Adsorptive Cathodic Stripping Voltammetric Determination of Theophylline at a Hanging Mercury Drop Electrode

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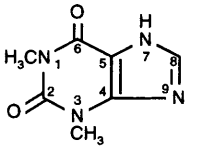
Adsorptive stripping voltammetry was used for the determination of trace amounts of theophylline. Theophylline in pH 7.5 Britton–Robinson buffer gives an adsorptive stripping voltammetric peak at a hanging mercury drop electrode at −0.38 V at accumulation potential 0.0 V *versus* Ag–AgCl. The optimum conditions of pH, accumulation potential and accumulation time were studied using differential-pulse voltammetry. The calibration graph for the determination of theophylline was linear in the range 0.6–3.6 μg l^{-1} with a relative standard deviation of 2%. The detection limit was 0.6 μg l^{-1} after 180 s accumulation at 0.0 V. The effect of some purine compounds and metal ions on the peak height of theophylline was studied. The presence of Cu^{II} ions enhanced the theophylline peak and the behaviour of the theophylline–copper complex was investigated. The method was applied to the determination of the drug in commercially available dosage forms. A comparison of the proposed method with previously reported methods was also made.

Keywords: Theophylline determination; adsorptive cathodic stripping voltammetry; hanging mercury drop electrode

Introduction

Theophylline (1,3-dimethyl-1*H*-purine-2,6-dione) has been used in the treatment of asthma as an alternative to prophylatic drugs[1] and acts as a stimulator of the central nervous system. Several methods for the determination of theophylline in biological samples have been reported, including non-aqueous titrimetry,[2] spectrophotometry,[3] colorimetry[4] and chromatographic methods.[5–10] A few studies have been reported on the electrochemical behaviour of theophylline.[11,12] Palecek *et al.*[11] found that theophylline did not yield a cathodic stripping voltammetric peak at the hanging mercury drop electrode (HMDE) in borate buffer solution. Wang *et al.*[12] studied the differential-pulse polarographic behaviour of theophylline and its reduction on a dropping mercury electrode in the presence of copper(II)–tartrate solutions.



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Differential-pulse cathodic stripping voltammetry (DPCSV) is a very sensitive method for the determination of substances that can be accumulated and then reduced at the electrode surface.

Several studies have been reported for the determination of some pharmaceutical compounds based on the adsorption of their copper complexes on an HMDE.[13–15] In this work, the optimum conditions for the CSV determination of theophylline in pure sample and tablets were investigated. The electrochemical behaviour of the copper–theophylline complex was also investigated.

Experimental

Apparatus

Adsorptive DPCSV was carried out using a Metrohm E506 Polarecord with a Model 663 VA stand with a multi-mode electrode operated in the HMDE mode. The three-electrode system was completed using a glassy carbon auxiliary electrode and an Ag–AgCl reference electrode. Polarographic measurements were carried out using a Metrohm E505 stand with an E506 Polarecord. A pulse amplitude of 50 mV was generally used with a scan rate of 4 mV s^{-1} and a pulse interval of 1 s. pH measurements were made with a Metrohm 632 pH meter. Cyclic voltammetry was carried out using a Metrohm E611 VA-detector and E612 scanner connected to a Houston *X–Y* recorder.

Reagents

Theophylline was obtained from Boehringer through a local pharmaceutical company (Julphar, United Arab Emirates) and a solution was prepared freshly every 3 d. The supporting electrolyte was a Britton–Robinson (BR) buffer solution. All solutions were prepared from doubly distilled water and analytical-reagent grade chemicals (BDH and Merck).

Procedure

The general procedure for adsorptive DPCSV was as follows. A 14 ml aliquot of BR buffer solution was placed in a voltammetric cell and deoxygenated with nitrogen for 10 min (and for an additional 2 min before each adsorptive stripping cycle). The accumulation potential (usually 0.0 V) was applied to the working electrode for an accumulation time of 60 s while the solution was stirred. The stirring was then stopped and after a 15 s rest period a negative-going differential-pulse scan was initiated between 0.0 and −0.7 V. The operational parameters of the scan were scan rate 4 mV s^{-1}, pulse amplitude 50 mV and the adsorptive stripping cycle was repeated using a new drop. After background

stripping voltammograms had been obtained, aliquots of the required amounts of standard theophylline solution were added.

Analysis of Tablets

The theophylline drug was commercially available as Nuelin (Riker, Loughborough, UK) and Lasma (Pharmax Ltd., Bexley, Kent, UK). Nuelin has an average mass of 493 mg per tablet and is claimed to contain 250 mg of theophylline per tablet. The average mass of Lasma tablets is 776 mg per tablet and are claimed to contain 300 mg of theophylline per tablet.

