Electrochemical determination of Cephalothin antibiotic by adsorptive stripping voltammetric technique

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Abstract

A sensitive and reliable stripping voltammetric method was developed to determine Cephalothin antibiotic drug. This method is based on the adsorptive accumulation of the drug at a hanging mercury drop electrode and then a negative sweep was initiated, which yield a well defined cathodic peak at $-625\,mV$ versus Ag/AgCl reference electrode. To achieve high sensitivity, various experimental and instrumental variables were investigated such as supporting electrolyte, pH, accumulation time and potential, drug concentration, scan rate, convection rate and working electrode area. The monitored adsorptive current was directly proportional to the concentration of Cephalothin and it shows a linear response in the range from 4×10^{-7} to $1.2 \times 10^{-6}\,mol\,l^{-1}$ (correlation coefficient $= 0.9995$) and the detection limit (S/N $= 3$) is $3.3 \times 10^{-9}\,mol\,l^{-1}$ at an accumulation time of 3 min. The developed AdSV procedure shows a good reproducibility, the relative standard deviation R.S.D.% ($n = 10$) at a concentration level of $5 \times 10^{-7}\,mol\,l^{-1}$ was 0.94%. Possible interferences by other pharmaceutical drugs and surfactants have been also evaluated. The applicability of this approach was illustrated by the determination of Cephalothin in pharmaceutical preparation and biological fluids such as serum and urine.

1. Introduction

Of the most commonly used instrumental techniques, electroanalytical approach is the one of choice, and stripping voltammetric method has provoked particular interest because it is currently the most sensitive and wide used electrochemical technique. Its possibility of applications cover many fields ranging from environment, pharmaceutical and clinical to food and industrial samples. Many of the adsorptive stripping voltammetric (AdSV) approach features such as sensitivity, selectivity, simplicity and versatility attributed to the combination of an effective preconcentartion step based on non-electrolytic adsorptive accumulation process with an advance measurement procedures such as DP or SW [1–5]. Unlike conventional stripping approaches (anodic and cathodic stripping voltammetry), which based on an electrolytic nature of preconcentration step, AdSV approach in contrast is based on adsorptive accumulation of the analyte on the electrode at open circuit with no charge transferred. Consequently, for a wide

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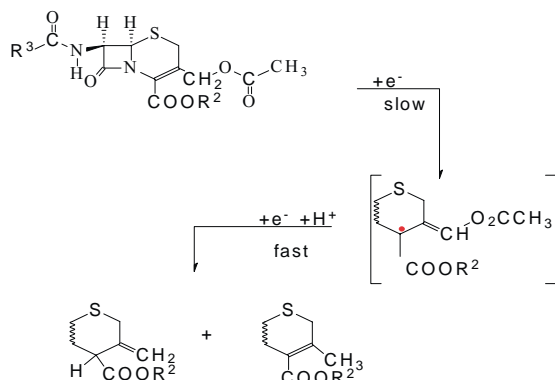
Scheme 1. The mechanism of the electrochemical reduction process.

range of surface-active organic and inorganic species, which cannot be preconcentrated electrolytically, the adsorption approach allows these analytes to be interfacially accumulated on the electrode and hence analysed. There have been many reviews devoted to emphasize and illustrate the wide spectrum and scope of AdSV applications and potentialities in the analysis of metal ions [6,7] organic analytes [8] and pharmaceutical drugs and biomedical compounds [9,10].

Cephalothin is a broad-spectrum antibiotic, which considered as one of the oldest known cephalosporin antibiotic drugs. It is a β-lactam antibiotic which is active against a wide variety of bacterial infections [11,12]. Its structure is shown in Scheme 1 and it has been analysed in pharmaceutical formulations and biological samples by various analytical methods such as spectrophotometry [13,14], chemiluminescence [15], fluorescence [16], chromatography [17,18], and various electroanalytical techniques such as potentiometry [19] polarography [20], coulometry and cyclic voltammetry [21] and square-wave voltammetry [22].

