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Determination of azathioprine and rocuronium in biological fluid samples by voltammetry

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ABSTRACT

Cyclic voltammetry and differential pulse voltammetry methods were used to determine Azathioprine (AZP) and Rocuronium (RCR) in biological fluid samples. To enhance the sensitivity of the proposed method, a clay modified carbon paste electrode (CMCPE) was used which showed increased peak currents compared to bare carbon paste electrode (CPE). The linearity was observed in the concentration ranges of 2.2×10^{-9} to 1.2×10^{-5} M (AZP) and 0.2×10^{-9} to 1.2×10^{-5} (RCR) with detection limits of 2.1×10^{-9} M (AZP) and 0.4×10^{-9} M (RCR). From the experimental results, it is found that the proposed method exhibited good repeatability and reproducibility. The present method has been successfully applied for the determination of AZP and RCR in spiked human serum samples and urine samples.

Key words: Cyclic voltammetry , differential pulse voltammetry, Azathioprine, Rocuronium, Clay modified carbon paste electrode, serum samples and urine samples.

INTRODUCTION

Azathioprine [6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)sulfanyl]-7*H*-purine] (AZP) is a 6-mercaptopurine derivative used as an immunosuppressive agent for prevention of transplant rejection and for the treatment of rheumatoid arthritis and various autoimmune diseases.

Rocuronium [[3-hydroxy-10,13-dimethyl-2-morpholin-4-yl-16- (1-prop-2-enyl-2,3,4,5-tetrahydropyrrol-1-yl)-2,3,4,5,6,7,8,9,11,12, 14,15,16,17-tetradecahydro- 1*H*-cyclopenta[a] phenanthren-17-yl] acetate] (RCR) is a non-depolarizing agent neuromuscular blocking agent used as an adjunct in general anaesthesia and as skeletal muscle relaxant during surgery.

AZP was determined by 1H NMR spectroscopy[1], spectrophotometric[2] and chromatographic[3-6] methods. RAN was determined by HPLC[20], GC-MS[22] and LC[23] methods which are expensive and time consuming. In the present work, a cheaper and selective voltammetric determination of AZP and RCR has been reported.

EXPERIMENTAL

Voltammograms were recorded with Metrohm 757 VA computrace (Herisau, Switzerland). AZP and RCR were purchased from Sigma. Graphite powder (l-2 mm particle size), paraffin oil and Clay from Aldrich India Ltd., Bangalore. All chemicals used for the preparation of buffers and supporting electrolytes are of reagent grade.

Recommended Procedure

A suitable amount of analyte is transferred into the electrolytic cell containing Britton-Robinson buffer solution. To remove oxygen, the solution is purged with nitrogen gas for 10 min. The voltammograms are recorded after each aliquot of the standard solution is added.

RESULTS AND DISCUSSION

Cyclic voltammetry

Figs. 1 and 2 illustrate the cyclic voltammograms recorded for AZPand RCR at carbon paste (CPE) and clay modified carbon paste electrode (CMCPE). On scanning towards a negative potential on a bare carbon paste electrode, only a much smaller cathodic peak is observed. When CMCPE is used a large increase in the peak currents is observed. No peaks are observed in the anodic sweep indicating that the reduction of AZP and RCR is of irreversible process.

Differential Pulse Voltammetry

Figs. 3 and 4 illustrate differential pulse voltammograms obtained at bare carbon paste electrode and clay modified carbon paste electrodes