Ten tablets each of Nuelin and Lasma were weighed and finally powdered separately. An accurately weighed 178 mg amount of Nuelin was transferred quantitatively into a 500 ml calibrated flask, 200 ml of doubly distilled water were added and the mixture was shaken well for 90 min, then diluted to volume with doubly distilled water. A 5 ml volume of the supernatant liquid was diluted to 100 ml with doubly distilled water. A 5 ml volume of this solution was transferred into the voltammetric cell containing de-aerated BR buffer (pH 7.5). The voltammograms were recorded under the optimum experimental conditions. An accurately weighed 233 mg amount of Lasma was treated in the same way as Nuelin.

Results and Discussion

The variations of the peak current and potential of the theophylline peak as a function of pH and constituents of the buffer solution were measured by DPCSV. The response was examined in the presence of different buffer solutions, *e.g.*, borate, carbonate, phosphate and BR. BR buffer was selected as the optimum because it gave the highest response and good reproducibility (Table 1).

The DPCSV response was compared with that obtained in differential-pulse polarographic (DPP) measurements. A significant peak-current enhancement was obtained with the DPCSV method. Therefore, the possibility of increasing the sensitivity of determination of theophylline using its adsorptive accumulation on the surface of the HMDE was investigated.

The adsorptive stripping response of theophylline is strongly dependent on the pH of the solution. No adsorptive stripping signal was observed at pH <4.0 and a gradual increase in current was observed when the solution pH was increased from 4.0 to 7.5. A sharp decrease in the peak response was observed at pH >10.5 (Table 2), hence pH 7.5 was selected in subsequent work.

Table 1 Effect of buffer constituents on the height and potential of the theophylline peak. Conditions: pH, 7.5; accumulation time, 60 s; accumulation potential, 0.0 V; theophylline concentration, 5.0×10^{-6} mol l^{-1}

Buffer (pH 7.5)	Peak current/nA	Peak potential/V
Carbonate	6.7	−0.48
Borate	5.2	−0.31
Phosphate	1.1	−0.22
Britton–Robinson	8.8	−0.38

Table 2 Effect of pH on the height of the theophylline peak in BR buffer solutions. Other conditions as in Table 1

pH	Current/nA	pH	Current/nA
4.0	2.4	8.0	7.8
5.0	4.3	8.5	6.8
6.5	5.5	9.5	4.9
7.0	5.7	10.5	0.7
7.5	8.8		

The effect of accumulation potential on the peak current for different concentrations of theophylline was studied. The dependence of the stripping peak current on the accumulation potential was examined over the range 0.0 to −0.4 V. At potentials more negative than −0.2 V the peak current decreased. An accumulation potential of 0.0 V offered the best signal-to-background characteristics and was used in all subsequent work.

The variation of peak current with accumulation time for 2.4 and 24 μg l^{-1} theophylline solution was investigated. A rectilinear relationship up to accumulation times of about 120 and 60 s were obtained, respectively. Above this time, saturation of the mercury drop was observed. Hence the choice of the accumulation time depends on the range of analyte concentration being determined.

Cyclic voltammetric studies showed that the height of the theophylline peak was directly proportional to scan rate within the range 10–100 mV s^{-1} and the peak potential shifted linearly to more negative potential when the scan rate was increased, indicating that the reduction is that of an adsorbed species.[16] The DPASV peak current is linearly related to the pulse amplitude between 20 and 100 mV; a value of 50 mV was chosen as optimum as there is a decrease in resolution at higher values.

The peak height of theophylline is linearly dependent on the theophylline concentration. A linear calibration graph from 0.6 to 3.6 μg l^{-1} was obtained after a 120 s accumulation time at 0.0 V accumulation potential, pulse amplitude 50 mV, scan rate 4 mV s^{-1} and pH 7.5. The reproducibility of the method was checked by measurements on 20 2.4 μg l^{-1} theophylline solutions. A relative standard deviation (s_r) of 2% was obtained. The limit of detection was calculated to be 0.6 μg l^{-1} with an accumulation time of 180 s at an accumulation potential of 0.0 V, scan rate 4 mV s^{-1}, pulse amplitude 50 mV and pH 7.5. The sensitivity of the proposed method was compared with those of previously reported methods and the results are summarized in Table 3.

Electrochemical Behaviour of Theophylline in the Presence of Copper(II)

The effect of the presence of Cu^{II} ions, which might affect the stripping peak of theophylline, was investigated. It was found that the addition of micro-amounts of Cu^{II} ions to theophylline solution enhanced its stripping peak, as shown in Fig. 1.

