Department of Analytical Chemistry[1], Faculty of Pharmacy, and Department of Analytical Chemistry[2], Faculty of Science, Ankara University, Ankara, Turkey

Determination of olsalazine sodium in pharmaceuticals by differential pulse voltammetry

B. Uslu[1], S. Yilmaz[2] and S. A. Özkan[1]

The electrochemical oxidation of olsalazine sodium was investigated by cyclic, linear sweep, differential pulse and square wave voltammetry using glassy carbon disc electrode in different buffer systems. Best results were obtained for the determination of olsalazine using the differential pulse voltammetric technique in phosphate buffer at pH 7.0. The electroactive species exhibits a diffusion-controlled voltammetric wave and its differential pulse peak current shows a linear dependence on olsalazine concentration in the range between 2×10^{-6} M and 2×10^{-4} M. This relationship has been applied to the determination of olsalazine in commercial capsule dosage forms. The recovery study shows good accuracy and precision for the assay developed. A UV spectrophotometric assay is also reported for comparison.

1. Introduction

Olsalazine sodium (olsalazine; disodium, 3,3′-azobis(6-hydroxy-)benzoate), which consists of two molecules of mesalazine linked with an azo bond, is being investigated for use as the sodium salt in ulcerative colitis.

It has been efficacious in the treatment of inflammatory bowel disease, in particular ulcerative colitis. Olsalazine also has been used in the treatment of rheumatoid arthritis and ankylosing spondylitis [1–3]. Because olsalazine itself is physically difficult to handle, the sodium salt has been used clinically. Electroanalytical techniques, especially modern pulse techniques, such as differential pulse and square wave voltammetry, have been used for the determination of a wide range of pharmaceuticals [4–9], advantages being that there is no need for derivatization in most instances, and that these methods are less sensitive to matrix effects than other analytical techniques.

Only a few methods have been employed for the determination of olsalazine, including HPLC with UV [10, 11] and electrochemical [12] detection.

No report seems to have appeared in the literature so far describing the analytical use of differential pulse voltammetry for the determination of olsalazine. However, to our knowledge, there is at present no published information about the electrochemical redox properties of olsalazine. This work reports the electrochemical behaviour of olsalazine at a glassy carbon disc electrode in different buffer systems with different pH values using various potential sweep techniques such as cyclic, linear sweep, square wave and differential pulse voltammetry. The present study deals with the quantitative determination of olsalazine in commercial pharmaceutical formulations using a differential pulse voltammetric method. This technique is simple, rapid, sensitive, reproducible and easy to apply in routine practice.

2. Investigations, results and discussion

The electrochemical behaviour of olsalazine was studied over a wide pH range (2–11.1) at a glassy carbon disc electrode in buffered aqueous media using cyclic, linear sweep, differential pulse and square wave voltammetric techniques.

The cyclic voltammetric behaviour of the compound yielded two oxidation processes between pH 3.0 and 8.0 (Fig. 1). Above pH 8, the two oxidation peaks combined.

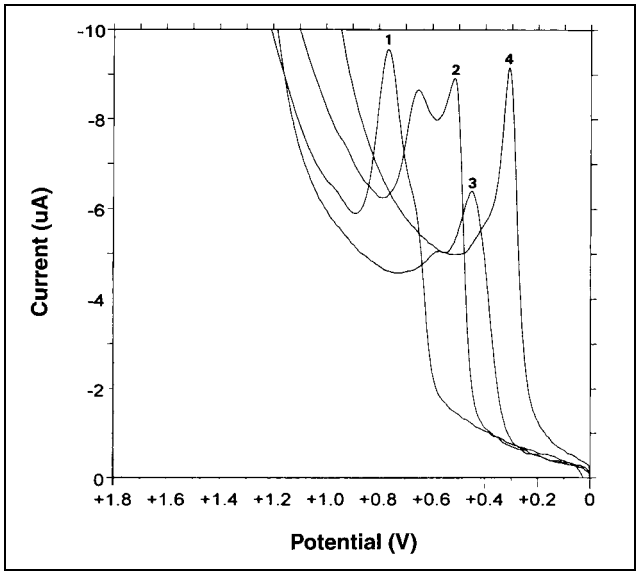

Fig. 1: Linear sweep voltammograms of 2×10^{-4} M olsalazine in Britton-Robinson buffer. Scan rate, 100 mVs^{-1}. (1) pH 4; (2) pH 6; (3) pH 8 ; (4) pH 11.1