1.1. Aim of the study

Although molecular spectroscopic methods were widely used for the analysis of various pharmaceutical drugs, yet most of the applied procedures request a separation and/or pretreatment steps which obviously retain and delay their adequate use for routine clinical analysis. In contrast, based on the wide variety of the practical applications of AdSV in this field, this electrochemical approach was found to be

suitable for analyzing pharmaceutical compounds in body fluids either with moderate sample preparation or even with direct analysis of these complex samples when applying the adsorptive-extractive accumulation procedure at carbon paste electrode. Moreover, since most of cited electrochemical studies dedicated for the analysis of Cephalothin were quit old and carried out with relatively old fashion electroanalytical approaches, the aim of present work is to investigate the adsorptive stripping behavior of Cephalothin antibiotic in order to develop a simple, rapid, sensitive and suitable stripping voltammetric procedure for the determination of this drug in biological fluids and pharmaceutical preparations with only limited sample pretreatment. For this purpose, all factors that may influence the AdSV performance were investigated to find out the most sensitive instrumental conditions.

2. Experimental

2.1. Apparatus

All adsorptive stripping measurements were carried out with 757 AV computrace (Metrohm, Herisau, Switzerland) in connection with Dell computer and controlled by (VA computrace 2.0) control software. Stripping voltammograms were obtained via a Hewlett–Packard laser jet printer. A conventional three electrode system was used in the hanging mercury drop electrode (HMDE) mode. pH values were measured with Metrohm 744 pH meter. Oxford adjustable micropipette (Ireland) was used to measure microliter volumes of the standard solutions.

2.2. Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Cephalothin stock solution of $1 \times 10^{-2}\,mol\,l^{-1}$ were prepared by dissolving the appropriate amount of Cephalothin sodium salt in distilled water in 25 ml volumetric flask. This stock solution was stored in the dark and under refrigeration in order to minimize decomposition. Standard solutions of this antibiotic with lower concentration were prepared daily by diluting the stock solutions with distilled water. Britton–Robinson supporting buffer (pH ≈ 2, 0.04 M in each

constituent) was prepared by dissolving 2.47 g of boric acid in 500 ml distilled water containing 2.3 ml of glacial acetic acid and then adding 2.7 ml of ortho-phosphoric acid and diluting to 1 l with distilled water.

2.3. Procedure

The general procedure adopted for obtaining ad-sorptive stripping voltammograms was as follows: a 20 ml aliquot of Britton–Robinson supporting buffer (unless otherwise stated) at desired pH was pipetted in a clean and dry voltammetric cell and the required standard solutions of Cephalothin were added. The test solutions were purged with nitrogen for 8 min initially, while the solution was stirred. The accumulation potential of 0.0 V versus Ag/AgCl was applied to a new mercury drop while the solution was stirred for 180 s. Following the preconcentration period, the stripping was stopped and after 20 s had elapsed, cathodic scans were carried out over the range 0.0 to −1.3 V. All measurements were made at room temperature.

3. Preliminary observations

When the differential pulse polarographic behavior was investigated for Cephalothin in Britton–Robinson buffer at pH 11, a broad polarographic wave at

$E_p = -1.065$ V was observed. Based on the previous electrochemical studies carried out on Cephalothin and series of analogy Cephalosporins, this obtained polarographic wave is probably due to the electrochemical reduction of CH_2R group (located at the 3-position), where R is a good leaving group [21,23–25]. A proposed mechanism for the electrochemical cleavage of this electroactive group is given in Scheme 1. This mechanism suggesting that the electrochemical reaction is an irreversible process, an assumption which was confirmed by cyclic voltammetric measurement at a scan rate of $50 \, mV^{-1}$ of Cephalotin in Britton–Robinson buffer (pH 11). As can be seen from Fig. 1, no anodic peak was observed on the measured cyclic voltammogram, indicating the irreversibility nature of the cathodic reduction process. These polarographic and cyclic voltammetric results are in good agreement with previous electrochemical observations for this drug [21,23].

