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Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 364–369
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Adsorptive stripping voltammetry of trimethoprim: Mechanistic studies and application to the fast determination in pharmaceutical suspensions

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Received 8 July 2004; received in revised form 10 January 2005; accepted 10 January 2005
Available online 6 February 2005

Abstract

The adsorptive stripping voltammetric behaviour of trimethoprim (TMP) was studied at pH 3.8 and 7.0 by linear-sweep (LS) and cyclic voltammetry at the hanging mercury drop electrode. The charges and surface concentrations of the protonated TMP species were determined at both pH values. Taking advantage of the adsorption features of TMP fast voltammetric techniques (LS and square-wave (SW) voltammetry) were applied to the determination of TMP at the 10^{-7} mol dm^{-3} concentration level (pH 3.8). For these concentrations the relative standard deviations were <2% ($N = 8$) and the detection limit was 10 nM (3 ng/mL) for the SW-AdCSV (3 s; accumulation time 10 s, frequency 100 Hz). The use of SW-adsorptive cathodic stripping voltammetry originated a very fast and sensitive method for the direct analysis of TMP in pharmaceutical suspensions without any matrix effects or interference from sulfamethoxazole. No sample pre-treatments or solvent extraction procedures were needed. The quantitative results were in agreement with the data supplied by the manufacturer.

Keywords: Trimethoprim; Adsorption; Mercury electrode; Square-wave cathodic stripping voltammetry; Pharmaceutical suspensions

1. Introduction

Trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine) (TMP) is a well-known antibacterial agent used as a potentiator in combination with several sulfonamides, e.g. sulfamethoxazole (SMX), for the treatment of a number of bacterial infections [1–4]. Binary mixtures of TMP and SMX are current in commercial pharmaceutical preparations, like Bactrim (ratio 1:5), but TMP can be also supplied as a single agent. Analytical methods for the determination of TMP range from liquid chromatography [1,5,6] spectrophotometry [7–9], potentiometry [10], capillary zone electrophoresis [4] and capillary electrophoresis with amperometric detection at carbon electrodes [11,12], NMR [13] and electroanalysis [3,14–17]. In the earlier electrochemical methods [3,14] the determination of TMP was achieved by polarographic reduction in acidic solutions. However, these polarographic methods were intrinsically time-consuming and involved handling high quantities of mercury. Also, the proposed methods required sample pre-treatments, e.g. solvent extraction of the TMP, whenever the determination of TMP proceeded from pharmaceutical suspensions [14] or from other complex media like blood and urine [3]. Kotoucek et al. [15] evaluated the mechanism of the polarographic reduction of TMP. In concentrated sulphuric acid ($c > 0.1$ mol dm^{-3}) the protonized TMP was reduced in a two-electron process. But for pH > 3.5 the polarographic cathodic wave of TMP corresponded to an overall four-electron/four-proton reduction process producing the tetrahydro derivative of TMP with the final irreversible released of ammonia (or ammonium cation, depending on pH). In neutral solutions, the reduction current of TMP decreased and in alkaline medium TMP was not electroactive. Those authors studied also the reduction of TMP at the hanging mercury drop electrode (HMDE) and concluded that the electrode process (in 10^{-4} M TMP solutions for pH < 3.7) involved significant adsorption on the HMDE. Taking advantage of that property, the authors exploited the use of adsorptive cathodic stripping voltammetry (AdCSV)

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doi:10.1016/j.jpba.2005.01.005

for the differential pulse determination of TMP in Biseptol tablets, at pH 3.8, without any interference from sulfamethoxazol. The limit of detection was ca. 1 nM for an accumulation time within the interval 20–80 s and a scan rate of 50 mV/s. However, the method involved a previous extraction of the TMP with methanol from the tablets. On the other hand, the square-wave (SW) AdCSV was used together with a partial least squares method for the analysis of sulphamethoxypyridazine and TMP in veterinary formulations [16]. Though, the information given on the adsorption features of TMP and on the optimisation of the SW parameters was scarce. The SW methodology was also used in a study of the reduction of TMP and other electroreducible drugs [17] where the detection limits were of the order of 10–100 nM.

Adsorptive stripping methods are intrinsically very sensitive, allowing working with very diluted samples, consequently decreasing interferences due to surface-active components that would compete with the analyte and hinder its signal. This is extremely important whenever the sample matrix is complex, e.g. in pharmaceutical or biological media. In adsorptive voltammetry, square-wave and linear-sweep techniques allow using fast run times, i.e. high scan rates, unless the electrode reaction is kinetically sluggish.