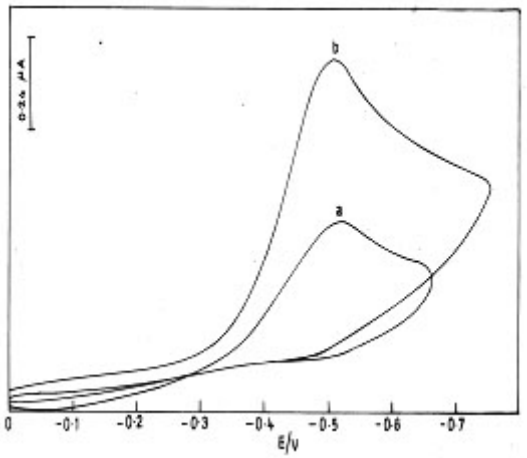


Fig. 1: Typical CV of 1.2 x 10^{-7} M AZP at (a) bare CPE (b) CMCPE

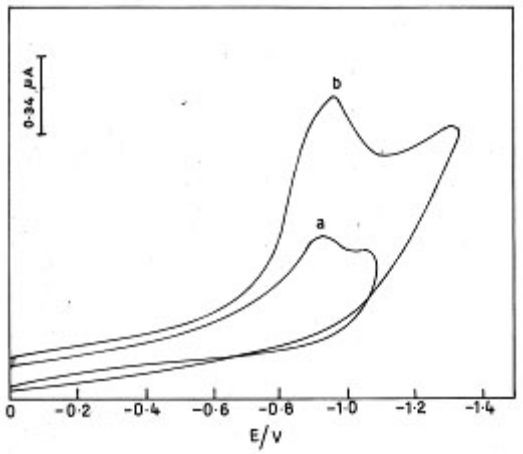


Fig. 2: Typical CV of 3.5×l0^{-6} M RCR at (a) bare CPE; (b) CMCPE

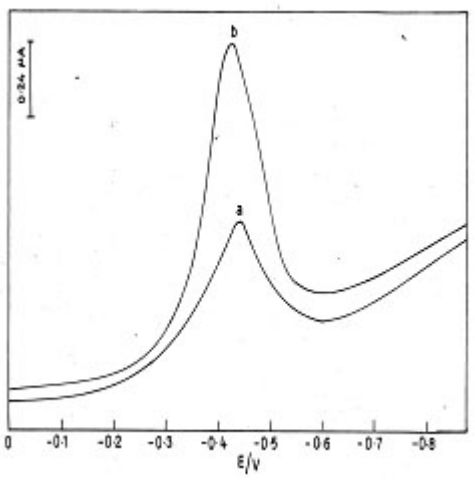


Fig. 3:Typical DPAdSV of 1.4×10^{-9} M AZP at (a) bare CPE (b) CMCPE

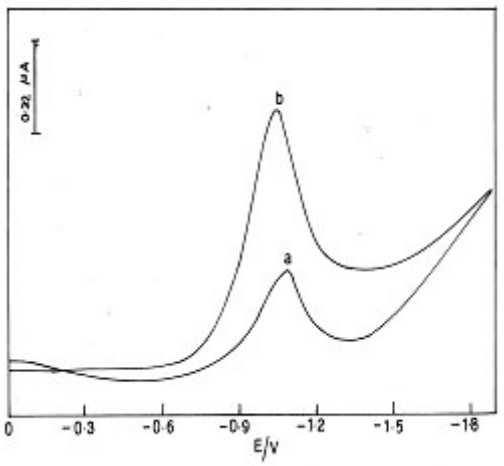
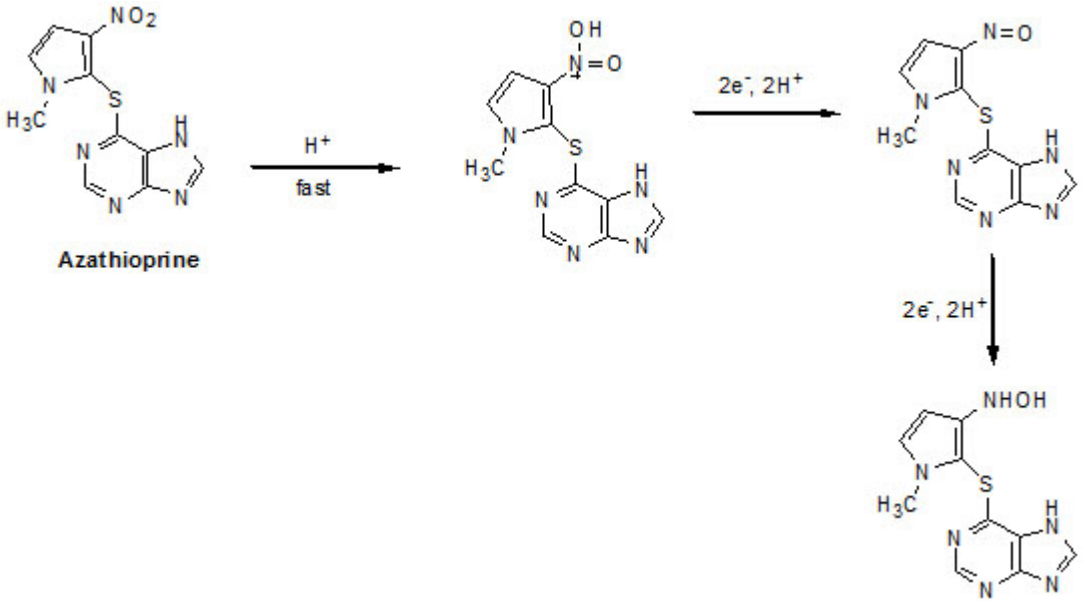


