Differential Pulse Polarographic Assay of Azathioprine in Serum and Urine

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Abstract

The polarographic behaviour of imuran (azathioprine) have been studied , the drug give a well sensitive defined double peaks at about (-0.4V, -0.96V) in carbonate buffer solution at pH 7.4 and at (-0.54V, -1.07 V) in serum – carbonate buffer and at (-0.42V, - 1.03 V) in urine – carbonate buffer.

The lowest determined concentration was ($8.7x10^{-7}$ M) in serum-carbonate media and ($15.2x10^{-7}$ M) in urine-carbonate media. The method can be successfully applied to determination of azathioprine in vivo. The relation between concentration and diffusion current (I_p) was linear for all solutions.

الخلاصة

تم دراسة بعض الخواص البولاروكرافية لدواء الاميوران (الازوثايوبرين) حيث اعطى الدواء موجتي اختـزال قوية و حساسة عند -0.4 ، -0.96 فولت في محلول منتظم هو الكاربونات عند الرقم الهيدروجيني 7.4, وعند-0.54 ، -1.07 فولت بوجود مصل الدم مع الكاربونات, وعند -0.42, -1.03 فولت بوجود الإدرار مع الكاربونات.

إن اقل تركيز أمكن تحديده عند 8.7×10^{-7} مولاري في محلول الكاربونات مع مصل الـدم، وفـي محلـول الكاربونات مع الادرار هو 15.2×10^{-7} مولاري. وكانت العلاقة خطية بين تيار الانتشار والتركيز لكافة المحاليل في هذه الطريقة.

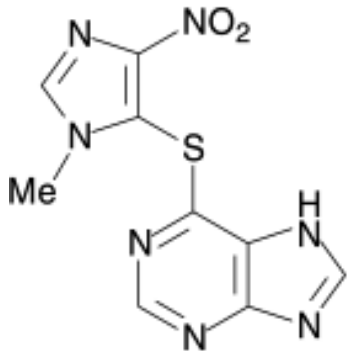
Introduction

The polarography and other voltammetric methods of analysis are successfully applied to the determination of pharmaceutical compounds (1-5). Azathioprine is an imidazole derivative of 6-mercaptopurine [6(5-methyl-3-nitromitazol-4-yl)] sulfonyl 7-purine](6). It is used as an immuonosuppr assant anti-metabolite, chiefly active against crohns disease and eraties calitio, it is converted in body to 6-mercaptopurine and 6-thioionsinic acid (7).

A number methods for the determination Azathioprine drug , Quantitative determination for this drug has been reported using 1HNMR spectroscopy (8). Spectrophotometric methods used for pharmaceutical formulation (9), while electrophoresis applied in bulk, commercial tablets and powder for injection (10),

The converted of drug in buddy to 6-mercaptopurine studied by polarography in 0.1 molar Britton-Robinson media pH4, the peak potential was found at -0.41 V vs SCE (11).

The electro chemical reduction studied, the peak reduction attributed to four electron Process (12). In the present paper and continouslly our interest in the field ,its worthwhile to determine this compound by polarographic technique. the structure of azathioprine is shown in I.



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Experimental

Apparatus:

A Metrohm polarecord model E506 polarographic analyser was used in the three electrode mode. A model PW 9420 pH meter (Philips) was also used.

Reagents:

All chemicals used were of analytical reagent grade. All solutions were prepared with deionized distilled water. The stock solution of (10^{-3} M) azathioprine was prepared by appropriate amount of azathioprine dissolved in a (25 ml) of pH (7.4) carbonate buffer solution (0.025 M) sodium bicarbonate and (0.025M) sodium carbonate.

Procedure:

Differential pulse mode was used with a (100 mV) pulse amplitude, a (2 s) drop time and (3 mV/s) scan rate, the

solution was de-aerated by passing a slow steam of purified (N_2) for (15 min) to remove the dissolved oxygen. For polarographic measurements appropriate amount of azathioprine stock solution was added to the pH (7.4) carbonate buffer solution to yield the desired concentration (calibration curve was then constructed). The same procedure was also followed in serum-arbonate and urine-carbonate media. In case of serum-carbonate 0.5 ml of normal serum was added to polarographic cell containing buffer (20 ml) at pH 7.4. In urine-carbonate media, 2 ml of normal urine was added to polarographic cell together with the carbonate buffer (10 ml) at pH 7.4.