In order to obtain a voltammetric peak with better definition and higher sensitivity, a HMDE was used to study the adsorptive prosperities of Cephalothin. The AdSV behavior of Cephalothin was investigated in various supporting electrolytes at different pH values. This drug yielded a well-developed and defined AdSV peak corresponding to the substituted CH_2R electroactive group at peak potential of −0.631 V, where as the peak potential for differential pulse polaroghraphic
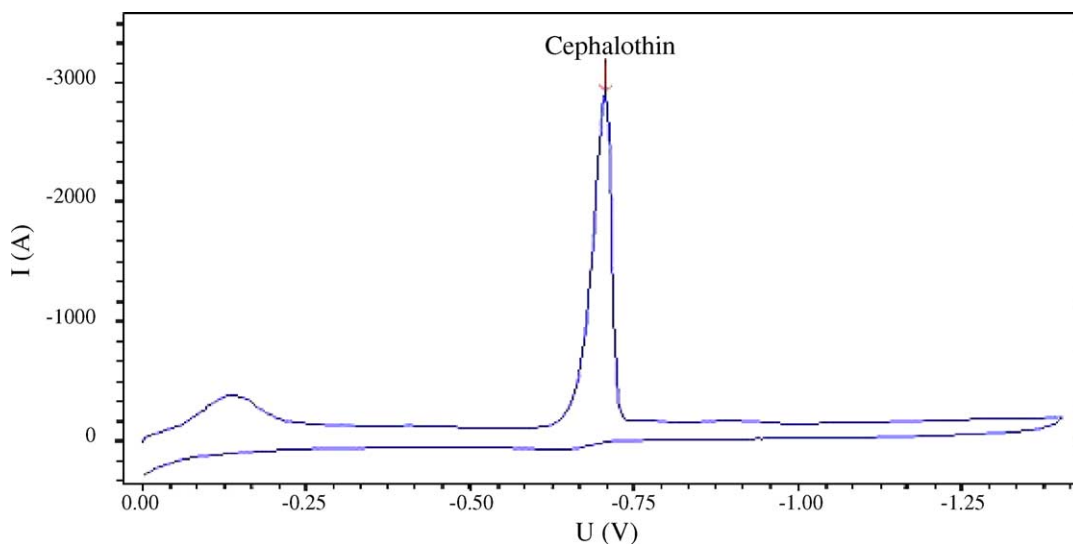

Fig. 1. Cyclic voltammogram of $1 \times 10^{-4} \, mol \, l^{-1}$ Cephalothin in pH 11 Britton–Robinson buffer, scan rate $100 \, mV \, s^{-1}$.
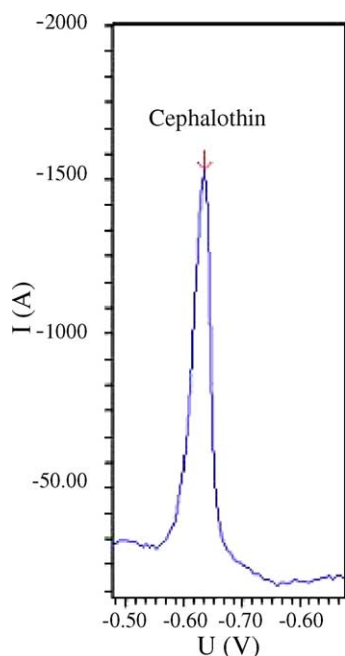
Fig. 2. DP AdSV voltammogram of 5×10^{-7} mol l^{-1} Cephalothin in pH 11 Britton–Robinson buffer. Accumulation time 3 min, accumulation potential 0.0 V and scan rate 15 mV s^{-1}.

measurement was at much more negative value near to the supporting electrolyte cut-off emphasizing the superiority of the stripping voltammetric approach in terms of resolution at least. A typical adsorptive stripping voltammogram for 5×10^{-7} mol l^{-1} Cephalothin in Britton–Robinson buffer is shown in Fig. 2, which illustrates a well observed electrochemical peak indicating a strong and readily adsorption process at the surface of the working electrode.