Olsalazine also gave two cathodic waves at pH below 8.0, which may be due to the reduction of the oxidized products (Fig. 2). With a decrease in pH below 3.0, the peak was poorly defined and quantitation of the peak was not possible.

The effects of potential scan rates between 5 and 750 mVs^{-1} on the peak current and the peak potential of olsalazine were evaluated. The linear increase in the peak current with the square root of the scan rate with a slope of 0.89 (correlation coefficient 0.999), showed a diffusion control process. A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.54 (correlation coefficient 0.998). Slopes of 0.50 and 1.0 are expected for ideal reactions of solution and surface species, respectively [13].

Various electrolytes, such as sulphuric acid, and Britton-Robinson, acetate and phosphate buffers were examined (Table 1). For analytical purposes the best response (with regard to peak current sensitivity and morphology) was obtained with a phosphate buffer of pH 7.01 (Fig. 3).

The influence of different percentages of methanol in the solvent was evaluted and it was found that for a 0.2 M

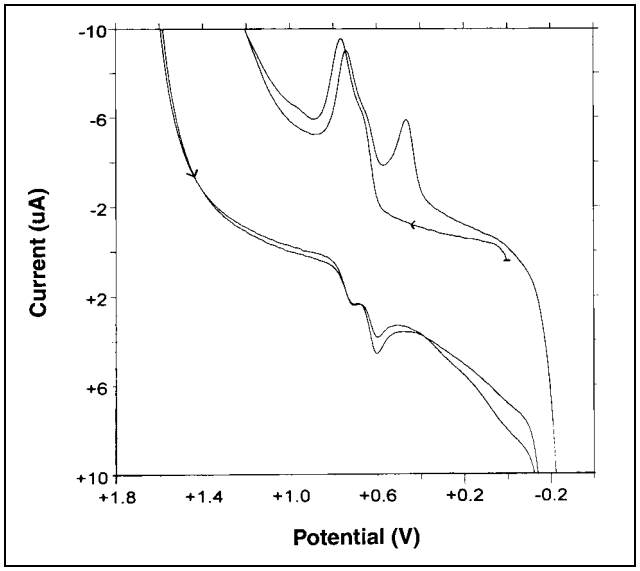

Fig. 2: Repetitive cyclic voltammogram of 2×10^{-4} M olsalazine in Britton-Robinson buffer at pH 4.01. Scan rate: 100 mVs^{-1}

phosphate buffer at pH 7.01, 20% methanol (v/v), led to the best improvement of peak definition and height. This supporting electrolyte was chosen for the subsequent experiments. The application of the differential pulse wave form (pulse amplitude: 50 mV) yielded voltammograms in which the peak currents were greater than those obtained by linear sweep and square wave voltammetric currents. For this reason, the differential pulse voltammetric technique was chosen for the quantitative application.

