Full Paper

Electroanalytical Characteristics of Amisulpride and Voltammetric Determination of the Drug in Pharmaceuticals and Biological Media

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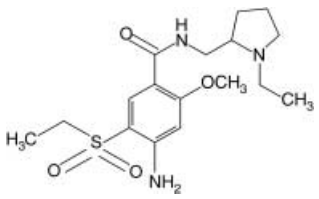
Abstract
The electrochemical oxidation of antipsychotic drug amisulpride (AMS) has been studied in pH range 1.8 – 11.0 at a stationary glassy carbon electrode by cyclic, differential pulse and square-wave voltammetry. Two oxidation processes were produced in different supporting electrolyte media. Both of the oxidation processes were irreversible and exhibited diffusion controlled. For analytical purposes, very resolved voltammetric peaks were obtained using differential pulse and square-wave modes. The linear response was obtained in the range of 4×10^{-6} to 6×10^{-4} M for the first and second oxidation steps in Britton-Robinson buffer at pH 7.0 and pH 3.0 (20% methanol v/v), respectively, using both techniques. These methods were used for the determination of AMS in tablets. The first oxidation process was chosen as indicative of the analysis of AMS in biological media. The methods were successfully applied to spiked human serum, urine and simulated gastric fluid samples.

Keywords: Amisulpride, Cyclic voltammetry, Differential pulse voltammetry, Square-wave voltammetry, Glassy carbon electrode, Pharmaceuticals, Biological samples.

1. Introduction

Amisulpride (AMS), a substituted benzamide derivative, belongs to the relatively new class of second-generation (atypical) antipsycotics. The drug is effective mainly in the management of psychoses such as schizophrenia, with fewer adverse effects than conventional high-potency anti-psychotic drugs [1]. It is reported that AMS binds selectively and with high affinity to dopamine D_2 and D_3 receptors. Amisulpiride is absorbed from the gastrointestinal tract however bioavailability and plasma protein binding are reported to be only about 48 and 17%, respectively. The elimination half-life is approximately 12 h. It is predominantly eliminated in the urine as the parent compound [2 – 4]:



Scheme 1. Chemical structure of AMS.

A survey of the literature revealed that there have been very few methods for its determination in biological fluids. The analysis of AMS in human plasma and urine using HPLC- fluorimetric [5, 6] and UV [7, 8] detection including solid phase extraction step has been reported in the literature. The drug takes the only part as a raw material in European Pharmacopoeia [9]. AMS determination from the pharmaceutical dosage forms is not yet official in any pharmacopoeia.

No literature data were found on the electrochemical behavior of AMS in general or its voltammetric determination in particular. Therefore the aim of this study is to establish the experimental conditions to investigate the oxidation behavior of AMS, and to optimize the conditions for determination of this compound in pharmaceutical dosage forms, human serum, human urine and simulated gastric fluid samples using cyclic, differential pulse (DPV) and square wave (SWV) voltammetric techniques.

2. Experimental

2.1. Apparatus

The cyclic, differential pulse and square-wave voltammetric experiments at a stationary electrode were performed using a BAS 100 W Electrochemical Analyser. A three electrode cell system incorporating a glassy carbon working electrode ($\phi = 3$ mm, BAS), a Ag/AgCl (KCl 3 M, BAS) reference electrode and a platinum wire auxiliary electrode (BAS), was used. Before each measurements, the glassy carbon

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electrode was polished manually with alumina ($\phi =$ 0.01 μm) in the presence of bidistilled water on a smooth polishing cloth.

For analytical applications, the following parameters were employed: DP voltammetry: pulse amplitude, 50 mV; pulse width, 0.05 s; scan rate, 20 mV s^{-1}; SW voltammetry: pulse amplitude, 25 mV; frequency, 15 Hz; scan increment, 4 mV.