4. Parameters affecting the adsorptive stripping response

4.1. Effect of supporting electrolyte and pH

The nature and acidity of the supporting buffer are some of the most important factors which strongly influence the stability of the analyte of and its cathodic reduction and adsorption processes. Among the various investigated buffers (Britton–Robinson, acetate, carbonate and phosphate) the best voltammetric signal in terms of sensitivity (peak height)

and resolution (peak shape) have been secured using Britton–Robinson buffer. In addition, when the AdSV peak current was measured as a function of pH over 8–13 range, the stripping voltammetric signal increased steadily over the alkaline region and the peak current reached its maximum value at pH 11 which was selected as optimal value for subsequent studies. It is noteworthy that when acidic Britton–Robinson supporting electrolyte was used, Cephalothin drug was barely detectable and nearly no stripping voltammetric signal was observed. The variation of AdSV peak current with pH, obtained for 5×10^{-7} mol l^{-1} Cephalothin concentration accumulated for 2 min is exhibited in Fig. 3. However, the peak potential was shifted gradually to more negative values from -542 to -738 mV, when pH increased over the range 8–13, which indicates that E_p was pH dependent as expected for an electrochemical reduction process consuming proton ions (see the provided mechanism).

4.2. Effect of accumulation time and potential

Preconcentration of the analyzed drug on the surface of HMDE is one of the essential conditions for highly sensitive determinations. Variation of the accumulation time over 0–9 min period for 5×10^{-7} mol l^{-1} Cephalothin solution at a preconcentration potential of 0.0 V, showed a gradual enhancement for the monitored peak current. The dependence of peak current on accumulation time is presented in Fig. 4. The proportional relationship was nearly observed up to 7 min and then it becomes virtually curved and leveled off owing to the saturation of the hanging mercury drop by the analyte. For further experiments an accumulation time of 3 min was selected as optimal because it provided relatively high peak current with adequate practical time. The variation of accumulation time did not produce significant shifts in peak potential value.

In addition, as can be seen from Fig. 5, when the influence of accumulation potential on the monitored electrochemical response was examined over the -0.4 to $+0.2$ V range at 3 min preconcentration time, the peak current increased steadily over the positive direction till it reached it maximum value at $E_p = 0.0$ V where it decreased sharply after this inflection point. Thus, $E_{acc} = 0.0$ V will be adopted as optimum op-
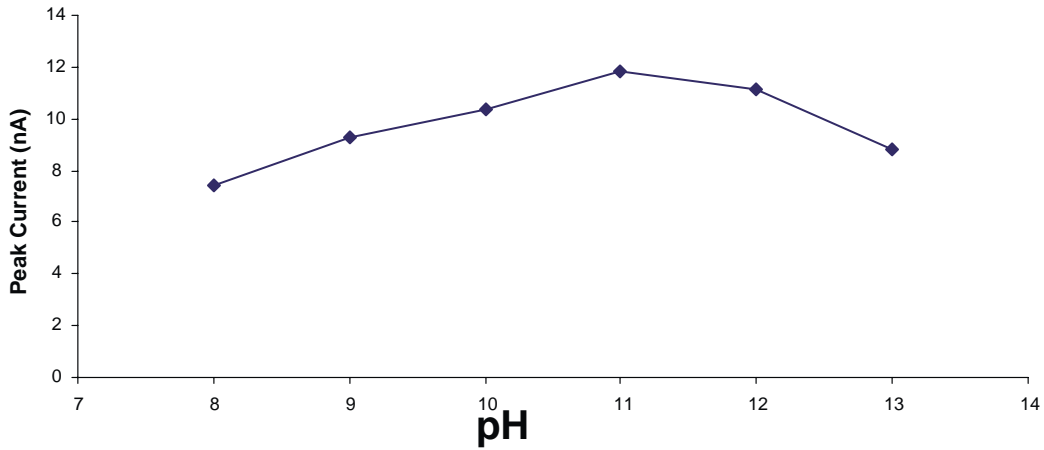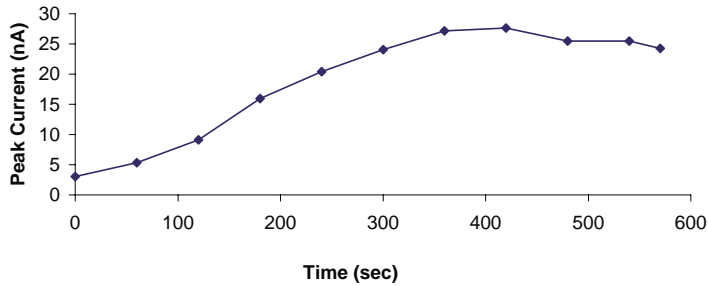
Fig. 3. Effect of pH on AdSV peak current.