As far as we know, the direct voltammetric analysis of TMP in pharmaceutical suspensions, like the Bactrim syrup, was not yet exploited. Also, the application of fast voltammetric methods, like square-wave or linear-sweep voltammetry has not been fully investigated.

In the present work, we examined further the mechanism of the voltammetric reduction of trimethoprim, namely the effect of pH on its adsorption characteristics at the HMDE. The influence of the instrumental parameters and of the accumulation potential on the TMP stripping signal was also assessed. Lastly, a simple procedure for the fast analysis of TMP in a pharmaceutical suspension was developed using square-wave cathodic stripping voltammetry as the analytical technique.

2. Experimental

2.1. Apparatus and reagents

The voltammetric experiments were performed with a computer-controlled potentiostat (PGSTAT-12 controlled by the GPES software, EcoChemie, Netherland) connected, via an IME-663 module, to an electrode stand (663 VA-Stand, Metrohm, Switzerland). The three-electrode configuration was used comprising the hanging mercury drop electrode, HMDE (medium drop size) as the working electrode, a GC rod counter electrode and a double junction Ag/AgCl (3 mol dm^{-3} KCl, saturated AgCl, and 3 mol dm^{-3} KCl in the bridge) reference electrode. All potentials quoted are relative to this Ag/AgCl reference electrode. The electrochemical surface area of the HMDE was determined by chronoamperometry (with a 8.89×10^{-3} mol dm^{-3} Cd^{2+} solution in 1.0 mol dm^{-3} KCl; diffusion coefficient of Cd^{2+} 7.8×10^{-6} cm^2 s^{-1} [18]) and was $(3.48 \pm 0.06) \times 10^{-3}$ cm^2 ($N = 4$). All experiments were conducted at room temperature (ca. 20 °C). A combined glass electrode (Orion 9104SC) connected to a pH meter (Cole Parmer, Model 05669-20) was used for pH measurements.

All solutions were prepared from ultra pure or analytical-reagent grade chemicals. Stock solutions of TMP (Sigma) with a concentration within the 1×10^{-3} mol dm^{-3} level were prepared in methanol, stored at 4 °C, and used without further purification. Supporting electrolytes, ammonium acetate buffer (1 mol dm^{-3} CH_3COO NH_4/0.5 mol dm^{-3} HCl; pH 3.8) and phosphate buffer (0.04 mol dm^{-3} Na_2HPO_4/0.026 mol dm^{-3} KH_2PO_4; pH 7.0) were prepared with deionised water (18.2 MΩ cm, Milli-Q system, Millipore–waters, USA).

Three diluted syrup solutions (Bactrim, paediatric formulation, 40 mg TMP/5 mL) were prepared for the analysis of TMP. A certain amount of the Bactrim suspension (0.1503; 0.2155 and 0.1409 g) was weight and dissolved in water/methanol (1:4, v/v) and diluted to 50 mL. An estimate of the syrup density was determined as 1.22 ± 0.01 g/cm^3 ($N = 4$).

2.2. Procedures

In the voltammetric experiments a suitable volume of TMP stock solution was added to 20 mL of a given supporting electrolyte in the voltammetric cell and purged with pure nitrogen for 5 min. For the linear-sweep and cyclic voltammetry (staircase techniques) the scan rate varied between 10 and 1000 mV/s (potential step 5 mV). For the determination of the voltammetric charge under the TMP reduction peak the scan rate was 250 mV/s and the charge values were calculated by electronic integration. In all these experiments four replicate scans were recorded for each experimental conditions and mean values are presented. In the optimisation of linear-sweep and square-wave stripping voltammetry, the experiments were performed with a 5.2×10^{-7} mol dm^{-3} cell solution of TMP at pH 3.8. The accumulation step lasted 20 s at an accumulation potential, E_{acc}, of −0.6 V whilst the solution was stirred (rotational frequency 25 s^{-1}). After 5 s quiescent time the stripping step was performed from −0.6 to −1.4 V. Calibration curves were done within the TMP concentration interval 1 to 10×10^{-7} mol dm^{-3} at pH 3.8, using both linear-sweep and square-wave stripping voltammetry. The accumulation step lasted 10 s at $E_{acc} = -0.6$ V.