2.2. Reagents

Amisulpride and its pharmaceutical formulation were kindly provided by Sanofi-Synthelabo Pharm. Ind. (Istanbul, Turkey). All other chemicals used were of reagent grade quality (Merck or Sigma) and they were employed without further purification.

AMS stock solutions, renewed daily, were prepared in methanol. The working solutions were prepared by serial dilution of the stock solution with selected supporting electrolytes and contained 20% methanol. Four different supporting electrolytes, namely sulfuric acid (0.1 M and 0.5 M), phosphate buffer (0.2M; pH 3.1 – 10.2), Britton-Robinson buffer (0.04 M; pH 2.0 – 11.0) and acetate buffer (0.2M; pH 3.5 – 5.7) were used.

All solutions were protected from light and were used within 24 h to avoid decomposition.

2.3. Tablet Assay Procedure

Ten tablets of Solian (each one contains 200 mg AMS) were weighed and ground to a homogeneous fine powder in a mortar. Portion equivalent to a stock solution of a concentration about 1×10^{-3} M was accurately weighed and transferred into a 50 mL calibrated flask and completed to volume with methanol. The contents of the flask were sonicated for 10 min to effect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with methanol:buffer solution in order to obtain a final solution of $20 + 80$ methanol:Britton-Robinson buffer, pH 3.0 or 7.0. Each solution was transferred to a voltammetric cell and DP and SW voltammograms were recorded as in pure AMS.

2.4. Recovery Experiments from Tablets

To study the accuracy of the proposed methods, and to check the possible interferences from common excipients such as potato starch, lactose monohydrate, methyl cellulose, colloidal hydrate silica (Aerocil), magnesium stearate used in tablet dosage forms, recovery experiments were carried out. For this procedure, known amounts of the pure drug were added to the earlier analyzed tablet formulation of AMS. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug.

2.5. Recovery Studies in Spiked Human Serum, Human Urine and Simulated Gastric Fluid Samples

Serum samples, obtained from healthy individuals, (after obtaining their written consent), were stored frozen until assay. An aliquot volume of sample was fortified with AMS dissolved in methanol to achieve final concentration of 1×10^{-3} M, and treated with 0.7 mL of acetonitrile, then the volume was completed to 2 mL with the same serum sample. The tubes were vortexed for 1 min and then centrifuged 10 min at 4000 rpm. Appropriate volumes of this solution were analyzed in the voltammetric cell containing methanol-Britton-Robinson buffer, pH 7.0 ($20 + 80$).

Urine samples were analyzed as for serum samples.

Simulated gastric fluid was prepared according to USP 24 using by purified pepsin and other reagents [10]. The required amount of AMS (dissolved in methanol) was added to this sample to give a final concentration of 1×10^{-3} M. After sonicating for 10 min., appropriate volumes of this solution were analyzed in the voltammetric cell containing methanol-Britton-Robinson buffer, pH 7.0 ($20 + 80$).

3. Results and Discussion

AMS was electrochemically oxidized at the glassy carbon electrode in two steps over the pH range investigated. Cyclic and DP voltammetric measurements show that the first oxidation process was less distinct in strong acidic media, while the second process became more pronounced as pH decreased (Fig. 1 and 2).

Due to the well-resolved signal obtained by DPV, the effect of solution acidity on peak potential and peak intensity were studied using this technique. For pH values lower than 3.0, the first peak is poorly defined, which

