential pulse technique. The developed voltammetric method was applied to the analysis of the drug in commercial pharmaceutical formulation (delayed-release tablets). The official high performance liquid chromatographic (HPLC) method [20] was chosen as the comparative method in evaluating the technique. The obtained data were analyzed statistically.

2. Experimental

2.1. Materials and reagents

5-ASA supplied by Merck (Darmstadt, Germany) was used without further purification. All other chemicals were of analytical grade (Merck and Sigma). Stock solution of 5-ASA (2×10^{-3} M) was prepared by dissolving appropriate amounts of the compound in doubly distilled water. The stock solution was stabilized by acidification with small volumes of 1 M HCl to minimize the risk of 5-ASA oxidation [21] and stored in the dark under refrigeration. Spectrophotometric measurements revealed no significant stock solution degradation after 5 days of storage.

Standard solutions were prepared by diluting the stock solution with a selected supporting electrolyte. The supporting electrolytes solutions, 0.1 M sulphuric acid, acetate buffer (pH 3.5–6; 0.02 M), phosphate buffer (pH 5.1–7.5, 0.02 M) and Britton–Robinson buffer (pH 1.8–11.8, 0.04 M) were used for the voltammetric studies. All solutions were prepared using doubly distilled water.

2.2. Apparatus

The voltammetry experiments were performed using an EG&G Princeton Applied Research Model 273A potentiostat controlled by the Model 270/250 Research Electrochemistry Software v. 4.30. A three-electrode system was composed of a glassy carbon working electrode ($\varnothing = 2$ mm, EG&G/PAR), Ag/AgCl reference electrode and a platinum auxiliary electrode.

To provide a reproducible active surface and improve the sensitivity and resolution of the voltammetric peaks, the working electrode was polished with 0.5 μm alumina powder on a polishing cloth prior to each electrochemical measurement. The electrode cleaning procedures require only 2 min. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper. All the solutions examined by electrochemical techniques were purged for 10 min with water-saturated argon, after which a continuous stream of argon was passed over the solutions during the measurements. All measurements were carried out at room temperature.

The pH measurements were made with a Radiometer PHM 85 pH-Meter (Radiometer, Copenhagen, Denmark) using combined glass electrode (Radiometer PHC 2406L).

2.3. Analysis of pharmaceutical dosage form

Salofak® delay-released tablets, each containing 500 mg of 5-ASA, was obtained from commercial sources (Dr. Falk Pharma, Germany). Excipients,

such as Eudragit-L/M, calcium stearate, cellulose, polyvinyl-2-pyrrolidone, talc, titanium dioxide and iron oxide, are added to dosage form. Twenty tablets were weighted accurately and finely powered. A portion of the powered, equivalent to the average weight of one tablet was transferred to a 500 ml volumetric flask and dissolved in 100 ml 0.1 M HCl. After sonicating and shaking the mixture for 10 min, it was diluted with double distilled water to the mark, mixed and passed through a 0.5 μm mash filter. An aliquot of the filtrate was then transferred into a calibrated flask and a series of dilutions was made with a supporting electrolyte.

3. Results and discussion

3.1. Voltammetric study

5-ASA shows one well-defined voltammetric oxidation peak in aqueous solutions over the pH range 1.0–12.0. Successive cyclic voltammograms of 5-ASA obtained in Britton–Robinson buffer of pH 1.81 at scan rate of 100 mV s^{-1} are shown in Fig. 1. 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 (Fig. 1 inset). This indicates that the initial oxidation product undergoes a chemical reaction to yield a second product that can be reduced at more negative potentials. The peak current dependence of the scan rate suggests diffusion controlled oxidation followed by irreversible chemical step. With scan rate increase the oxidation becomes quasi-reversible (Fig. 1). This complies with previous findings that 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 [22].

In general, pH is one of the variables that commonly and strongly influences the shapes of voltammograms, and therefore it is important to investigate the effects of pH on electrochemical systems. The pH dependence of the oxidation wave peak potential (E_p) for 5-ASA, obtained by linear sweep voltammetry, is shown in Fig. 2. The pH increase generated a shift in the oxidation peak potential to less positive values together with a decrease in peak current (i_p), implying the involvement of protons in current-limiting electrode process (Fig. 3). The variation of E_p with pH exhibited two regions of linearity with a −60.9 mV per pH unit slope of the first linear region, thus indicating the consumption of an identical number of protons and electrons. The intersection point observed at pH 5.9 can be attributed to the pK_a value of 5.78 reported in the literature for the $-NH_3^+$ group of 5-ASA molecule [23].