For the AdCSV-SW determination of TMP, 100 μL of each diluted syrup solution was added to 20 mL of degassed supporting electrolyte (acetate buffer, pH 3.8) in the voltammetric cell and the voltammogram was recorded (five replicate runs) using the above mentioned conditions (accumulation time 10 s at −0.6 V). The SW instrumental parameters were: frequency 100 Hz, amplitude 25 mV and step potential 5 mV (the corresponding scan

rate was 500 mV/s). Calibration curves were carried out with TMP standard solutions (five additions of 5.0 μL of a 1.757×10^{-4} mol dm^{-3} TMP stock solution) and all peak currents were mean values of five replicate measurements.

3. Results and discussion

3.1. Linear-sweep and cyclic voltammetry of trimethoprim: mechanistic studies

The nature of the TMP reduction process was studied by cyclic and linear-sweep (LS) voltammetry. Fig. 1 shows typical cyclic voltammograms of 1.0×10^{-5} mol dm^{-3} TMP solutions at pH 3.8 and 7.0, where the effect of pH, both on the peak potential and on the peak current is clearly seen and agrees with the expected behaviour for a proton-dependent process coupled to a final irreversible chemical (homogeneous) reaction [15]. This chemical irreversibility leads to the absence of any oxidation peak in the reverse scan, even if the scan rate v is increased to 1000 mV/s. Accordingly, the peak potential becomes more negative with the increase in v. At this concentration level and pH 3.8, the electrode process is controlled not only by diffusion of TMP but adsorption is also significant: within the scan rate interval 10–1000 mV/s, the log–log plot of the peak current versus scan rate, v, gave a slope of 0.7. In fact, there is a marked dependence of the current of the reduction peak on the initial potential of the reduction scan, as can be seen from the inset in Fig. 1. So, for potentials in the range −0.6 to −1 V, the reduction peak attains its maximum intensity, meaning that TMP is being accumulated by adsorption on the mercury electrode surface. The behaviour for pH 7.0 was similar.

Lowering the concentration of TMP to 5.2×10^{-7} mol dm^{-3} (pH 3.8) the LS voltammograms, initiated at $E_{acc} = -0.6$ V with an accumulation time of 20 s, showed a rather symmetrical peak, characteristic of a predominant reduction of the adsorbed protonated TMP species. A linear dependence of the peak current from the scan rate was now obtained, as expected for the reduction of an adsorbed species [18] (the log–log plot gave a slope of 0.9, corresponding to I_p(nA) = 443 [v(V/s)] + 2.6; $r = 0.998$ ($N = 5$)). Also, the peak potential depends linearly on log v with a slope of −47.9 mV ($N = 5$, $r = 0.998$). Additionally, the peak potentials undergo a significant displacement towards more positive potentials comparing with the values for $c = 1.0 \times 10^{-5}$ mol dm^{-3} (e.g. for 250 mV/s, the peak potential shifted from −1.36 to −1.27 V by lowering the concentration of TMP), indicating that the reduction of the adsorbed protonated TMP is energetically easier than that of the diffusing TMP species. Further, for concentrations lower than 10^{-6} mol dm^{-3} and for both pH values 3.8 and 7.0, the current of the stripping peak of TMP increased with the accumulation time, t_{acc} at $E_{acc} = -0.6$ V, reaching a plateau for higher times where the saturation of the electrode surface occurred. For a diffusion-controlled adsorption of the reactant, the peak current, and therefore the charge, are dependent of the square root of the accumulation time (or of a corrected time, t_p) regardless of the reversibility of the reaction [19,20]. The time t_p is the accumulation time corrected for the scanning time between the initial potential, E_{in}, (identical to E_{acc}) and the peak potential, E_p, and is given by Eq. (1) [19]

$$t_p = \frac{t_{acc} + (E_p - E_{in})}{v} \quad (1)$$

This phenomenon can be illustrated by plots of the voltammetric charge corresponding to the LS stripping peak of TMP, Q, versus the square root of t_p (Fig. 2).

