

Original Article

SIMULTANEOUS DETERMINATION OF SULFAMETHOXAZOLE AND TRIMETHOPRIM IN PHARMACEUTICAL FORMULATIONS BY SQUARE WAVE VOLTAMMETRY

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ABSTRACT

Objective: To develop a simple, fast and low-cost square wave voltammetric (SWV) method for simultaneous determination of sulfamethoxazole (SMX) and trimethoprim (TMP) using a glassy carbon electrode (GCE).

Methods: The SWV parameters were optimized by 2³ Full Factorial Design and the statistical treatment of the data showed that the main effects and two interaction effects were significant at 95% confidence level, the best analytical signal is obtained at $a=30$ mV, $f=100$ s⁻¹ and $\Delta E_s=5$ mV.

Results: Two well defined oxidation peaks were obtained at 0.96V (SMX) and 1.12V (TMP) in Britton–Robinson buffer (pH 6.0). Under optimized conditions, SWV measurements showed excellent linear response, ranging from 5.5×10^{-5} to 3.95×10^{-4} mol L⁻¹ (R=0.9971) and 1.05×10^{-5} to 1.04×10^{-4} mol L⁻¹ (R=0.9974) for SMX and TMP, respectively. The detection limits were found to be 8.52×10^{-6} mol L⁻¹ for SMX and 9.31×10^{-7} mol L⁻¹ for TMP.

Conclusion: The proposed method was successfully applied to the determination of these antibiotics in commercial pharmaceutical formulations (tablets, oral suspension and injection), without any sample pretreatment. The obtained results are in good agreement with that obtained by the standard HPLC method at a 95% confidence level.

Keywords: Sulfamethoxazole, Trimethoprim, Glassy carbon electrode, Square wave voltammetry, Pharmaceutical formulations.

INTRODUCTION

Sulfonamides are synthetic bacteriostatic drugs used for several decades in the treatment of many human and animal infectious diseases, because of its low cost and high efficiency against many gram-positive and gram-negative bacteria [1, 2]. The action mechanism of sulfonamides is based on inhibiting folic acid synthesis, essential for bacteria growth, by competition with the structure resembling 4-aminobenzoic acid (PABA) [3].

Sulfamethoxazole (SMX) or 4-amino-N-(5-methyl-1,2-oxazol-3-yl)-benzenesulfonamide is one of the most effective sulfa drugs in the treatment of urinary infections. Because of its slow absorption and excretion in humans, SMX produce excessive sanguine levels and cause crystalluria [4]. To reduce the incidence of crystalluria and increase the bacteriostatic effect, SMX is commonly associated with trimethoprim in pharmaceutical formulations that usually consist in a 5:1 ratio [SMX: TMP] [5].

Trimethoprim (TMP), chemically known as (2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine), belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors [6]. Sulfamethoxazole and trimethoprim combination have a synergetic effect, due to the inhibitor effect that occurs in more than one step during the obligatory sequence of enzymatic reactions in the bacteria [7]. The association is widely used in the treatment of many common infections such as urinary, respiratory and gastrointestinal tract infections [8]. Therefore, it is important to develop rapid, simple and low cost analytical methods for the simultaneous quantification of these compounds for a good quality control. According to the United States Pharmacopeia (USP) [9], the official method for the simultaneous analysis of sulfamethoxazole and trimethoprim in pharmaceutical formulations implicates to use High Performance Liquid Chromatography (HPLC). A considerable number of analytical papers for determination of association in commercial formulations and biological samples have been reported in the literature, these include mainly chromatography [4, 8, 10-12], spectrophotometric [13-16] and electrophoresis [17-19] methods.

Simultaneous quantification of these antibiotics using alternative voltammetric methods are less frequent, there are a few reports available in the literature, some examples using mercury electrode [3], boron-doped diamond electrode [20, 21] and modified composite electrode (in environmental samples) [22]. However, the use of low cost and environmentally friendly electrode would be more attractive which could facilitate the implantation of this method in fast routine analysis. In this work, an unmodified glassy carbon electrode was used to develop a rapid, simple and low cost analytical method for simultaneous square wave voltammetric determination of sulfamethoxazole and trimethoprim in pharmaceutical formulations, without any sample pretreatment. The results are compared with those obtained from the pharmacopeic method (HPLC).