Fig. 4. Effect of time accumulation on AdSV peak current.

erational value for the following works as it ensured the highest AdSV signal.

4.3. Effect of drug concentration

In order to obtain a significant analytical utility, the developed adsorptive stripping procedure must exhibit linear concentration dependence. Hence, the influence of Cephalothin concentration on the electrochemical signal was evaluated. The AdSV peak current of this drug increased linearly as a function of its concentration over the range 4×10^{-7} to 2.2×10^{-6}. However, beyond the linearity point the monitored peak current reached a plateau (as illustrated in Fig. 6) owing to
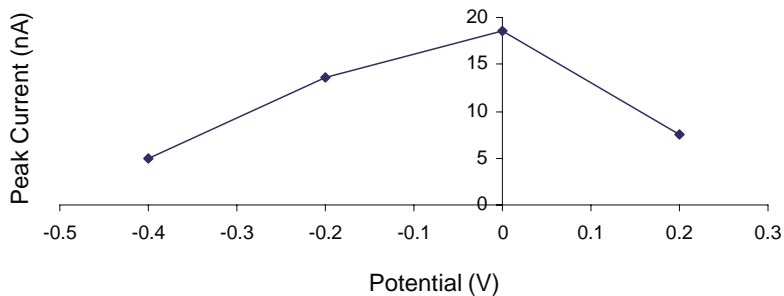

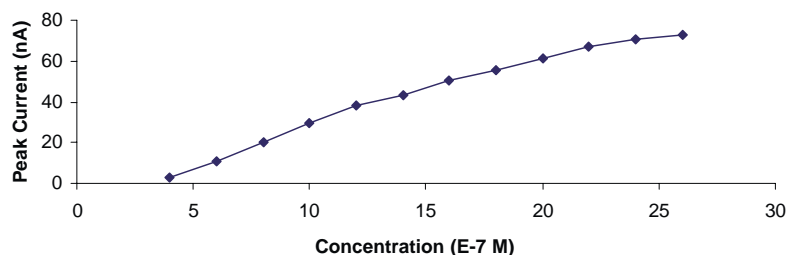Fig. 5. Effect of accumulation potential on AdSV peak current.

Fig. 6. Effect of drug concentration on AdSV peak current.

the saturation of the adsorption sites on HMDE. Fig. 7 demonstrate the variation in peak height at higher concentration with the continuous addition of the analyzed drug. These stripping voltammograms exhibit the level off stage for the peak height at higher concentration as can be deduced from the decline of size of the leap in the peak height at high concentrations. In view of that, determination of the drug in high concentration samples like pharmaceutical preparations is possible only by using the calibration method after previous dilution of the test sample solution. Drug concentration of 8×10^{-7} mol l^{-1} was found to be adequate for succeeding practical investigations.

4.4. Effect of scan rate

The cathodic peak current of Cephalothin was found to be directly proportional to the scan rate, particu-

larly at low scan rate values, a phenomenon characterized for adsorbed materials [26]. When the AdSV peak current of 5×10^{-7} mol l^{-1} Cephalothin in pH 11 Britton–Robinson buffer was measured over the range 2–30 mV^{-1}, it was found that peak height was linearly dependent on the scan rate up to15 mV^{-1}. However, after this maximum value the peak current start to decrease slightly with faster scan rates. Accordingly, 15 mV^{-1} scan rate value was adopted as optimum condition for further investigations.