The linear-sweep voltammetry (staircase) can be used for the estimation of the mechanistic parameter (αn_α) relative to the reduction an adsorbed species. For an irreversible process where a diffusion-controlled adsorption of the reactant occurs, the following relationships apply [19,20]:

$$Q = nFA\Gamma \quad (2)$$

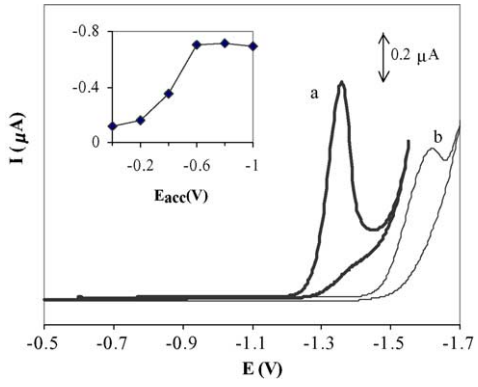

Fig. 1. Cyclic voltammograms of 1.0×10^{-5} mol dm^{-3} TMP solutions at pH 3.8 (a) and 7.0 (b). Scan rate: 250 mV/s, E_{in} = –0.5 V. Inset: effect of the accumulation potential, E_{acc} on the current of the reduction peak of TMP (pH 3.8): equilibration time 10 s, no stirring.

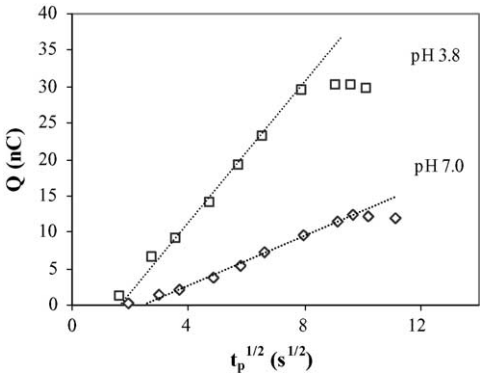

Fig. 2. Typical variation of the voltammetric charge of the stripping peak of TMP ($c = 2.8 \times 10^{-7}$ mol dm^{-3}) with the square root of t_p (corrected accumulation time) at −0.6 V for pH 3.8 and 7.0. Scan rate of the LS voltammograms: 250 mV/s.

$$I_p = n(\alpha n_\alpha)\frac{A\Gamma v F^2}{eRT} \quad (3)$$

$$W_{1/2} = \frac{62.5}{(\alpha n_\alpha)}\,\text{mV} \quad (4)$$

$$\frac{\partial E_p}{\partial \log v} = \frac{58.2}{(\alpha n_\alpha)}\,\text{mV}(20\,^\circ\text{C}) \quad (5)$$

where n is the total number of transferred electrons, α the transfer coefficient, n_α the number of electrons transferred in the rate determining step, Γ the reactant surface concentration and $W_{1/2}$ is the peak width at half height.

Combining Eqs. (2) and (3) one obtains $I_p/Q = (\alpha n_\alpha) vF/eRT$. So, the parameter (αn_α) can be estimated independently from I_p/Q and from $W_{1/2}$. Hence, for the reduction of the adsorbed protonated TMP, the following values were obtained at pH 3.8: 1.29 ± 0.04 and 1.26 ± 0.04, respectively, from I_p/Q and $W_{1/2}$ ($N = 6$, $v = 0.25$ V/s). These values agree with that obtained from Eq. (5), which was 1.21 (cf. the slope given previously in the text). For the reduction of TMP conducted at pH 7.0, the estimated values of (αn_α) were 1.41 ± 0.05 and 1.32 ± 0.07, respectively, from I_p/Q and $W_{1/2}$ ($N = 6$, $v = 0.25$ V/s). The (αn_α) values are similar at both pH values. Hence, one may assume that the reduction of TMP follows the same mechanism at pH 3.8 and 7.0, which agrees with the mechanistic pathway presented by Koutocek [15].