The effect of the accumulation potential on the peak height of the theophylline–copper complex was investigated at potentials between 0.0 and −0.4 V. The peak height decreased when accumulation potentials more negative than

Table 3 Comparison of methods for the determination of theophylline

Method	Detection limit/ μg l^{-1}	Reference
Liquid chromatography	0.75	19
Enzyme immunoassay	1	20
Flow injection immunoassay	7.02	21
Spectrophotometry and thermal lens spectrometry	0.2	22
HPLC (electrochemical detection)	0.2	10
GLC	10	23
HPLC	0.10	24
Automated fluoroimmunoassay with HPLC	<1	25
Spectrophotometry	1	4
DPCSV	0.6	This work
DPCSV	0.012 (in presence of Cu^{II} ions)	This work

−0.2 V were used. The accumulation was usually made at 0.0 V.

The peak current of the complex increased with increasing concentrations of both theophylline and copper. At higher concentrations of copper (1.0×10^{-5} mol l^{-1}), the peak height decreased markedly and a new peak for free copper was obtained at −0.18 V. Hence a Cu^{II} concentration of 3.0×10^{-7} mol l^{-1} was selected in subsequent work.

The signal enhancement associated with the adsorptive preconcentration of the theophylline–copper complex results in lowering of the detection limit for theophylline determination to 0.012 μg l^{-1} compared with that obtained in the absence of Cu^{II} ions (0.6 μg l^{-1}).

To verify the adsorptive behaviour of the theophylline–Cu^{II} complex at the electrode, electrocapillary curves were measured at a DME. The electrocapillary curve of a solution containing theophylline and Cu^{II} ions was similar to that containing theophylline itself. This behaviour supports the postulate of adsorption of both the theophylline complex and theophylline itself at the electrode.

The cyclic voltammetric behaviour of 1.0×10^{-5} mol l^{-1} theophylline at the HMDE at pH 7.5 is shown in Fig. 2(*a*). The ratio $I_{cathodic}:I_{anodic}$ is close to 1, confirming the reversibility of the reduction. The cathodic peak height increased with increasing scan rate, confirming the adsorptive

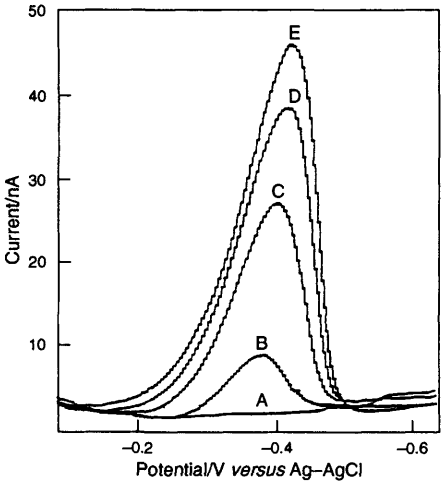

Fig. 1. Differential-pulse adsorptive stripping voltammogram obtained after accumulation for 60 s at 0.0 V in BR buffer (pH 7.5) containing (A) background; (B) 5.0×10^{-6} mol l^{-1} theophylline; (C) as (B) + 1.0×10^{-7} mol l^{-1} Cu^{2+}; (D) as (B) + 2.0×10^{-7} mol l^{-1} Cu^{2+}; and (e) as (B) + 3.0×10^{-7} mol l^{-1} Cu^{2+}.

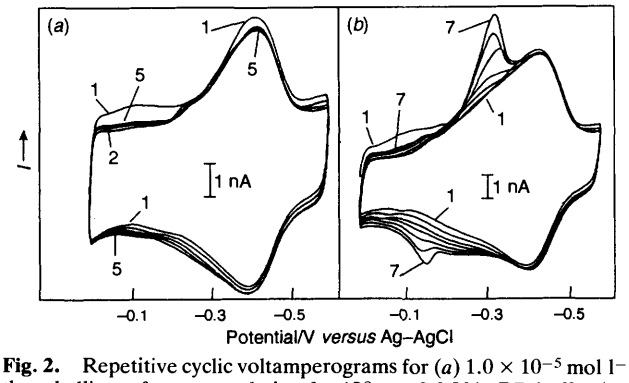

Fig. 2. Repetitive cyclic voltamperograms for (*a*) 1.0×10^{-5} mol l^{-1} theophylline, after accumulation for 120 s at 0.0 V in BR buffer (pH 7.5), and (*b*) 1.0×10^{-5} mol l^{-1} theophylline + 1.0×10^{-6} mol l^{-1} Cu^{2+} after accumulation for 60 s at 0.0 v.