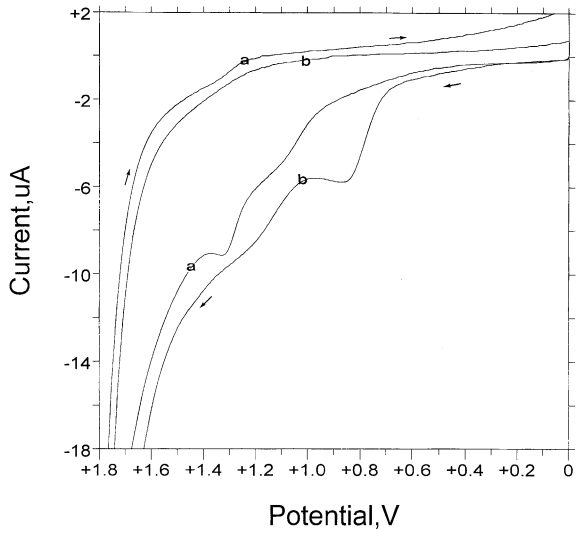

Fig. 1. Cyclic voltammograms of 2×10^{-4} M AMS in Britton-Robinson buffer (20% methanol). pH 3.0 (a); 7.0 (b). Scan rate 100 mV s^{-1}.

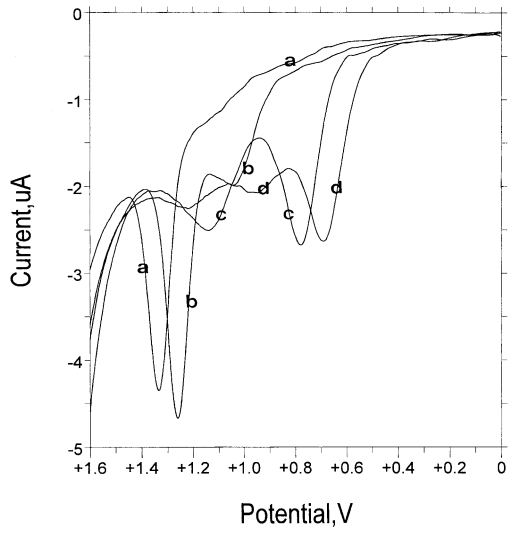

Fig. 2. Differential pulse voltammograms of 2×10^{-4} M AMS in Britton-Robinson buffer (20% methanol). pH 2.2 (a); pH 3.0 (b); pH 7.0 (c); pH 9.0 (d). For operating conditions, see Section 2.

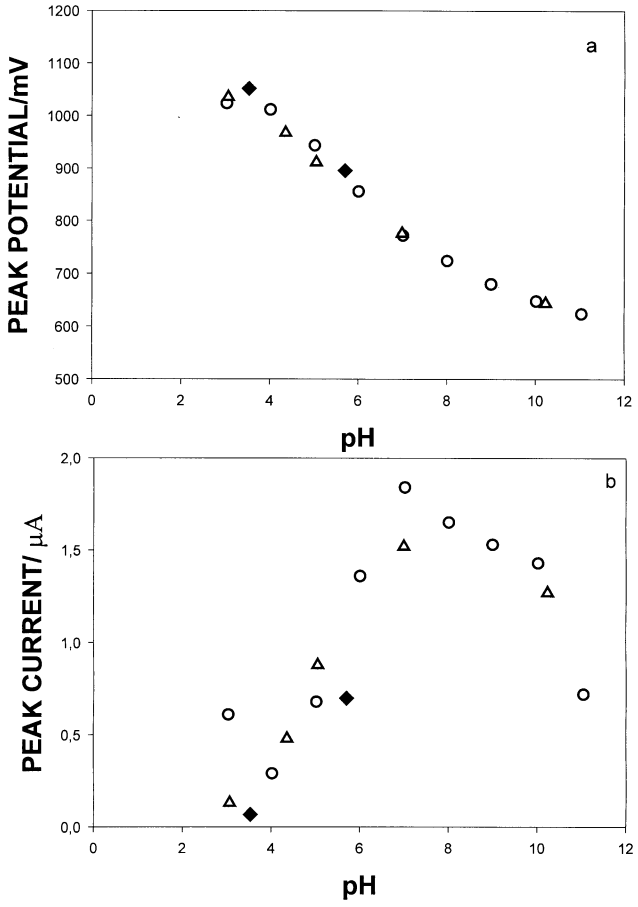

Fig. 3. Effects of pH on AMS peak potential (a) and peak current (b) for the first peak. AMS concentration, 2×10^{-4} M. Britton-Robinson buffer (○); acetate buffer (◆); phosphate buffer (△).