MATERIALS AND METHODS

Reagents and solutions

Sulfamethoxazole (Figure 1A) and Trimethoprim (Figure 1B) standards were kindly supplied by the Institute of Drug Technology Farmanguinhos/Fiocruz (Rio de Janeiro - Brazil), with purity greater than 99%.

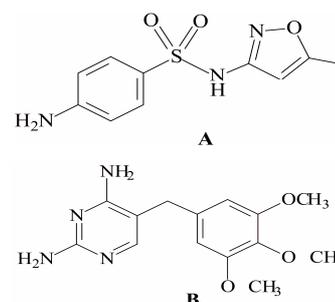


Fig. 1: Chemical structure of sulfamethoxazole (A) and trimethoprim (B).

Stock solutions were prepared daily, TMP (1.5×10^{-3} mol L⁻¹) in distilled water and SMX (5.0×10^{-3} mol L⁻¹) in 30% (v/v) ethanol solution. Working solutions were prepared by dilution with 0.04 mol L⁻¹ Britton-Robinson buffer solution. Acetonitrile and triethylamine were HPLC grade and used as received. All other chemicals used in the present work were of analytical grade.

Voltammetric measurements

All voltammetric measurements were carried out at room temperature ($\sim 25^\circ\text{C}$) using a potentiostat/galvanostat $\mu\text{Autolab}$ (Type III, Metrohm-Eco Chemie) connected to a computer with software GPES 4.9 (General Purpose Electrochemical System). A conventional three-electrode cell was employed containing a glassy carbon electrode (GCE) as working electrode ($A=7.07$ mm²), a Pt disc ($A=12.57$ mm²) as a counter electrode and Ag/AgCl (saturated aqueous KCl) as a reference electrode. Before use and between potential scans, the working electrode was cleaned by polishing with alumina paste on a polishing pad followed by a rinse in distilled water. After each treatment, the electrode was cleaned with isopropyl alcohol in an ultrasonic bath for 5 minutes to remove retained aluminum oxide particles on the electrode surface.

HPLC measurements

All the simultaneous HPLC determinations of TMP and SMX, adapted according to USP method [9], were carried out using a Varian 920-LC (Agilent Technologies) with a DAD detector set at 216 nm and 268 nm, respectively. The equipment was connected to a computer controlled by Varian Galaxie™ Chromatography Software (Version 1.9). A C18 column (microsorb 100-5, 250 mm x 4.6 mm, 5 μm) was used. The mobile phase consisted of a mixture of deionized water (1400 mL), acetonitrile (200 mL) and triethylamine (2 mL), with pH adjusted to 5.9 ± 0.1 with 0.2 mol L⁻¹ sodium hydroxide or 1% v/v acetic acid, at a flow rate of 1.0 mL min⁻¹. The column temperature was kept at 25°C , while the injection volume was 20 μL .

Analytical Curves

After optimizing the instrumental parameters for the square wave voltammetry (SWV) by 2³ Full Factorial Design, the analytical curves ($n=3$) were constructed by addition of aliquots of different volumes of the stock solution into the electrochemical cell containing the supporting electrolyte, 0.04 mol L⁻¹ Britton-Robinson (BR) buffer solution, at pH 6.0. All measurements were carried out in triplicate for each concentration. The limits of detection (LOD) and quantification (LOQ) were estimated according to IUPAC [23], as $\text{LOD}=3S_b/b$ and $\text{LOQ}=10S_b/b$, where S_b is the standard deviation of the blank ($n=10$) and b is the slope of the calibration curve.