Further, from the plots of Q versus square root of t_p the maximum charge, Q_{max} corresponding to the saturation of the electrode surface can be estimated by the charge at the plateau. For pH 3.8, Q_{max} was (34.3 ± 2.9) nC (mean value from four data sets similar to Fig. 2) to which corresponded a maximum accumulation time of ca. 60 s (calculated from the plots Q versus $t^{1/2}$ by the intersection of the linear branch for lower times with the line corresponding to the plateau). This means that at the present concentration (2.8×10^{-7} mol dm^{-3}), an accumulation time lower than 60 s must be used in order to obtain linear calibration curves (no saturation of the electrode surface). For pH 7.0, Q_{max} was lower than for pH 3.8, i.e. (12.0 ± 0.2) nC (mean values of three data sets), and the accumulation time corresponding to the saturation of the surface were higher, ca. 90 s. Estimates of the maximum surface concentration of the electroactive TMP, Γ_{max}, can be calculated from Q_{max} (Eq. (2)), considering $n = 4$ regardless of pH [15] and were 2.6×10^{-11} mol cm^{-2} and 8.94×10^{-12} mol cm^{-2} at pH 3.8 and 7.0, respectively. Some conclusions may be drawn out from these data. Firstly, considering the pK_a of trimethoprim as 7.2 [14], at pH 3.8 the distribution of the protolytic forms of TMP is shifted to the protonized ones, which are electroactive. Therefore, at pH 3.8 a monolayer of adsorbed protonated TMP shall be built up for longer accumulation times. The TMP molecule has a volume of 251.28 Å^3 to which corresponds a surface area of 291.15 Å^2 (calculated by the Cyrus 2 software from Molecular Simulations Inc.). Assuming that TMP adsorbs with a planar configuration and that there are no interactions between adsorbed molecules, one compact monolayer of adsorbed TMP should correspond to a surface concentration of 5.7×10^{-11} mol cm^{-2}. This value compares fairly with that obtained from the maximum charge at pH 3.8. Nevertheless, the lower experimental value might mean that a degree of repulsive interaction between the positively charged TMP species occurred. Another consideration involves the lower Γ_{max} obtained at pH 7.0. In fact, at pH 7.0, a lower proportion of the electroactive protonated TMP species shall be present at the electrode interface, and consequently the charge plateau was observed for higher accumulation times than at pH 3.8. Additionally, the Q_{max} value was approximately two times lower than the observed at pH 3.8, for a monolayer of electroactive TMP molecules, meaning that the uncharged TMP species, which are not reactive, adsorbed competitively at the accumulation potential of −0.6 V. It shall be noted that for the present supporting electrolytes no specific adsorption is expected and the potential of zero charge of the mercury electrode shall be around −0.5 V [21], i.e. for an accumulation potential of −0.6 V a predominant adsorption of the protonated TMP species shall occur, but the uncharged TMP species will also adsorb, at a lower extent.

3.2. Linear-sweep and square-wave stripping voltammetry of trimethoprim

In order to attain a fast analysis of TMP in pharmaceutical preparations, the application of linear-sweep and square-wave adsorptive stripping voltammetry was tested. As concluded in the previous section, the highest surface concentrations of TMP are obtained at pH 3.8. The choice of a rather low pH is thus important in order to maximize the adsorption of the protonated (electroactive) TMP relative to the neutral (non-electroactive) TMP, and also to other possible tensioactive species that could be present in the sample matrix. On the other hand, it was previously showed that sulfamethoxazole, although a major component in the common pharmaceutical preparations is only electroactive bellow pH 2 [15]. Therefore, the subsequent work was done only at pH 3.8.

Experiments were carried out with LS stripping voltammetry for different scan rates (10–250 mV/s) for TMP solutions of 5.2×10^{-7} mol dm^{-3} at pH 3.8, accumulating at −0.6 V for 20 s. As expected, the LS peak current increased with v, but the background current also increased, especially in the vicinity of the TMP stripping peak (which occurred near the negative limit of the working potential window), increasing the slope of the peak baseline. For $v > 100$ mV/s that increase was more marked. The step potential was varied between 1 and 10 mV; for the lowest values the background current noise increased and for a potential step higher than 5 mV the peak current decreased slightly. So, a scan rate of 100 mV/s and a potential step of 5 mV were selected for further work.

The application of square-wave (SW) voltammetry as the analytical technique for the stripping step was also tested,

using the same experimental conditions as above. The SW stripping peak current increased with the SW amplitude (from 10 to 100 mV) attaining a plateau for amplitudes higher than 50 mV. For these values the peak baseline also increased in height. However, the peak width at half height, $W_{1/2}$, remained constant. On the other hand, the peak current increased linearly with the frequency within the range 10–200 Hz (I_p(nA) = 3.78 [f] + 6.30; r = 0.999, N = 5), for an amplitude of 25 mV and a potential step of 7.5 mV. This trend is characteristic of an electrode process proceeding from an adsorbed reactant [19,22]. The peak width at half height was constant over the frequency interval. However, for f = 200 Hz the background current increased significantly (ca. 1.5 times that for f = 100 Hz). Further, I_p varied linearly with the step potential over the range (1–15 mV), but a steady increase of both $W_{1/2}$ and the slope of the peak baseline were observed for step potentials higher than 5 mV. Therefore, the selected optimal parameters were amplitude 25 mV, frequency 100 Hz, and step potential 5 mV (corresponding scan rate of 500 mV/s).