Results and discussion

In this work the differential pulse polarographic behaviour of azathioprine was studied in different media containing carbonate buffer 3.2x 10^{-5} M in azathioprine aqueous buffer solution (carbonate-serum and carbonate-urine solution). Typical differential pulse polarograms of 3.2x10^{-5} M azathioprine recorded in the aqueous media is shown in Figure (1).

The peak potential E_p was found to be almost the same in the case of aqueous carbonate buffer (-0.405 V, -0.94 V vs. Ag/AgCl electrode) while in the case of serum-carbonate solution the peaks potential shifted to the more negative value (-0.54 V, -1.075 V vs. Ag/AgCl electrode); this is due to the presence of albumin in serum which acts as surfactant and causes damping in current, while the shift in potentials to more negative due to the interaction of the peaks with albumin peaks.

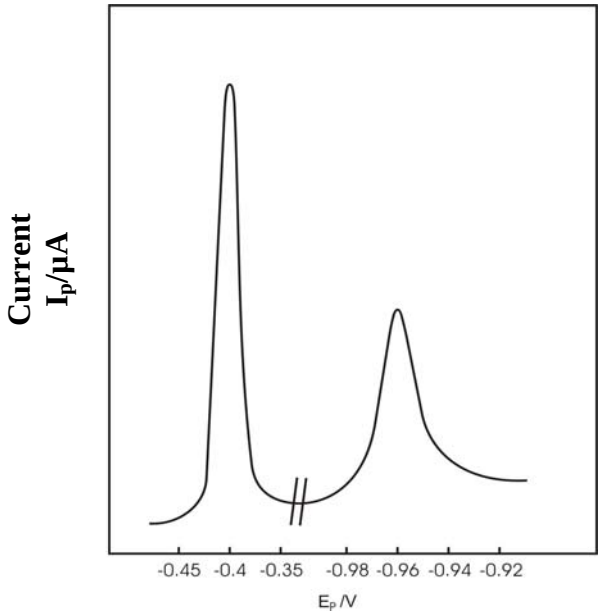


Fig. (1): Differential pulse polarogram of (3.2 x 10^{-5} M) azathioprine solution in carbonate buffer pH (7.4) in aqueous media.

Stability of azathioprine in aqueous-carbonate media pH (7.4) and serum-carbonate media pH (7.4).

The differential-pulse polarograms of (3.2 x 10^{-5} M) azathioprine solution pH (7.4) were recorded at different time for both aqueous-carbonate and serum-carbonate solution. It has been seen that azathioprine is stable for more than (100)

minutes. It has also been noticed that the value of the peak current in serum-carbonate medium is always less than that in aqueous-carbonate buffer because of albumin interaction with drug

Effect of buffers:

Different buffers at pH (7.4) were used, carbonate buffer gave the largest (I_p) as shown in Table (1), so it was chosen in the present study.

Table (1): Effect of buffer of ($3.2x10^{-5}$ M) on differential pulse peak at pH 7.4 in aqueous media

kind of Buffer		$Ipx10^{-2}$ (µA)	E_p (V)	$I_p x10^{-2}$ (µA)	E_p (V)
1	Phosphate.	17	-0.335	100	-0.93
2	Glycine.	87.5	-0.25	42	-0.76
3	B.R.B.*	160	-0.33	91	-0.49
4	Carbonate.	200	-0.4	36	-0.96

*Briton-Robinson buffer

Analytical consideration:

The degree of resolution and sensitivity of differential-pulse peak current are dependent on pH, drop time and pulse peak-amplitude. According to the differential pulse polarography of $3.2x10^{-5}$ M azathioprine investigated at various pulse amplitudes (20-100) mV, drop time (0.4-2) sec and pH values (2-11) using buffers. The largest drop time is 2.0 sec and the largest pulse amplitude is 100 mV are recommended for the procedure.