Real Samples

Commercial tablets, oral suspension and injection were obtained from local hospitals and drugstores (Ponta Grossa - Brazil). For SWV analysis, 20 tablets were accurately weighed and ground into finely powder. A portion of the powder was transferred to a volumetric flask with 30% ethanol solution and sonicate. Appropriated amount from oral suspension and injection were suitably diluted in 30% ethanol and sonicate. To obtain final concentrations in the range of the calibration curve, aliquots from real samples stock solutions were diluted with BR buffer solution at pH 6. No further sample treatment was done. In the HPLC analysis, real samples were pretreated according to USP procedure [9]. For real samples available as oral suspensions, the determination by HPLC was performed as follows: 2 mL of oral suspension was transferred in a 50 mL volumetric flask with about 30 mL of methanol and sonicated for 10 minutes with occasional shaking and diluted with methanol, mixed and centrifuged. Then, 5 mL of a supernatant solution was transferred to a second 50 mL flask, diluted with mobile phase, mixed and filtered through a 0.45 μm cellulose acetate membrane. For tablets analysis, an accurately weighed portion of the finely powder (20 tablets) was transferred to a 100 mL flask, added 50 mL of methanol, sonicated with intermittent shaking for 5 minutes and diluted with methanol, mixed and filtered. 5 mL of the clear filtrate was transferred to a 50 mL flask, diluted with mobile phase to volume and mixed. Finally, 1 mL of injection was transferred to a 50 mL flask with methanol, 5 mL of this solution was transferred to a 50

mL flask and diluted with mobile phase, mixed and filtered. SMX and TMP content was carried out in triplicate for each sample and quantified by peak area with reference to the calibration curve.

RESULTS AND DISCUSSION

Electrochemical behavior of SMX and TMP at a glassy carbon electrode

Figure 2 shows the cyclic voltammograms for sulfamethoxazole and trimethoprim (2×10^{-4} mol L⁻¹) in a 0.04 mol L⁻¹ Britton - Robinson buffer solution (pH 6.0) on glassy carbon electrode at a scan rate of 50 mV s⁻¹. As it can be seen, the SMX and TMP voltammograms exhibit a well-defined irreversible oxidation peak at approximately +0.96 V and +1.12 V vs. Ag/AgCl/KCl (3.0 mol L⁻¹), respectively.

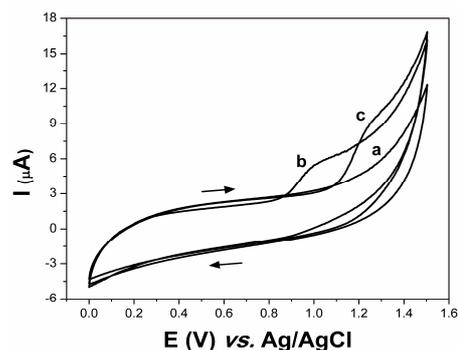


Fig. 2: Cyclic voltammograms for (a) blank (b) 2×10^{-4} mol L⁻¹ SMX and (c) 2×10^{-4} mol L⁻¹ TMP, in BR buffer pH 6.0 using a GCE, $v=50$ mV s⁻¹

According to previous investigations in the literature, sulfamethoxazole can be electrochemically oxidized at the amino ($-\text{NH}_2$) group with an irreversible two-electron pH dependent reaction in aqueous solutions. On the other hand, the reduction of the $-\text{SO}_2$ group is very difficult to achieve [24]. The trimethoprim oxidation process involves four electrons and occurs at the amino group ($-\text{NH}_2$) attached to C4 because its deprotonation is energetically favorable as compared to deprotonation of the C2 amino group, as reported by Rajith *et al* [25].

Effect of pH

The current response of sulfamethoxazole (1.7×10^{-4} mol L⁻¹) and trimethoprim (1.5×10^{-4} mol L⁻¹) was investigated over the pH range from 2 until 11, in 0.04 mol L⁻¹ Britton-Robinson buffer solution. Both antibiotics showed voltammetric response in all pH range studied, with maximum oxidation currents values observed at pH 6.0 (Figure 3), condition that was chosen to subsequent analytical applications.

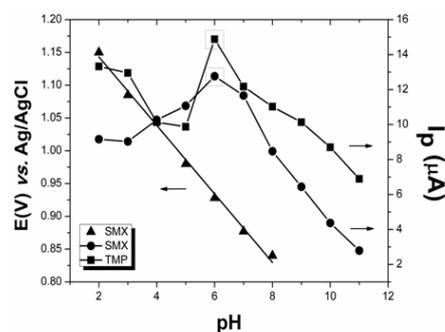


Fig. 3: Relationships between the pH value and the peak potentials for SMX (left y-axis) and anodic peak currents (right y-axis) for SMX and TMP using GCE and SWV technique in 0.04 mol L⁻¹ of BR buffer.