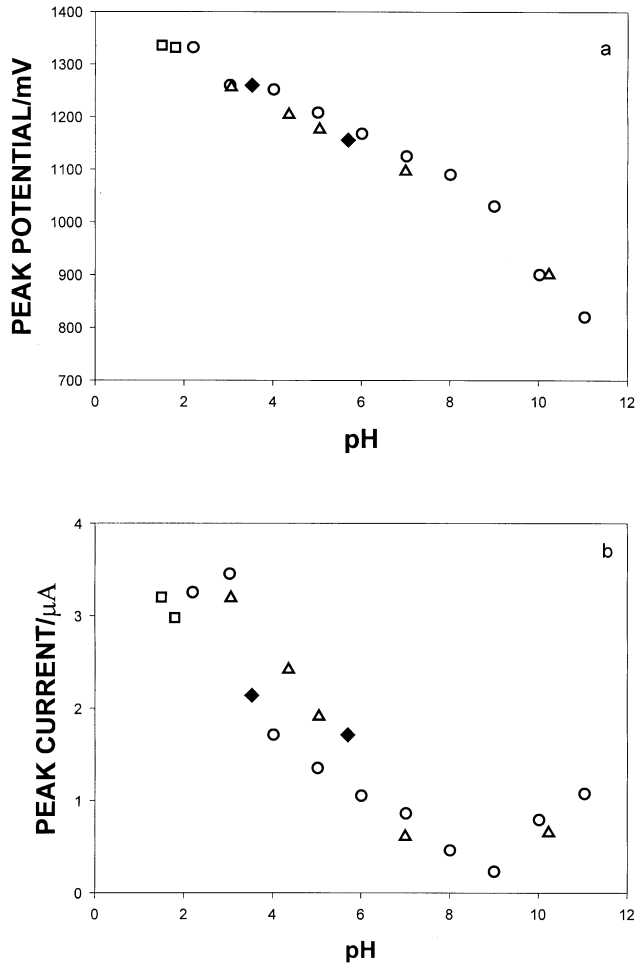

Fig. 4. Effects of pH on AMS peak potential (a) and peak current (b) for the second peak. AMS concentration, 2×10^{-4} M. H_2SO_4 (□); Britton-Robinson buffer (○); acetate buffer (◆); phosphate buffer (△).

prevents accurate measurements of the values of peak potential and peak current.

The plots of peak potentials (E_p) vs. pH (Fig. 3a and 4a) for both of the oxidation steps showed two straight lines, which can be explained by changes in protonation of the acid-base functions in the molecule. The results can be expressed by the following equations:

For the first process

$$\text{pH} \leq 8 \qquad E_p\,(\text{mV}) = 1242.9 - 63.8\ \text{pH} \quad (r = 0.989;\ n = 13)$$

$$\text{pH} \geq 8 \qquad E_p\,(\text{mV}) = 925.7 - 27.5\ \text{pH} \quad (r = 0.997;\ n = 4)$$

For the second process

$$\text{pH} \leq 9 \qquad E_p\,(\text{mV}) = 1398.4 - 40.5\ \text{pH} \quad (r = 0.987;\ n = 16)$$

$$\text{pH} \geq 9 \qquad E_p\,(\text{mV}) = 1945.7 - 102.6\ \text{pH} \quad (r = 0.990,\ n = 4)$$

The intersection point of the second process is close to the pK_a value of AMS obtained spectrophotometrically which is

reported to be 9.3 corresponding to loss of a proton from tertiary amine function in the pyrrolidine ring [11]. Analyzing the evolution of peak current (Fig. 3b and 4b) it is possible to observe that this parameter is affected by the pH value. The peak current reached the highest values at around pH 7.0 and 3.0 for the first and second processes, respectively, and these were selected as the optimum values for quantitative analysis.