Differential pulse voltammetry was used to optimize a rapid and sensitive electroanalytical

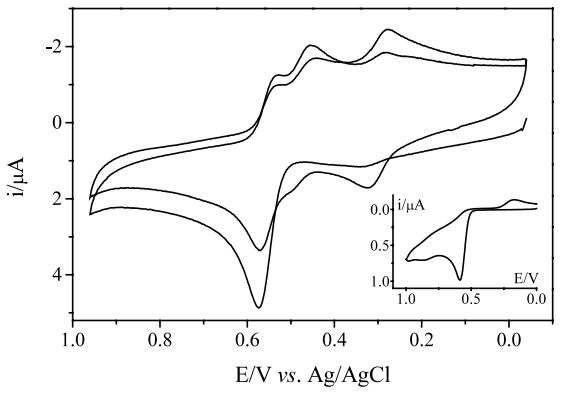

Fig. 1. Successive cyclic voltammograms of 5-ASA (2×10^{-4} M) in Britton–Robinson buffer of pH 1.81 at a scan rate of 100 mV s^{-1}. Inset: at a scan rate of 20 mV s^{-1}.

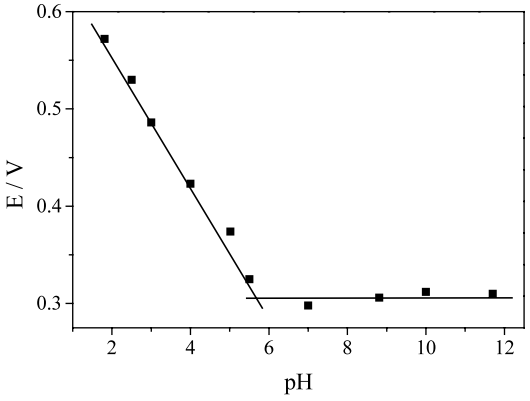

Fig. 2. Effect of pH on peak potentials for 2×10^{-4} M 5-ASA solutions in Britton–Robinson buffer by means of linear sweep voltammetry at a glassy carbon electrode. Scan rate 100 mV s^{-1}.

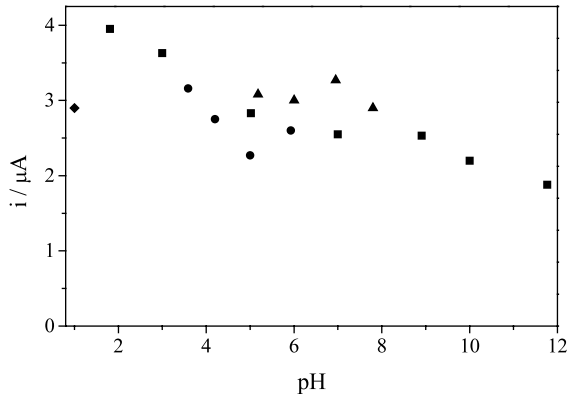

Fig. 3. Influence of pH on peak current for 5-ASA (2×10^{-4} M) by means of linear sweep voltammetry at a scan rate of 100 mV s^{-1} in 0.1 M H_2SO_4 (diamond), Britton–Robinson (squares), acetate (circles) and phosphate buffer (triangles).

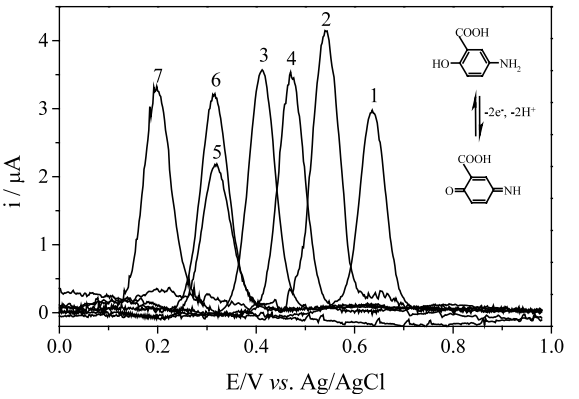

Fig. 4. Differential pulse voltammograms obtained for 1×10^{-4} M 5-ASA solutions in different electrolytes: (1) 0.1 M H_2SO_4; (2) Britton–Robinson buffer pH 1.81; (3) Britton–Robinson buffer pH 3.00; (4) acetate buffer pH 3.60; (5) acetate buffer pH 5.00; (6) phosphate buffer pH 5.18; (7) phosphate buffer pH 7.00. Scan rate: 20 mV s^{-1}; pulse amplitude: 50 mV; pulse width: 30 ms.