Moreover, the results showed that the oxidation potential of trimethoprim remained almost constant with increasing pH solution, in agreement with the literature [6]. On the other hand, sulfamethoxazole voltammograms like other sulfa drugs are known to be pH-dependent [26]. As can be seen in Figure 3, the SMX peak potential (E_p) shifts towards to less positive values as the pH increases, this is a consequence of the deprotonation in the oxidation process that is facilitated at higher pH [27]. A linear portion was observed in the range of pH from 2.0 to 8.0, with a slope of -52 mV pH^{-1} . The equation that represents the correlation between peak potential and pH is shown below, where values of potential are given in Volt.

$$E_p \text{ (vs. Ag/AgCl)} = 1.247 - 0.052 \text{ pH, } R=0.9978 \text{ (1)}$$

The slope value is very close to the theoretical slope -59 mV pH^{-1} of the plot of E_{pa} versus pH for a classical Nernstian two-electron and two-proton process [28]. According to the currently accepted mechanism, described by Momberg *et al* [26].

Optimization of SWV parameters

SWV parameters were optimized using a 2^3 full factorial design, including a central point assayed in quintuplicate (replicate was used to calculate standard deviation). In this way, the frequency of the pulse potential (f), the amplitude of the pulse (a) and the scan increment (ΔE_s) were simultaneously evaluated, making it possible to obtain the best analytical signal, in terms of sensitivity and selectivity.

Table 1: Factors, levels and response values for the 2^3 factorial design

Runs	Factor			Responses Corrente (μA)/Potencial (V)
	a	f	ΔE_s	
01	-	-	-	29.23
02	+	-	-	68.88
03	-	+	-	34.10
04	+	+	-	85.19
05	-	-	+	36.12
06	+	-	+	93.16
07	-	+	+	43.39
08	+	+	+	108.44
09	0	0	0	66.40
10	0	0	0	65.27
11	0	0	0	64.64
12	0	0	0	65.42
13	0	0	0	64.44

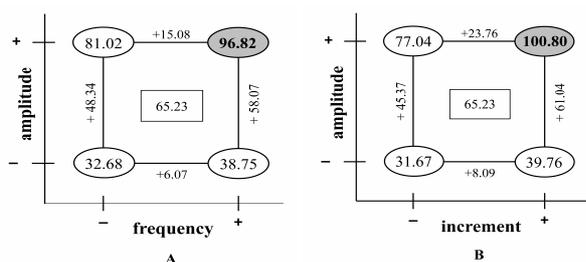


Fig. 4: Geometric representations of interaction effects (A) amplitude vs. frequency and (B) amplitude vs. increment.

The ratio between peak current (I_{pa}) and half-peak width ($\Delta E_{p/2}$) was evaluated to obtain the best SWV conditions for determination of trimethoprim, since it is at lower amount in the commercial formulation. The variables and studied levels were: $f = 80 \text{ s}^{-1}$ (-1) and 100 s^{-1} (+1); $a = 10 \text{ mV}$ (-1) and 30 mV (+1); $\Delta E_s = 3 \text{ mV}$ (-1) and 5 mV (+1). The results are shown in Table 1.

Considering the ratio between the peak current (I_{pa}) and half-peak width ($\Delta E_{p/2}$), the statistical treatment of the data showed that the main effects, increment (+15.93), frequency (+10.93), amplitude (+53.21) and interaction effects of amplitude vs. Frequency (+7.84)

and amplitude vs. Increment (+4.86) were significant at 95% confidence levels ($0.77 \times 2.78 \text{ } t_{95\%, \nu=4} = 2.14$). The geometric representations of interaction effects (Figure 4) shows that the best analytical signal is obtained at the ++ vertex (96.82 and 100.80 $\mu\text{A/V}$) for any interaction effect, according to following values: frequency of the pulse potential (f), 100 s^{-1} ; amplitude of the pulse (a), 30 mV and scan increment (ΔE_s), 5 mV .