The αn values of anodic reactions corresponding to both voltammetric peaks were obtained using Tafel plots ($\log i$ vs. E). The values of 0.31 and 0.24 were obtained in Britton-Robinson buffer pH 7.0 and 3.0 for the first and second process, respectively. These values together with the absence of cathodic waves in cyclic voltammetry (Fig. 1), indicated the irreversibility of the oxidation reactions.

The following experiments were made with the aim of elucidating the character of AMS both processes and observing the effect of potential scan rate (v) on analytical signal. When scan rate was varied from 5 to 500 mV s^{-1} and 5 to 250 mV s^{-1} for the first and second processes, respectively, in a 2×10^{-4} M solution of AMS, a linear dependence of the peak intensity upon the square root of the scan rate ($v^{1/2}$) was found, demonstrating a diffusional behavior.

The equations are noted below:

For the first peak $i_p(\mu A) = 0.23v^{1/2}$ (mV s^{-1}) $- 0.15$ ($r = 0.998$; $n = 8$)

For the second peak $i_p(\mu A) = 0.31v^{1/2}$ (mV s^{-1}) $+ 0.24$ ($r = 0.999$; $n = 7$)

A plot of logarithm of peak current ($\log i_p$) versus logarithm of scan rate ($\log v$) gave a straight-line with a slope of 0.53 and 0.43 for first and second peak, respectively, very close to the theoretical value of 0.5, which is expected for an ideal reaction of solution species [12], so in this case, both the processes had a diffusive component.

The equation obtained was:

For the first peak $\log i_p$ (μA) $= 0.53 \log v$ (mV s^{-1}) $-$ 0.73 ($r = 0.997$; $n = 8$)

For the second peak $\log i_p$ (μA) $= 0.43 \log v$ (mV s^{-1}) $+$ 0.24 ($r = 0.994$; $n = 7$)

Even though the exact oxidation mechanism was not determined, some conclusions about the potentially electroactive centers under working conditions, could be reached. The anodic voltammetric behavior of anisidines (methoxyanilines) at the tubular graphit electrode has been previously discussed [13]. While two distinct oxidation waves are obtained in the para isomers, only one oxidation wave is obtained in the case of ortho and meta anisidines. Taking into account that the E_p vs. pH relationship for the first peak is similar to that described for the oxidation of the amino group in the *m*-anisidine derivatives [13], we suggest that the first anodic reaction could be attributed to the oxidation of primary amine group on the *m*-anisidine ring. Wang et al. [14], using glassy carbon electrode, demonstrated that metoclopramide, a similar structure to that of AMS molecule, changing ethylpyrrolidinyl moiety and ethylsulfonyl group for the diethylaminomethyl and chloro group, respectively, gave only one oxidation peak, corresponding to the oxidation of amino group. Since ethylsulfonyl group is inactive anodically, we can conclude that the electroactivity was due to the pyrrolidine ring. Considering the above comparison and the position of the break in the E_p vs. pH plot for the second process, and bearing in mind the oxidative process of heterocyclic amines [15], we may assume that the second oxidation step is located on the pyrrolidine ring and attributed to the oxidation of tertiary nitrogen.

Under chosen experimental parameters described in Section 2., the variation of peak current with the AMS concentration was studied by means of DPV and SWV. In this study, SWV was proposed as an alternative method, since applying the wave form allowed very rapid determination. Both the peak height and the peak shape were taken in consideration during choosing the supporting electrolyte. The results showed that Britton-Robinson buffer solutions gave the best background and signal response. For quantitation, the calibration graph method, with concentrations ranging between 4×10^{-6} and 6×10^{-4} M, was used for both techniques. The calibration characteristics and related validation parameters were given in Table 1. The detection limit (LOD) and limit of quantification (LOQ) of the procedures (Table 1) were calculated according to the 3 *s*/*m* and 10 *s*/*m* criterions, respectively, where *s* is the standard deviation of the peak currents (five runs) and *m* is the slope of related calibration plot [16, 17].