procedure for 5-ASA determination and to use the procedure for the determination of the drug in its pharmaceutical dosage form. The differential pulse voltammograms showed improved peak current compared to the linear scan and square-wave ones. The solution conditions affect the enhancement of the peak current. Various electrolytes were examined, such as solutions of Britton–Robinson buffer, sulphuric acid, acetate and phosphate buffer. The best peak current sensitivity and morphology results for analytical purpose were obtained with 0.04 M Britton–Robinson buffer (Fig. 4). Therefore, this electrolyte was used in all subsequent studies. The peak current reaches its maximum value at pH 1.81, selected as optimum value for quantitative determination. There is no degradation of the analyte in solution under the conditions employed as indicated from the literature [21]. Therefore, the degradation products of 5-ASA do not interfere with assay and the proposed method can be regarded as a stability-indicating one.

The influence of electrochemical parameters known to affect the differential pulse voltammograms, viz. pulse amplitude, pulse width and scan rate were studied. During the study, each variable was changed while the other two were kept to constant. The variables of interest were studied over the ranges of 25–100 mV for pulse amplitude, 30–90 ms for pulse width and 10–50 mV s^{-1} for scan rate. It was found that the peak sensitivity rose with the increasing pulse amplitude and the increasing scan rate, however, the peak current decreased as the pulse width increased. To acquire voltammograms of relatively high sensitivity and well-shaped waves with relatively a narrow peak width, values of 50 mV, 30 ms and 20 mV s^{-1} were chosen for pulse amplitude, pulse width and scan rate, respectively.

Under these optimum conditions 5-ASA can be determined in a linear concentration range 1×10^{-4}–2×10^{-6} M. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of 5-ASA or its oxidation product at higher concentration. Moreover, the peak currents in the cyclic voltammograms increase linearly with scan rates and decrease with succeeding potential scans (Fig. 1), due to the adsorbed species formation on the electrode surface. However, the preconcentration onto the electrode surface by adsorption prior to the voltammetric measurements and the enhancement of the peak current can not be achieved by accumulation over the potential range 0.0 to +0.4 V for 240 s. After 10 min of 5-ASA oxidation at a constant potential in a stirred solution followed by the transfer of the working electrode to the pure buffer solution, the recording of the new voltammogram exhibits a

peak at about +0.9 V. This less defined peak can be attributed to an electroactive polymeric film produced during the oxidation of 5-ASA. The quinone imine formed upon oxidation of 5-ASA may also react with the starting material to produce polymeric species consisting of several aminosalicylic acid subunits [21].

The peak current of differential pulse voltammograms was linearly related to the 5-ASA concentration according to the regression equation of the calibration curve: i_{pa} (μA) = 41.089 C (μM)–0.031, $r = 0.9996$. The analytical parameters for the calibration graph are summarized in Table 1. The inter-day reproducibility of the method was evaluated for ten independent determinations of 1×10^{-4}, 6×10^{-5} and 1×10^{-5} M solutions, yielding relative standard deviations of 1.32, 1.44 and 2.68%, respectively. The RSD value for intra-day assay reproducibility at 6×10^{-5} M solution ($n = 12$) was found to be 0.78% indicating good repeatability and accuracy of the method. The detection limit estimated as $3s/m$ was 8.16×10^{-7} M, with s representing the standard deviation of the peak current of the sample ($n = 5$) and m representing the slope of the calibration curve. Based on the signal-to-noise ratio of 10, the quantitation limit was calculated to be 2.72×10^{-6} M.

3.2. Determination of 5-ASA in pharmaceutical formulation

The proposed voltammetric method was used for the determination of 5-ASA in commercial delayed-release tablet forms at different concentration levels. The amount of 5-ASA present in corresponding solution was calculated from the calibration equation. The analysis of 5-ASA in its pharmaceutical formulation exhibited the mean recovery of 101.23% and the relative standard deviation of 1.35%, indicating adequate precision and accuracy of the proposed method. There is no interference from any excipients, which are soluble in acidic aqueous solution, because they are electrochemically inactive at the potential of electrooxidation of 5-ASA. Therefore, the proposed method can be used as a selective method.

The results obtained with the described method for the analysis of 5-ASA in delayed-release tablets were compared with those obtained by reported HPLC method [8]. The results of the student t-test and variance ratio F-test show that there are no significant differences between the techniques with regard to accuracy and precision (Table 2). The proposed method is more simple, fast and less cost tool for 5-ASA analysis.