Fig. 4: Typical DPAdSV of 1.6×10^{-9} M RCR (a) bare CPE; (b) CMCPE

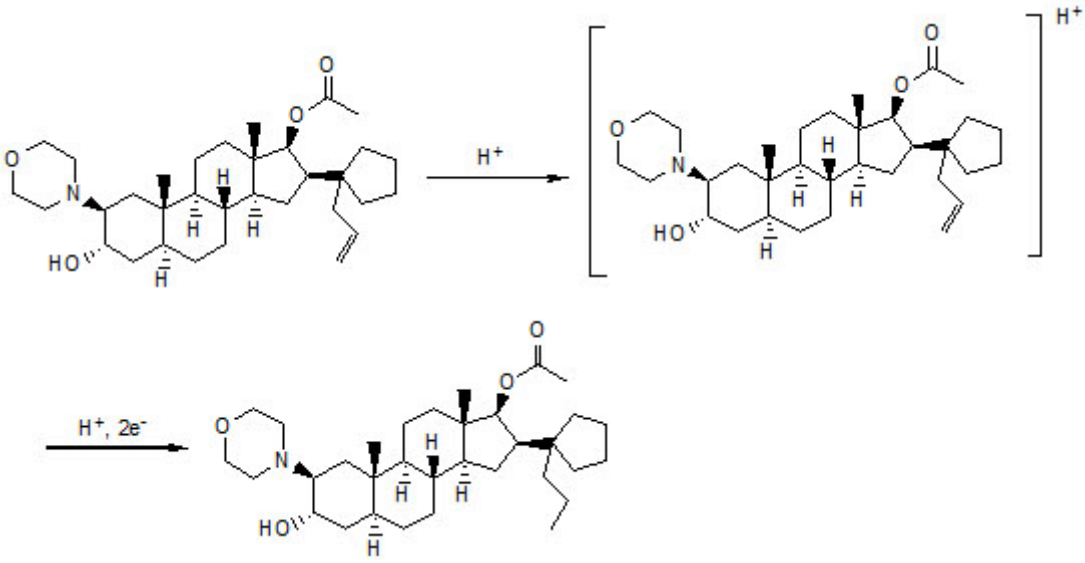
of AZP and RCR in BR buffer. From the obtained results the peak current obtained at clay modified carbon paste electrode are almost twice than those at carbon paste electrode of AZP and RCR.

This peak in the voltammogram of AZP is attributed to the four electron reduction of nitro group to the corresponding hydroxylamine group according to the currently accepted mechanism for electroreduction of nitro compounds[82-84].

The peak in the voltammogram of RCR is attributed to the reduction of carbon, carbon double bond according to the currently accepted mechanism for the electroreduction of carbon, carbon double bond containing compounds[25,26].



Scheme 1: Reduction mechanism of Azathioprine



Scheme 2: Reduction mechanism of Rocuronium

Table 1: Chosen Experimental Conditions

Variables	Chosen Value	
	AZP	RCR
pH	8.5	3.0
Buffer volume (ml)	10	10
Accumulation potential (V)	- 0.16	-0.4
Accumulation time (s)	160	150
Rest time (s)	15	25
Stirring rate (rpm)	2000	2000
Scan rate (mVs^{-1})	10	10
Pulse amplitude (mV)	50	50

Table 2: Experimental data of AZP and RCR

Parameters	AZP		RCR	
	CPE	CMCPE	CPE	CMCPE
Linearity range (M)	2.0×10^{-8} to 3.0×10^{-7}	2.2×10^{-9} to 1.2×10^{-5}	1.2×10^{-8} to 1.0×10^{-5}	0.2×10^{-9} to 1.2×10^{-5}
Calibration curve equation	Y(µA)=0.30214 ×+0.03535	Y(µA)=0.2985X+ 0.0442	Y(µA)=0.9815 ×+0.1139	Y(µA)= 0.9865 ×+0.1063
Correlation coefficient	0.9971	0.9995	0.9865	0.9815
L.O.D (M)	1.5×10^{-8}	2.1×10^{-9}	1.4×10^{-8}	0.4×10^{-9}
L.O.Q (M)	0.5×10^{-7}	0.667×10^{-8}	0.466×10^{-7}	0.133×10^{-8}
Repeatability of peak currents%RSD)	4.28	4.86	4.29	4.32
Repeatability of Peak potentials %RSD)	0.32	0.38	0.54	0.59
Reproducibility of peak currents %RSD)	3.92	4.02	4.91	4.98
Reproducibility of potentials%RSD)	0.51	0.53	0.32	0.38
Numbers of assays	12	12	12	12

Table 3: Determination of AZP and RCR in spiked human serum samples

Name of the drug	Amount labelld (µ .g/L)	*Average amount found (µ.g/L)	Recovery percentage (%)	±S.D	RSD
AZP	2	1.961	98.0	0.0200	1.02
	4	3.93	98.25	0.0461	1.173
	6	5.956	99.26	0.0208	0.3**49**
RCR	4	3.923	98.07	0.0513	1.307
	8	7.93	99.125	0.0655	0.825
	12	11.9167	99.30	0.0611	0.5127

* Each value is an average of three determinations

Table 4: Determination of AZP and RCR in spiked human urine samples

Name of the drug	Amount spiked (µ.g/L)	*Average amount found (µ.g/L)	Recovery percentage (%)	±S.D	RSD
AZP	2	1.97	98.50	0.004	0.2030
	4	3.94	98.5	0.0624	1.583
	6	5.71	95.31	0.07	1.17
RCR	2	1.98	99.0	0.0206	1.040
	4	3.903	97.57	0.032	0.81
	6	5.72	95.33	0.0670	1.17

* Each value is an average of three determinations.

CONCLUSION

Clays are complex micro porous media with an appreciable surface area helped in increasing the sensitivity of the proposed method. Due to CMCPE, stability, accuracy and low expensive, it offers a good possibility as a substitute for the previous approaches used in routine analysis, such as colorimetric, spectrophotometric and chromatographic methods. Results obtained in the developed method shows that drugs can be determined accurately and reliably at a lower expense.

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