The intra-day reproducibility of peak potentials and peak currents were tested by repeating five experiments on 4×10^{-5} M AMS for both media and using DPV and SWV techniques. The inter-day variation of same concentration of AMS was studied for analyses made during 4 consecutive days by performing five measurements on each day. Related parameters are given in Table 1. The results indicate an acceptable precision in measures made in same the day and separate days.

Sample solutions recorded after 72 h did not show any appreciable change in assay values.

In order to know the applicability of the proposed methods, a commercial tablet formulation containing AMS was studied (Table 2). Since there is no official method in any pharmacopoeias (e.g., USP, BP or EP) related to tablet dosage forms of AMS, the recovery studies specified in Experimental, were carried out. As Table 2 shows, good results demonstrate the validity of the proposed methods for the determination of AMS in commercial tablet dosage forms.

Consequently, application of DPV and SWV methods using glassy carbon electrode to pharmaceutical preparations is possible after a simple dilution step. The analyses were performed without any interferences from the additives present in tablet.

Both the proposed voltammetric techniques were also successfully applied for the determination of AMS in spiked

Table 1. Regression data of the calibration lines for quantitative determination of AMS obtained by DPV and SWV.

	BR buffer, pH 7.0 (20% methanol) (for the first peak)		BR buffer, pH 3.0 (20% methanol) (for the second peak)	
	DPV	SWV	DPV	SWV
Measured potential (V)	+0.80	+0.82	+1.26	+1.30
Linearity range (M)	$4\times10^{-6}-6\times10^{-4}$	$4\times10^{-6}-6\times10^{-4}$	$4\times10^{-6}-6\times10^{-4}$	$4\times10^{-6}-6\times10^{-4}$
Slope of calibration plot ($\mu A\ M^{-1}$)	6.73×10^{3}	8.16×10^{3}	1.03×10^{4}	8.22×10^{3}
Intercept (μA)	0.076	0.052	0.029	0.049
Correlation coefficient	0.998	0.999	0.999	0.999
RSD of slope, %	1.02	1.26	0.68	0.79
RSD of intercept, %	0.47	0.85	0.86	0.73
LOD (M)	2.2×10^{-8}	3.6×10^{-8}	7.3×10^{-8}	1.9×10^{-7}
LOQ (M)	7.4×10^{-8}	1.2×10^{-7}	2.4×10^{-7}	6.1×10^{-7}
Intraday reproducibility of peak current (RSD,%)	0.94	0.52	0.41	0.34
Intraday reproducibility of peak potential (RSD,%)	0.73	0.80	0.76	0.74
Interday reproducibility of peak current (RSD,%)	1.02	0.79	0.65	0.44
Interday reproducibility of peak potential (RSD,%)	0.98	1.08	0.85	0.90

Table 2. Assay results from AMS tablets (each tablet contains 200 mg AMS) and mean recoveries in spiked Solian tablets.

	BR buffer, pH 7.0 (20% methanol) (for the first peak)		BR buffer, pH 3.03 (20% methanol) (for the second peak)	
	DPV	SWV	DPV	SWV
Amount found (mg)	198.6	199.0	199.6	199.4
RSD, %	0.99	0.99	0.610	0.210
95% Confidence limit	2.44	2.44	1.51	0.52
Added (mg)	20.0	20.0	20.0	20.0
Found (mg) [a]	19.9	19.9	20.0	19.9
Recovered, %	99.5	99.6	99.7	99.7
RSD % of Recovery	0.41	0.36	0.34	0.30

[a] Each value is the mean of five experiments.

Table 3. Application of the DPV and SWV methods to the determination of AMS in spiked human serum, urine and simulated gastric fluid (SGF) samples.