Table 1
Analytical parameters of the calibration graph for the determination of 5-ASA by differential pulse voltammetry

Linearity range (M)	1×10^{-4}–2×10^{-6}
Slope (μA μM^{-1})	41.089
Intercept (μA)	−0.031
S.E. of slope (μA μM^{-1})	0.338
S.E. of intercept (μA)	0.018
Correlation coefficient	0.9996
Detection limit (M)	8.16×10^{-7}
Quantification limit (M)	2.72×10^{-6}

4. Conclusions

The proposed voltammetric method can be successfully used to determine 5-aminosalicyclic acid in pharamceutical formulation. It compares reasonably well with reported HPLC method. The advantage of the proposed method is that no prior separation procedure is required. It is simple, inexpensive, selective and precise and does not require any complex pre-treatment except polishing the electrode within a few minutes.

Table 2
Application of the proposed method to the determination of 5-ASA in commercial delay-released tablets

Method	% Recovery ± S.D.[a]	t-tests	F-tests
Differential pulse voltammetry	101.23 ± 1.37	2.18	4.94
Official HPLC method [8]	99.93 ± 0.52	2.31[b]	6.39[b]

[a] The results are the average of five separate determinations.
[b] The tabulated t and F values, respectively, at $P = 0.05$.

The developed electroanalytical technique may be applied to the body fluids analysis of 5-ASA and its metabolite, *N*-acetyl derivative, whose oxidation potential has shifted +300 mV relative to 5-ASA [21] and it will be the subject of the next report.

References

[1] S. Bondesen, S.N. Rasmussen, J. Rask-Madsen, O.H. Nielsen, K. Lauritsen, V. Binder, S.H. Hansen, E.F. Hvidberg, Acta Med. Scand. 221 (1987) 227–242.
[2] A. Prakash, A. Markham, Drugs 57 (1999) 383–408.
[3] B.J. Dull, K. Salata, A. von Langenhove, P. Goldman, Biochem. Pharmacol. 36 (1987) 2467–2472.
[4] J. Brynskov, N. Tvede, C.B. Andersen, M. Vilien, Gut 33 (1992) 55–58.
[5] W.H. Betts, M.W. Whitehouse, L.G. Cleland, B. Vernon-Roberts, J. Free Rad. Biol. Med. 1 (1985) 273–280.
[6] A. Tromm, T. Griga, B. May, Hepato-Gastroenterol. 46 (1999) 3124–3135.
[7] I. Cendrowska, M. Drewnowska, A. Grzeszkiewicz, K. Butkiewicz, J. Chromatogr. 509 (1990) 195–199.
[8] C. Gandini, G. Caccialanza, M. Kitsos, G. Massolini, E. De Lorenzi, J. Chromatogr. 540 (1991) 416–421.
[9] C. Fischer, K. Maier, U. Klotz, J. Chromatogr. Biomed. Appl. 225 (1981) 498–503.
[10] S.H. Hansen, J. Chromatogr. Biomed. Appl. 226 (1981) 504–509.
[11] E. Brendel, I. Meineke, D. Witsch, M. Zschunke, J. Chromatogr. 385 (1987) 299–304.
[12] E.J.D. Lee, S.B. Ang, J. Chromatogr. Biomed. Appl. 413 (1987) 300–304.
[13] E. Nagy, I. Csipo, I. Degrell, G. Szabo, J. Chromatogr. Biomed. Appl. 425 (1988) 214–219.
[14] M. De Vos, H. Verdievel, R. Schoonjans, R. Beke, G.A. De Weerdt, F. Barbier, J. Chromatogr. Biomed. Appl. 564 (1991) 296–302.
[15] G. Palumbo, G. Carlucci, P. Mazzeo, G. Frieri, M.T. Pimpo, D. Fanini, J. Pharm. Biomed. Anal. 14 (1995) 175–180.
[16] B. Bystrowska, J. Novak, J. Brandys, J. Pharm. Biomed. Anal. 22 (2000) 341–347.
[17] G. Norwitz, P.N. Keliher, Talanta 33 (1986) 311–314.
[18] T.J. Smith, M.A. Tucker, Anal. Lett. 22 (1989) 1703–1708.
[19] E.L. Beckett, N.S. Lawrence, R.G. Evans, J. Davis, R.G. Compton, Talanta 54 (2000) 871–877.
[20] The United States Pharmacopoeia, 24th rev., Easton, Rand McNally, Taunton, MA, 2000, Fourth Supplement, p. 3225.
[21] R.K. Palsmeier, D.M. Radzik, C.E. Lunte, Pharm. Res. 9 (1992) 933–938.
[22] A. Eriksson, L. Nyholm, Electroanalysis 10 (1998) 198–203.
[23] H. Allgayer, J. Sonnenbichler, W. Kruis, G. Paumgartner, Drug. Res. 35 (II) (1985) 1457–1459.