Analytical Responses

Under optimized conditions, Figure 5 shows the SWV voltammograms series carried out at different concentrations of SMX/TMP in BR buffer solution (pH=6). The analytical curves obtained are presented inset of Figure 5, with linear response ranging from 5.5×10^{-5} to $3.95 \times 10^{-4} \text{ mol L}^{-1}$ for SMX and 1.05×10^{-5} to $1.04 \times 10^{-4} \text{ mol L}^{-1}$ for TMP, in accordance with the following equations, with a correlation coefficient of 0.9974 and 0.9971 for SMX and TMP, respectively.

$$I_{pa} \text{ (A)} = 4.55 \times 10^{-6} + 0.0366 C_{\text{SMX}} \text{ (mol L}^{-1}) \text{ (2)}$$

$$I_{pa} \text{ (A)} = 2.66 \times 10^{-6} + 0.1070 C_{\text{TMP}} \text{ (mol L}^{-1}) \text{ (3)}$$

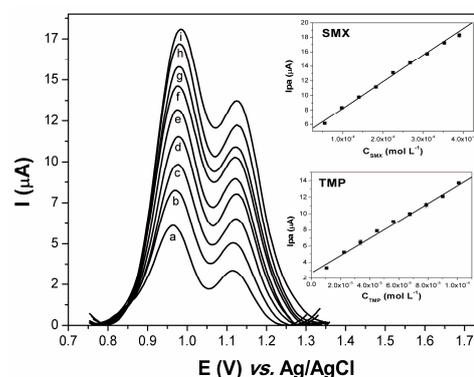


Fig. 5: SWV responses (simultaneous oxidation) obtained on a GCE at different concentrations of SMX/TMP in a BR buffer solution (pH 6), with $a=30 \text{ mV}$, $f=100 \text{ s}^{-1}$ and $\Delta E_s=5 \text{ mV}$. SMX/TMP concentrations, in mol L^{-1} : a) $5.50 \times 10^{-5}/1.05 \times 10^{-5}$; b) $9.75 \times 10^{-5}/2.25 \times 10^{-5}$; c) $1.40 \times 10^{-4}/3.38 \times 10^{-5}$; d) $1.83 \times 10^{-4}/4.50 \times 10^{-5}$; e) $2.25 \times 10^{-4}/5.63 \times 10^{-5}$; f) $2.68 \times 10^{-4}/6.75 \times 10^{-5}$; g) $3.10 \times 10^{-4}/7.88 \times 10^{-5}$; h) $3.53 \times 10^{-4}/9.00 \times 10^{-5}$; i) $3.95 \times 10^{-4}/1.01 \times 10^{-4}$. Inset: the respective analytical curves for SMX and TMP.

The quantification and detection limits, determined according to IUPAC recommendations [23], were found to be $2.84 \times 10^{-5} \text{ mol L}^{-1}$ (7.19 mg L^{-1}) and $8.52 \times 10^{-6} \text{ mol L}^{-1}$ (2.16 mg L^{-1}) for SMX and $3.10 \times 10^{-6} \text{ mol L}^{-1}$ (0.9 mg L^{-1}) and $9.31 \times 10^{-7} \text{ mol L}^{-1}$ (0.27 mg L^{-1}) for TMP, respectively. Despite the LOD and LOQ have been higher than found in literature [3, 20-22] for simultaneous determination of SMX and TMP, these are not so relevant in drugs determination, because of their high concentration in commercial formulations [29]. Besides, the glassy carbon electrode shows advantages, like low cost, environmentally friendly and did not need any modification for the purpose.

Recovery studies were performed by the addition of known amounts of SMX and TMP in supporting electrolyte solution (BR buffer, pH 6) followed by SWV analysis. Five determinations were carried out, and recovery values ranged from 97.4% to 103.5% for SMX (RSD 2.37%) and from 97.5% to 103.2% for TMP (RSD 2.19%) were observed, suggesting the viability of the proposed method for simultaneous determinations of SMX and TMP.

In addition, the intra-assay precision (repeatability) determined by ten successive SWV voltammograms measurements ($n=10$) in the same solution, were good, with RSDs of 0.99% and 1.23% for sulfamethoxazole and trimethoprim, respectively.