behaviour of theophylline. As the theophylline concentration increased (>1.0×10^{-5} mol l^{-1}), the irreversibility of the electrode reaction might increase, which is indicated by the greater deviation of the $I_c:I_a$ ratio from unity.[17] On the other hand, the cyclic voltammogram of a 1.0×10^{-5} mol l^{-1} solution of theophylline in the presence of 1.0×10^{-6} mol l^{-1} copper(II) solution obtained by successive scans with the same drop are shown in Fig. 2(*b*). Subsequent repetitive scans yielded a peak in the cathodic scan at −0.28 V, which may be owing to the reduction of Cu^{II}–theophylline complex produced at the electrode surface. The peak height increased on increasing the scan number as a result of the increasing amount of copper amalgam produced during the scan at more negative potentials.[18] Another anodic peak at −0.15 V [Fig. 2(*b*)] was obtained owing to the oxidation of amalgamated copper to Cu^{II}–theophylline complex.

Interferences

Possible interferences in the determination of 2×10^{-6} mol l^{-1} theophylline by the proposed method were investigated. Guaphenisin (glyceryl guaiacolate) is used as an expectorant and it is used in combination with theophylline in some pharmaceutical preparations.[7] Theophylline could be determined with no significant interference from 1×10^{-5} mol l^{-1} guaiphenesin. No peak was observed for 1×10^{-5} mol l^{-1} guaiphenesin in BR buffer (pH 7.5) with accumulation for 60 s at a 0.0 V accumulation potential. Serious interference was observed on addition of aminophylline, which enhanced the theophylline peak markedly owing to the formation of a reduction peak at the same potential as for theophylline. No interference was observed on addition of 2.0×10^{-6} mol l^{-1} xanthine, 1,7-dimethylxanthine or 1,3-dimethyluric acid. Hence the determination of theophylline in the presence of paraxanthine (1,7-dimethylxanthine) is possible, whereas the separation of theophylline from paraxanthine, the main metabolite of caffeine, remains the most serious interference in any LC assay.[26] The effects of the presence of some purine derivatives on the peak height of theophylline are summarized in Table 4.

Other metal ions can interfere in the determination of theophylline because they form reducible and adsorbable chelates with the reagent. Measurement of 1.0×10^{-6} mol l^{-1} theophylline was not affected by the addition of 1.0×10^{-6} mol l^{-1} Zn^{II}, Co^{II}, Cd^{II}, Au^{III}, and Pb^{II}; 50% and 90% depressions of the peak height were observed after addition of 1.0×10^{-4} mol l^{-1} La^{III} and Au^{III}, respectively. Addition of 1.0×10^{-6} mol l^{-1} Mn^{II}, Ni^{II} and Ag^{I} yielded a 10% enhancement of the peak height.

Table 4 Recovery of theophylline (2.0×10^{-6} mol l^{-1}) in the presence of some purine derivatives at concentrations of 5.0×10^{-7} and 2.0×10^{-6} mol l^{-1}. Conditions as in Table 1

	Effect on recovery (%)	
Purine	5.0×10^{-7} mol l^{-1}	2.0×10^{-6} mol l^{-1}
Guanine	+20	+70
1-Methylguanine	+30	+110
3-Methylguanine	+15	+80
7-Methylguanine	+15	+10
9-Methylguanine	+12	+25
Xanthine	NE*	NE
7-Methylxanthine	−10	−20
1,7-Dimethylxanthine	NE	NE
6-Chloropurine	+8	+8
1,3-Dimethyluric acid	NE	NE
Dimenhydrinate	−20	−15

* NE = no effect.

Application to Analysis of Formulations

The validity of the proposed method for the determination of theophylline in pharmaceutical preparations was investigated by assaying Nuelin (labelled to contain 250 mg per tablet of theophylline) and Lasma (labelled to contain 300 mg per tablet of theophylline). Ten analyses of five different samples of each drug gave a mean recovery of 99.5% for Nuelin (range 97.5–102.5% with s_r = 3%) and 100.8% for Lasma (range 97.5–104% with s_r = 4.2%).

Conclusion

Adsorptive DPCSV is a useful technique for the determination of theophylline with no interferences from paraxanthine. The sensitivity and accuracy of the method allow its use for the determination of theophylline in pharmaceutical formulations using relatively short accumulation times and inexpensive instrumentation.

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Paper 4/00113C,
Received January 10, 1994
Accepted April 5, 1994