Technique	Medium	AMS added		Level determined	Average recovery	RSD
		(M)	n	(M)	(%)	(%)
DPV	Serum	6×10^{-5}	3	5.9×10^{-5}	98.6	1.06
		8×10^{-5}	3	7.9×10^{-5}	98.8	0.90
		1×10^{-4}	3	9.9×10^{-5}	98.9	0.99
SWV	Serum	6×10^{-5}	3	5.9×10^{-5}	98.7	0.93
		8×10^{-5}	3	8.0×10^{-5}	99.5	0.43
		1×10^{-4}	3	9.9×10^{-5}	99.3	0.35
DPV	Urine	6×10^{-5}	3	6.0×10^{-5}	99.6	0.54
		8×10^{-5}	3	8.0×10^{-5}	99.4	0.38
		1×10^{-4}	3	1.0×10^{-4}	99.7	0.27
SWV	Urine	6×10^{-5}	3	6.0×10^{-5}	99.6	0.26
		8×10^{-5}	3	8.0×10^{-5}	99.4	0.22
		1×10^{-4}	3	1.0×10^{-4}	99.5	0.55
DPV	SGF	6×10^{-5}	3	5.9×10^{-5}	99.9	0.64
		8×10^{-5}	3	7.9×10^{-5}	99.3	0.23
		1×10^{-4}	3	9.9×10^{-5}	99.1	0.85
SWV	SGF	6×10^{-5}	3	6.0×10^{-5}	99.3	0.58
		8×10^{-5}	3	8.0×10^{-5}	99.6	0.38
		1×10^{-4}	3	1.0×10^{-4}	99.4	0.11