The inter-assay precision was carried out by five experiments for fresh solution over a period of 5 days, the RSDs were found to be 2.28% for SMX and 2.74% for TMP. These tests were performed in

0.04 mol L⁻¹ BR buffer solution at pH 6.0 containing 2.0x10⁻⁴ mol L⁻¹ of SMX or 5.5x10⁻⁵ mol L⁻¹ of TMP. Prior to each experiment, the electrode surface was cleaned.

Sulfamethoxazole and trimethoprim determination in real samples

Finally, the square wave voltammetric method was applied to simultaneous determination of SMX and TMP in six real samples, including three different pharmaceutical formulations (tablets, oral suspension and injection) (Figure 6). As can be seen, there is no significant difference between the voltammetric profile of SMX/TMP standard solution and the different commercial samples, indicating that the matrix effect does not present any significant interference.

The results of each commercial drug are presented in Table 2. The precision of the proposed method was compared to the HPLC method by statistical examination of the values obtained from *F*-tests (95% confidence level). *F*_{exp} value for both of analytes in all determinations, was lower than the *F*_{critical} (19.00), indicating no difference between the standard deviations or precision of the two methods. The *t*-tests to compare the average obtained using the standard and the proposed method showed that the calculated values of *t* were lower than the theoretical value (*t*_{critical}=2.78, $\alpha=0.05$).

As can be seen, there is no difference between the obtained results at a confidence level of 95%.

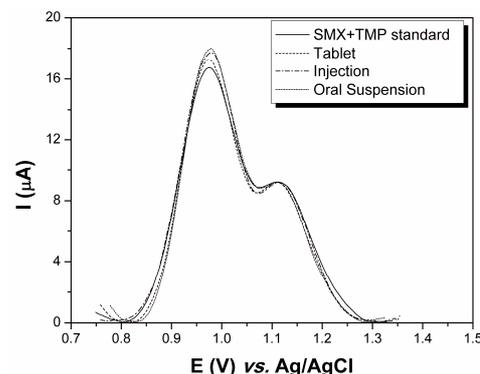


Fig. 6: SWV responses (simultaneous oxidation) for different samples and standard of SMX/TMP (at the same concentration of label) in a BR buffer solution (pH 6) obtained on GCE. SWV conditions: $\alpha=30$ mV, $f=100$ s⁻¹ and $\Delta E_s=5$ mV.

Table 2: Results obtained in simultaneous determination of SMX and TMP in different formulations by standard method (HPLC) and the proposed method (SWV).

SMX / TMP Samples	Label Value (mg)	HPLC ^a (mg)	SWV ^a (mg)	<i>F</i> -test ^b	<i>t</i> -test ^c	Relative error (%)
Injection A	400/80	414/81.0	418/79.3	3.17/4.06	0.29/1.93	0.97/-2.10
Injection B	400/80	425/80.4	433/81.2	10.06/16.03	1.19/1.65	1.88/1.00
Tablets A	400/80	414/79.3	404/80.8	3.69/1.06	1.49/1.38	-2.41/1.89
Tablets B	400/80	380/78.1	382/80.6	1.31/15.04	0.14/2.12	0.53/3.20
Oral suspension A	200/40	217/39.6	218/39.6	10.90/4.06	0.24/1.07	0.46/0
Oral suspension B	200/40	204/41.9	219/43.6	6.93/3.05	1.48/1.68	7.35/4.06

^a Average of three measurements. ^b Critical *F*-value for $\nu_1 = 2$ and $\nu_2 = 2$ at 95% confidence level is 19.00. ^c Critical *t*-value for $\nu = 4$ at 95% confidence level is 2.78. Relative error 1 (%): [(voltammetric value - HPLC value) / HPLC value] x 100.

CONCLUSION

The developed voltammetric method showed excellent performance for simultaneous determinations of SMX and TMP in pharmaceutical formulations. The glassy carbon electrode provides good sensitivity and selectivity for SMX and TMP quantification in pharmaceutical matrixes, without any surface modification. Moreover, the measured concentrations were statistically similar to those obtained by the standard chromatographic method, at a 95% confidence level. The proposed method is an excellent alternative for the quality control of these samples, with the advantage that it did not require any sample pretreatment.

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CONFLICT OF INTEREST

None

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