The effect of pH is summarized in Table 2, the results indicate that peak current (I_p) is dependent on pH, an intermediate pH value 7.4 has been chosen for the present study because it is quite similar to the blood pH value 7.4. It was found that the $E_{1/2}$ more to negative potential in the range (2.4-7.4), after that the $E_{1/2}$ was found next constant.

Table (2) : Effect of pH on the differential pulse peak current I_p at $3.2x10^{-7}$ M.

pH	I_p (10^{-2} µA)	E_p (V)	I_p (10^{-2} µA)	E_p (V)
2.4	267	-0.115	------	------
3.4	315	-0.35	120	-0.975
4.4	345	-0.3	172	-0.98
5.4	375	-0.33	223	-0.98
6.4	397	-0.355	224	-0.985
7.4	382	-0.4	225	-0.96
8.4	385	-0.4	203	-0.99
9.4	387	-0.405	162	-0.99
10.4	400	-0.405	167	-1.055

Using the above optimum conditions, the calibration curves were constructed using a serial dilution of a standard azathioprine in aqueous-carbonate buffer Serum-carbonate buffer and urine-carbonate buffer (pH 7.4). The results are listed in Tables (3), (4), (5,) .

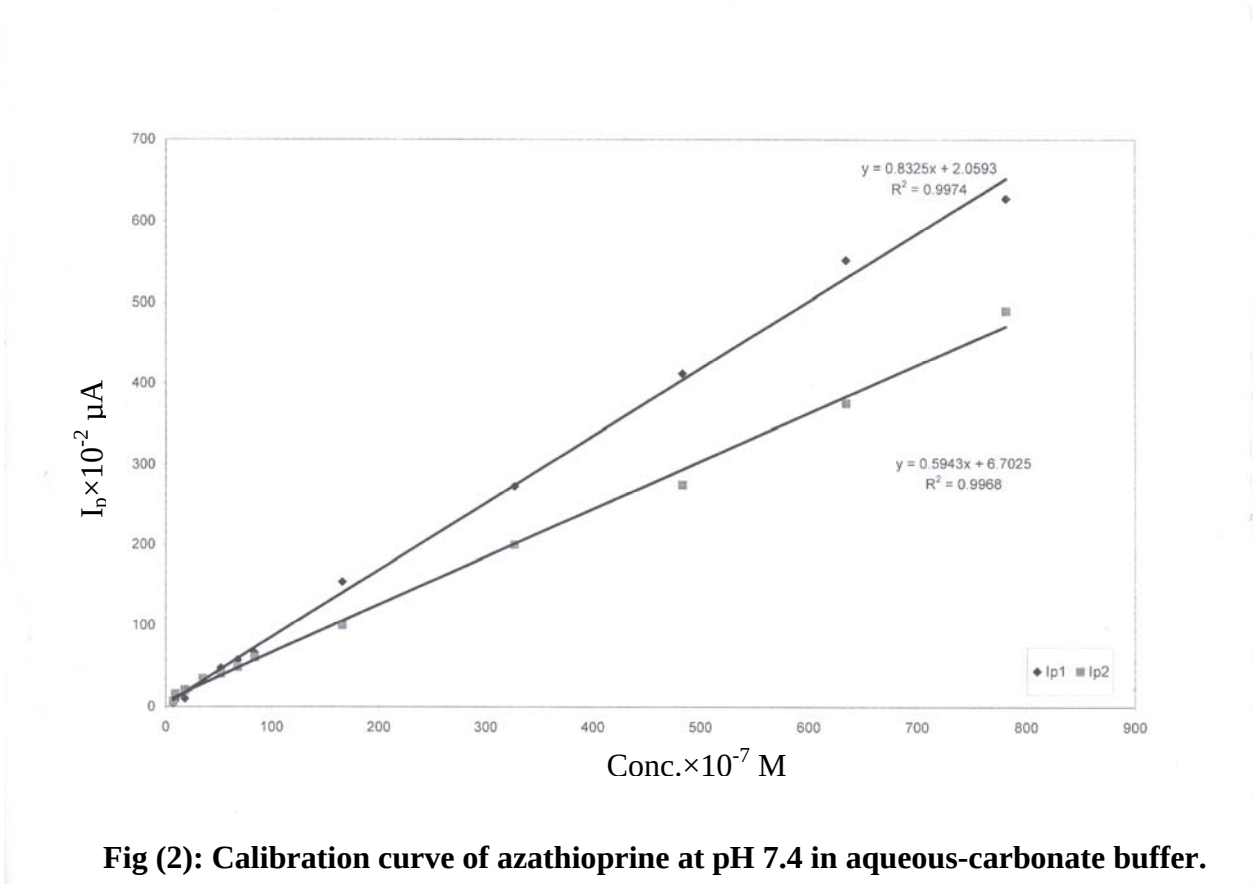


Fig (2): Calibration curve of azathioprine at pH 7.4 in aqueous-carbonate buffer.

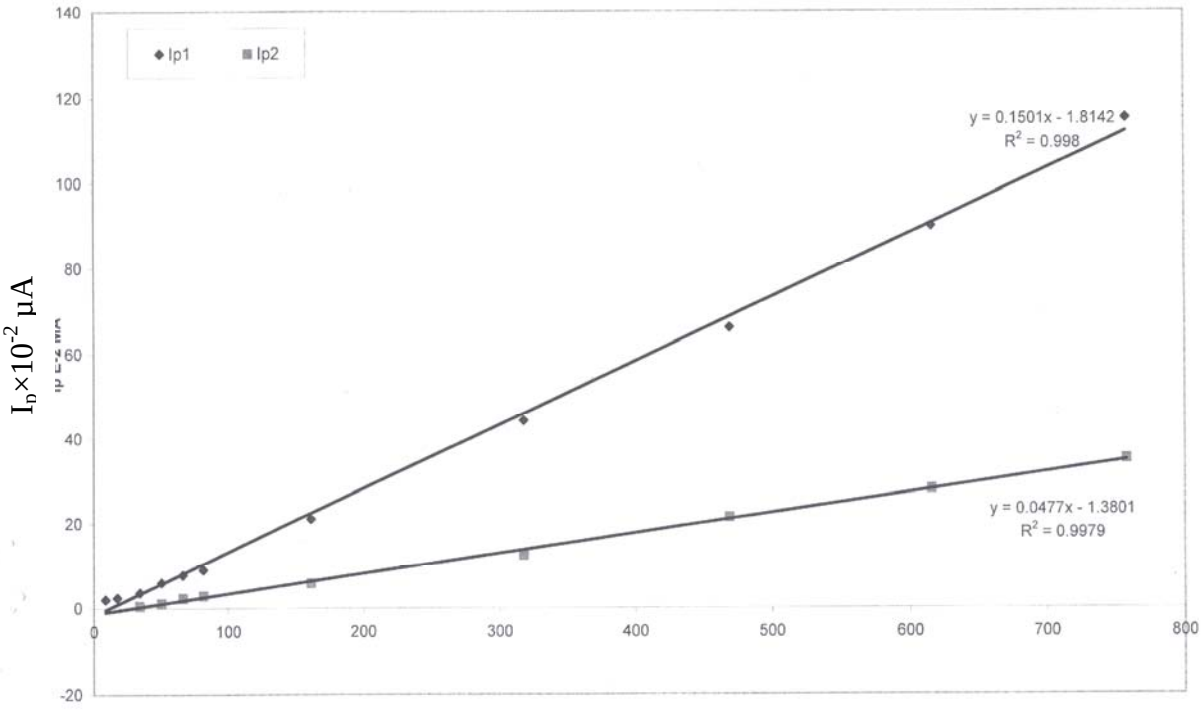
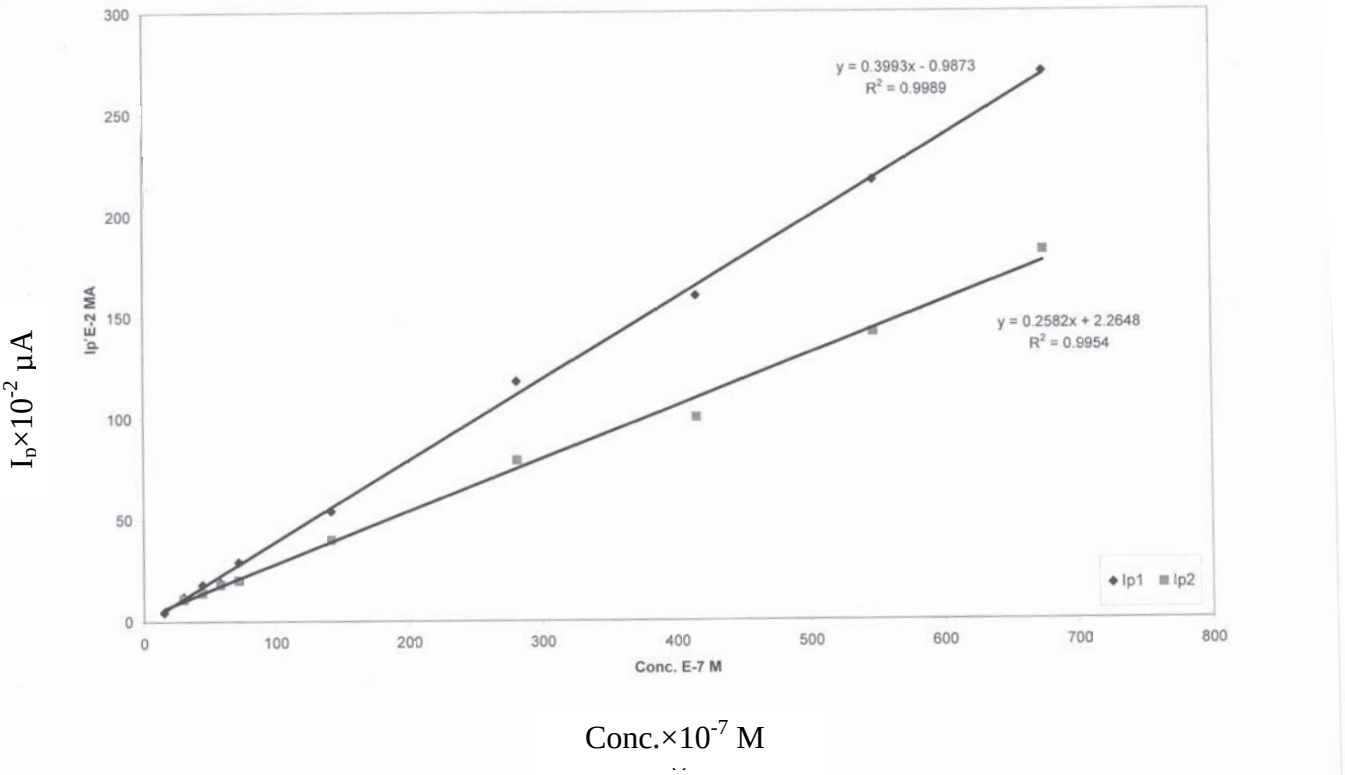


Fig (3): Calibration curve of azathioprine at pH 7.4 in presence of serum-carbonate buffer*.

* The solution was prepared by adding a 0.5 ml of normal serum to 20 ml carbonate buffer inside polarographic cell.



$I_D \times 10^{-2}$ µA

Conc.$\times 10^{-7}$ M

Fig (4): Calibration curve of azathioprine at pH 7.4 in presence of urine-carbonate buffer*.

* The solution was prepared by adding a 2 ml of normal urine to 20 ml carbonate buffer inside polarographic cell.

Regression analysis of the standard curves indicated :

1- linear relationship between peak current and concentration for the drug in the three different media .

2- Correlation coefficient (R) of the plots are shown in Tables no. (3, 4, and 5).

3- The lowest determined concentration for drug was found to be $7x10^{-7}$ M in aqueous-carbonate buffer while in serum-carbonate buffer the lowest concentration of drug was $8.7x10^{-7}$M, and $15.2x10^{-7}$M in urine-carbonate buffer.

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