4.5. Effect of instrumental parameters

The monitored AdSV peak height can be further maximized by optimizing other experimental factors that can affect the adsorption process of the analyzed drug. The influence of both the surface size of the mercury drop working electrode and electrode convection
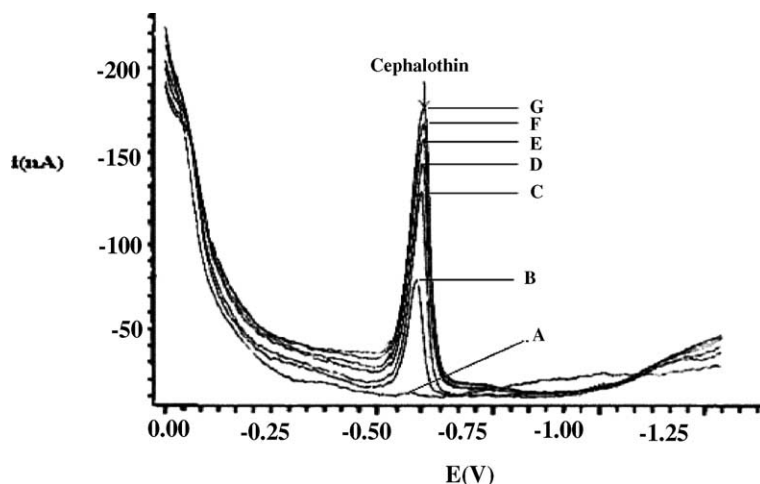

Fig. 7. AdSV voltammograms for Cephalothin in Britton–Robinson buffer (pH 11). (A) blank, (B) 1×10^{-6} Cephalothin mol l^{-1}, (C) 2×10^{-6} Cephalothin mol l^{-1}, (D) 3×10^{-6} Cephalothin mol l^{-1}, (E) 4×10^{-6} Cephalothin mol l^{-1}, (F) 5×10^{-6} Cephalothin mol l^{-1}, (G) 6×10^{-6} Cephalothin mol l^{-1}.

rate was also evaluated. An increase in the surface of the working electrode (over $0.15–60\,mm^2$) or in the stirring rate (rasing it from 0 to 3000 rpm) yielded, as expected, a linear enhancement in the analytical signal and did not affect the value of the stripping voltammetric potential. Thus, for optimal sensitivity, $60\,mm^2$ drop size and 3000 rpm stirring speed were chosen for subsequent practical works.

5. Analytical performance (method validation)

Once the most ideal and suitable chemical conditions and instrumental parameters for the adsorptive determination were established, a calibration plot for the analyzed drug was recorded to estimate the analytical characteristics of the developed method.

5.1. Calibration graph

Under the optimum conditions a very good linear correlation was obtained between the monitored voltammetric peak current and Cephalothin concentration in the range 4×10^{-7} to 12×0^{-7} M. Least-square treatment of the calibration graph yielded the following regression equation:

$$i_p\,(A) = -1.56 \times 10^{-8} + 0.045C\,(mol\,l^{-1})$$
$$r = 0.9995 \quad n = 5$$

where i_p is the adsorptive stripping peak current, C the analysed drug concentration and r the correlation coefficient.

5.2. Detection limit

The lowest detectable concentration of this drug was $3.3 \times 10^{-9}\,mol\,l^{-1}$ ($1.38\,ng\,ml^{-1}$), which was estimated based on the signal-to-noise ratio (S/N = 3) of the response of $1 \times 10^{-7}\,mol\,l^{-1}$ Cephalothin. Such remarkable enhancement for the sensitivity, clearly demonstrates the superiority of this electroanalysis approach over conventional spectrophotometric method which only succeeded to achieve $0.29\,\mu g\,ml^{-1}$ [27] or $1.0\,\mu g\,ml^{-1}$ [13] concentration levels.