Calibration graphs were obtained using both voltammetric techniques within the concentration interval 0.1 to 1.0×10^{-6} mol dm^{-3} for t_{acc} of 10 s at E_{acc} = –0.6 V at the above mentioned optimised instrumental parameters. Table 1 presents the calibration data. Both voltammetric techniques afforded good quality results, with low limits of detection and very good relative standard deviations. Still, the square-wave technique provides higher sensitivities and faster run times.

3.3. Application

The present SW-AdCSV method was tested in the determination of TMP in a pharmaceutical suspension, the Bactrim syrup. A 100 μL of each diluted syrup solution (see Section 2.1) was added to the voltammetric cell and the voltammogram was recorded under the optimal conditions. The mean content of TMP calculated from the three syrup aliquots using a calibration curve was 7.70 (± 0.18) mg/mL, which compares very well with the declared value of 8 mg/mL. There were no effects of the suspension matrix due to the high dilution factor. Also, the major Bactrim syrup component, sulfamethoxazole, did not have an effect on the TMP signal, indicating that this sulfonamide is not electroactive and especially it does not adsorb at the HMDE under the present experimental conditions. Fig. 3 shows typical SW-AdCSV voltammograms used for the calibration curve and also that of TMP in a diluted Bactrim sample (inset).

Table 1
Data for the calibration curves for the adsorptive stripping voltammetry of TMP (pH 3.8) using linear-sweep (LS) and square-wave (SW) analysis

	LS-AdCSV	SW-AdCSV
Slope (A mol^{-1} dm^{-3})[a]	0.074 ± 0.009	0.45 ± 0.02
Correlation coefficient (N = 6)	0.999	0.999
Limit of detection[b]	8 nM	10 nM
R.S.D. (N = 8; $c = 6.5 \times 10^{-7}$ M)	0.9%	1.5%
Scan rate (V/s)	0.1	0.5

Accumulation time 10 s at −0.6 V.
[a] Slope and the corresponding 95% confidence limits for four degrees of freedom.
[b] Calculated for 3 s.

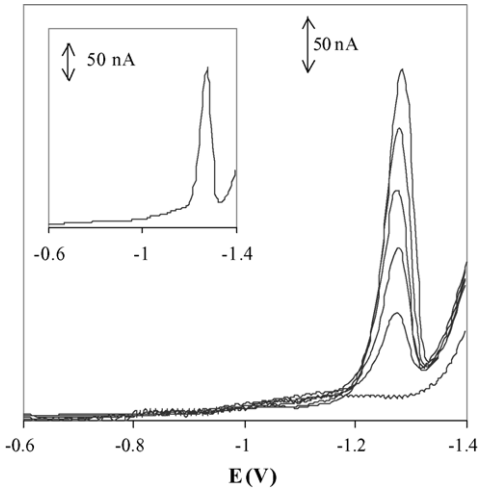

Fig. 3. Square-wave adsorptive stripping voltammograms of TMP standard solutions (pH 3.8) at various concentrations (0 to 7×10^{-7} mol dm^{-3}). Inset: SW-AdCSV of TMP in an aliquot of the diluted Bactrim syrup. Accumulation at −0.6 V for 10 s. SW parameters: f = 100 Hz; a = 25 mV and potential step 5 mV.

4. Conclusion

The adsorption features of TMP were studied at the HMDE for different solution pH (3.8 and 7.0). The results pointed to a predominant adsorption of the protonated TMP, though the neutral molecule (electroinactive) might adsorb competitively, namely at pH 7.0. The adsorption of protonated TMP is rapid (it is diffusion-limited) and independent of the electrode potential in the range −0.6 to −1.0 V. The voltammetric features are in accordance with the expected for an irreversible four-electron reduction process with protonation. The estimated values for (αn_α) ranged between 1.21 and 1.29 at pH 3.8 and 1.32 and 1.41 at pH 7.0. At pH 3.8, the surface concentration of the electroactive TMP species attains its maximum value, envisaging high sensitivity in the adsorptive voltammetric determination. Linear-sweep and square-wave techniques were therefore applied and optimised for the AdCSV analysis of TMP.

A procedure for the fast analytical determination of TMP in a pharmaceutical suspension (Bactim syrup) was developed using square-wave cathodic stripping voltammetry as the analytical technique. No sample pre-treatments or solvent extraction procedures were needed. The present method resulted in a simple, fast and reliable methodology for the determination of TMP in a pharmaceutical suspension taking advantage of the high sensitivity of the adsorptive stripping method.

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