Table 1: Voltammetric characteristics of olsalazine in the buffer system (20% MeOH) by cyclic voltammetry

Buffer	Studied pH range	Selected pH	E_p/(V)	i_p/(μA)
Acetate	3.5–4.7	4.7	0.59	7.43
Phosphate	5.06–8.0	7.01	0.47	8.18
Britton-Robinson	2.0–11.1	7.03	0.50	6.32

Olsalazine concentration 2×10^{-4} M

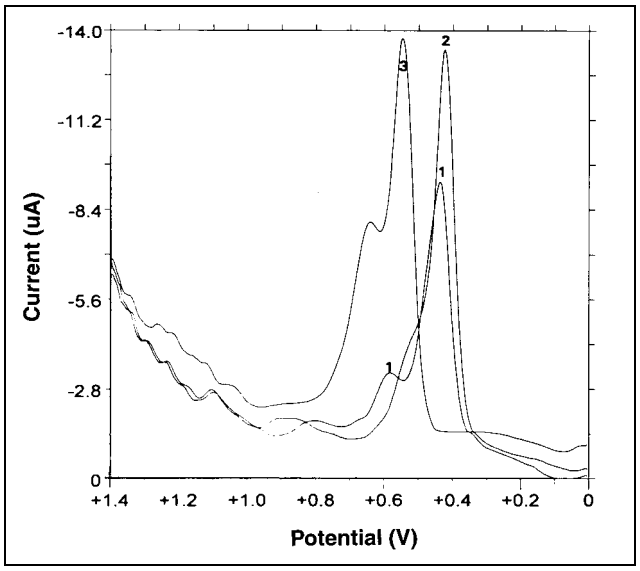

Fig. 3: Differential pulse voltammograms of 2×10^{-4} M olsalazine in different buffer systems. Scan rate: 100 mVs^{-1}. (1) Britton-Robinson buffer pH 7.03; (2) Phosphate buffer pH 7.01; (3) acetate buffer pH 4.7

The peak potential decreased almost linearly with increasing pH, with a slope of 58.6 mV/pH unit. Thus, the oxidation potential of olsalazine is pH dependent (Fig. 4a). The effect of the peak current is illustrated in Fig. 4b. As shown in Fig. 4b the peak current of olsalazine is also pH dependent.

A pulse interval of 0.25 s gave rise to the sharpest and most symmetrical peak shape. The optimum scan rate was found to be 20 mVs^{-1}.

The intra-day reproducibility of peak potential and peak current was tested by repeating four experiments on 1×10^{-4} M olsalazine. The relative standard deviations were calculated to be 0.33% and 0.51% for peak current and peak potential, respectively.

The inter-day reproducibility of peak potential and peak current was also tested by repeating four experiments on four different days with 1×10^{-4} M olsalazine. The relative standard deviations were calculated to be 0.64% and 0.91% for peak current and peak potential, respectively.

Using the optimum conditions described in the Experimental section, a linear calibration curve was obtained for olsalazine in the range of 2×10^{-6}–2×10^{-4} M. The characteristics of the calibration plot are listed in Table 2. The limit of detection (LOD) and the limit of quantitation (LOQ) of the procedure are shown in Table 2, which was calculated for the peak current using the following equations:

$$LOD = 3s/m \qquad LOQ = 10s/m$$

Where s, the noise estimate, is the standard deviation of the peak currents (five runs) of the sample, m is the slope