5.3. Reproducibility

The high sensitivity of adsorptive voltammetry is accompanied by very good reproducibility. This

analytical performance was evaluated from 10 repeated measurements of electrochemical signal of $5 \times 10^7\,mol\,l^{-1}$ Cephalotin solution. The precision of the electrochemical developed method in terms of the relative standard deviation (R.S.D.%) was 0.94%, which was once again preferable than its spectrophotometric counterpart analytical method which yielded 1.7% R.S.D. [13].

5.4. Accuracy

The accuracy of the proposed method was checked by calculating the recovery of known amount of Cephalothin ($7.5 \times 10^{-7}\,mol\,l^{-1}$) added to Britton–Robinson buffer solution and analyzed via the optimized stripping voltammetric procedure. The value of the recovery obtained by the standard addition method was 101%.

5.5. Stability

Under the optimum conditions, the stability of $5 \times 10^{-7}\,mol\,l^{-1}$ Cephalothin solution was evaluated by monitoring the changes in the height of AdSV peak over a period of 80 min. The electroanalytical signal was gradually decreased with time. The basic media (pH 11) of the Britton–Robinson electrolyte solution probably initiated a slow degradation process for the antibiotic drug.

6. Interference studies

In order to evaluate the selectivity of the developed AdSV procedure, the influence of various interferences was examined. Considerable interference can be caused by co-existing surface-active compounds capable of competing with the analyte of interest for the adsorption site on the electrode surface, resulting in decreased peak height. Triton X-100 non-ionic surfactant was used as a model compound for surface-active materials. The stripping voltammetric signal was decreased gradually by additions of Triton X-100 over the range 1–4 mg/100 ml and the degree of Cephalothin peak current damping reached 60% depression of the monitored signal. Apparently, this inhibition effect was caused by the working electrode surface blockage due to adsorption of Triton X-100

surfactant. In contrast, this surfactant has practically negligible influence on the adsorptive stripping peak shape and potential.

Furthermore, in view of the fact that in some clinical circumstances a group of antibiotic drugs might be administrated to sick patient suffering serious infections, hence, the selectivity of the proposed method was evaluated in the presence of other cephalosporin antibiotic medicines. The competitive effect of these related cephalosporin antibiotic drugs was modulated by addition of Cephaloridine (its structure is similar to Cephalothin and it have a CH_2R electroactive group) to the test solution. The presence of 1×10^{-6} and 5×10^{-6} mol l^{-1} Cephaloridine in the test solution containing 5×10^{-7} mol l^{-1} Cephalothin, caused the AdSV peak current to increase by about 16 and 115%, respectively, of its original peak current. In contrast, the addition of electro-inactive Cefadroxil drug, which lacks the CH_2R group, caused virtually no effect for the voltammetric signal at relatively low interfering concentrations in the range $5–10 \times 10^{-7}$ mol l^{-1}. However, when adding higher Cefadroxil concentration (5×10^{-6} mol l^{-1}) the AdSV response slightly increased by nearly 8%.

7. Practical applications

The reliability of the proposed AdSV method for the determination of Cephalothin was investigated by assaying this drug in some real samples. Following the developed electroanalytical procedure described above, Cephalothin was analysed in a synthetic injection solution. Five aliquots of the test sample were diluted to the optimal concentration level and determined via the standard additions approach. The results obtained by this recovery test for Cephalothin have a recovery mean of 101.3% with standard deviation of ±0.92%. In addition, the applicability of the AdSV procedure for the analysis of Cephalothin in biological samples was also evaluated by estimating its recovery from spiked human urine and serum samples. A simple and fast pretreatment (clean-up) procedure, which is in fact a slight modification of the sample preparation method develop for the determination of some antagonist drugs [28] was used. By adding a small amount of 5% $ZnSO_4$ solution and ethanol to the urine or serum samples and centrifuging the mixture, most of the in-

terfering substances (mainly proteins) were simply removed and eliminated by precipitation. As a result, the recoveries obtained for urine and serum samples spiked with 8×10^{-7} mol l^{-1} Cephalothin concentration were 102 and 98%, respectively.

Acknowledgements

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