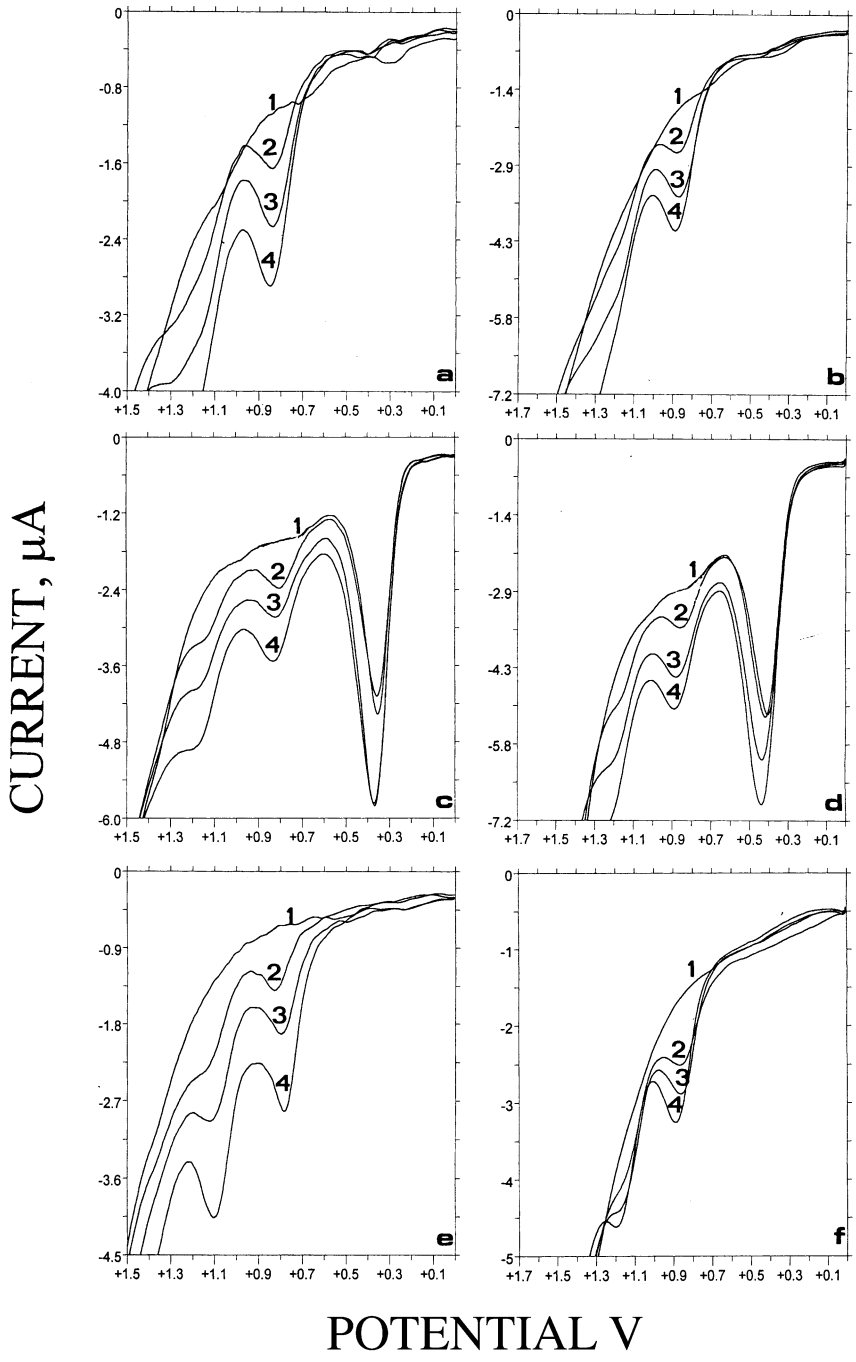

Fig. 5. Differential pulse (a, c,e) and square-wave (b, d,f) voltammograms in Britton Robinson buffer pH 7.0 (20% methanol) obtained for the determination of AMS in spiked serum (a, b); spiked urine (c, d) and simulated gastric fluid (e, f) samples. 1) blank; 2) sample spiked with 6×10^{-5} M AMS; 3) sample spiked with 8×10^{-5} M AMS; 4) sample spiked with 1×10^{-4} M AMS. For operating conditions, see Section 2.

human serum, urine and simulated gastric fluid samples under the present optimized conditions. The first oxidation process was chosen as indicative of the analysis of AMS in biological media. For the determination of drug in spiked serum, urine and simulated gastric fluid samples, three replicate samples at levels of 6×10^{-5}, 8×10^{-5} and 1×10^{-4} M for AMS were run through the procedures. Typical DPV and SWV curves of AMS examined in biological fluids are shown in Figure 5. As can be seen from the figure, no oxidation of compounds present in serum and simulated gastric fluid occured. However, a large peak at about $+0.35$ and $+0.45$ V using DPV and SWV, respectively, was recorded in a blank urine. This peak was well differentiated from that of AMS. Thus the peak did not interfere with the determination of AMS. The amount of the drug in these biological samples was calculated from the related linear

regression equations given in Table 1. Both the voltammetric methods were performed directly in gastric fluid samples that contain the enzyme pepsin, without sample preparation. As in case of urine and serum, the latter is especially more complex biological matrice, the extraction procedure by acetonitrile [18], that was both quick and easy, was applied to eliminate the influence interfering substances. The results of the determination of AMS in all biological media are summarized in Table 3. Good recoveries of AMS were achieved from these types of matrices.

As a consequence, application of the proposed voltammetric methods to the analysis of AMS in biological fluids have been easily assessed. The methods do not require filtration, degassing and expensive grades of solvents that are needed for HPLC procedures recommended for AMS analysis in body fluids in literature [5 – 8].

It is reported that metabolism of AMS is limited, with most of a dose appearing in the urine and feces as unchanged drug. For this reason, the proposed studies were found selective for AMS in each type of biological samples. Impurity peak or metabolites could not be obtained during the parent drug determination.

This paper is not intended to be a study of the pharmacodynamic properties of AMS, since only healthy volunteers were used for the sample collection and results may be of no significance. It only shows that the possibility of monitoring this drug makes the method useful for pharmacokinetic and pharmacodynamic purposes.

4. Acknowledgements

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