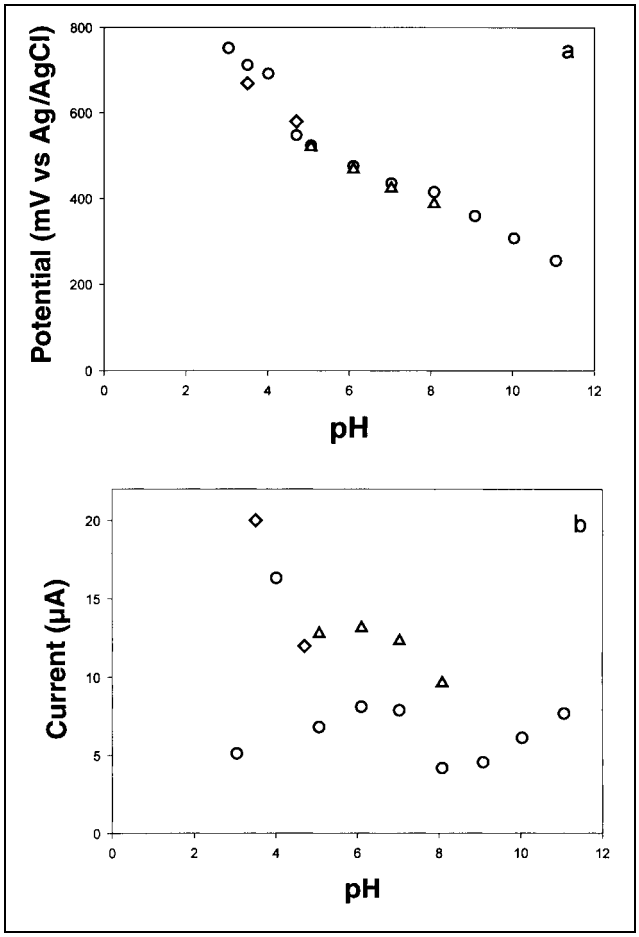

Fig. 4: Effects of pH on olsalazine peak potential (a) and peak current (b); olsalazine concentration, 2×10^{-4} M; scan rate 100 mVs^{-1}, (◇) acetate buffer; (△) phosphate buffer; (○) Britton-Robinson buffer

Table 2: Analytical parameters for the determination of olsalazine by differential pulse voltammetric and UV-spectrophotometric method

	Differential pulse voltammetry	UV-Spectrophotometry
Linearity range (M)	2×10^{-6}–2×10^{-4}	2×10^{-5}–8×10^{-5}
Slope	5.95×10^{4}	9.2×10^{3}
Intercept	0.64	0.38
Correl. Coeff. (r)	0.999	0.999
St. error of slope	9.1×10^{2}	2.4×10^{2}
St. error of intercept	0.068	0.01
LOD (M)	5.75×10^{-7}	3.52×10^{-6}
LOQ (M)	1.92×10^{-6}	1.17×10^{-5}

of the calibration curve. The same equations were also used for spectrophotometric results.

The olsalazine content of commercially available capsules, prepared as described in the Experimental section, was determined directly using the differential pulse voltammetric technique. The amount of olsalazine in the pharmaceutical formulation was calculated by reference to the calibration plot. The assay results for the formulation are given in Table 3.

In order to validate the proposed procedure, we applied the USP XXIV UV spectrophotometric method [14] which is given for the similar compound sulfasalazine. The corresponding linear regression equation and related parameters of the UV spectrophotometric method are shown in Table 2.

Experimental results obtained from our proposed differential pulse voltammetric method were compared with those determined by the UV spectrophotometric method [14].

The results of the determinations are summarized in Table 3. The olsalazine content obtained by our proposed method was slightly lower than that obtained by the UV-spectrophotometric method. In general, good precision and accuracy of determination were demonstrated. Table 3 shows that the calculated t-value did not exceed the tabulated value. This result indicates that there is no significant difference between the population means for the two procedures. In comparison to the spectrophotometric method, the proposed differential pulse voltammetric method was simpler and more rapid. No sample treatment was needed for the proposed method.

In order to establish whether the excipients in the capsule show any interference with the analysis, known amounts of the pure drug were added to the same aliquot portions of the same powdered capsules and the mixtures were analysed by the proposed method. The recovery study shows an average recovery of 99.6% with a RSD of 0.39%, indicating adequate precision and accuracy of the proposed method.

The principal advantage of the proposed method over the previously published HPLC procedure and UV spectrophotometric method is that it involves no sample preparation other than dissolving and transferring an aliquot to the supporting electrolyte, and does not require separation procedures such as filtration, extraction and expensive grades of solutions. Based on all the above results, we consider that the method developed is a good tool for use in olsalazine determination in pharmaceutical products, with adequate reproducibility and recovery. Furthermore, the voltammetric analysis was not time-consuming and the excipients did not interfere in the analysis, again avoiding separation steps.

Table 3: Olsalazine content of Dipentum® capsules obtained by differential pulse voltammetric and UV-spectrophotometric methods

Method	Labelled amount (mg per capsule)	Amount found*	RSD %	Confidence limit (95%)	t-test of (p = 0.05)
Differential pulse voltammetry	250	250.62	1.30	4.07	0.327**
UV-spectrophotometry	250	251.26	1.15	3.60	

* Each value is the mean of five experiments.
** $t_{theoretical}$: 2.306

3. Experimental

3.1. Apparatus

A model BAS 100 W electrochemical analyser (Bioanalytical System, USA) was used for the cyclic, linear sweep, differential pulse and square wave voltammetry, with a three-electrode system consisting of a glassy carbon electrode as working electrode, an Ag/AgCl (NaCl 3 M, BAS) reference electrode and a platinum wire auxiliary electrode (BAS). Before each experiment the glassy carbon electrode was polished manually with alumina ($\phi = 0.01$ μm), in the presence of bidistilled water on a smooth polishing cloth.

Operating conditions for square wave voltammetry were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step 4 mV; and for differential pulse voltammetry: pulse amplitude, 50 mV; pulse width 50 ms; scan rate, 20 mVs^{-1}.

Spectrophotometric measurements were carried out using a Shimadzu UV 1601 spectrophotomer with a 1 cm quartz cell.

3.2. Reagents

Olsalazine was kindly provided by Eczacıbaşı Pharmaceuticals Inc. (Istanbul, Turkey). A stock solution of olsalazine (1×10^{-3} M) in methanol was prepared, and kept in the dark in a refrigerator. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution by the selected supporting electrolyte and contained 20% methanol. Four different supporting electrolytes, namely sulphuric acid (0.1 M, 0.5 M); phosphate buffer (pH 5.06–8.0; 0.2 M), Britton-Robinson buffer (pH 2–11.1; 0.04 M) and acetate buffer (pH 3.5–4.7; 0.2 M) were used. For the spectrophotometric study, 0.1 N sodium hydroxide and 0.1 N acetic acid were used.

3.3. Capsule assay procedure

Olsalazine determination was performed on commercial capsule dosage forms. The whole content of ten capsules was thoroughly ground to a fine powder. The required amount of sample corresponding to a stock solution of ca 1×10^{-3} M was accurately weighed and transferred into a 100-ml calibrated flask containing 80 ml methanol. The contents of the flask were stirred magnetically for 10 min to effect complete dissolution and then diluted to volume with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with methanol: selected buffer solution in order to obtain a final solution of 20 : 80, respectively. Each solution was transferred to the voltammetric cell and recorded as for the pure drug.

The required amount of finely powdered sample, corresponding to a stock solution of ca 1×10^{-3} M, was accurately weighed and transferred into a 100-ml calibrated flask containing 80 ml 0.1 N sodium hydroxide. The contents of the flask were stirred magnetically for 10 min to effect complete dissolution and then diluted to volume with the same solvent and filtered through a fine pore filter paper. Appropriate amounts of the solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with water after addition of 2 ml 0.1 N acetic acid. The sample solution absorptivity was measured at 363.3 nm. The mg of olsalazine in the sample solution were calculated from a prepared standard calibration curve.

3.4. Recovery experiments

In order to know whether the excipients show any interference with the analysis, known amounts of the pure sample were added to the different pre-analysed formulations of olsalazine and the mixtures were analysed by the proposed differential pulse voltammetric method. The recovery experiments were performed to assess the reliability and suitability of the proposed method. After four repeated experiments, the recoveries were calculated.

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Assoc Prof. Dr. Sibel A. Özkan
Ankara University
Faculty of Pharmacy
Department of Analytical Chemistry
06100, Ankara
Turkey
ozkan@pharmacy.ankara.edu.tr