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Differential- Pulse Polarographic Determination of Doxycycline in Serum and Urine

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ABSTRACT

The polarographic behavior of doxycyline has been studied in phosphate buffer at different pH (2-9). The compound shows four reduction peaks at pH 2-4 , three reduction peaks are observed at pH 5&6 and only two reduction peaks at pH 7-9.

The best well-defined two peaks observed at pH 7.0, appearing at-1.09 V and -1.26 V versus Ag/AgCl, sat.KCl are used in this study for microdetermination of doxycycline. The lowest experimental determination limit is 1.85×10^{-7} M . The method is successfully applied to the determination of doxycycline in vivo (serum and urine).

تقدير الدكسوسايكلين في مصل الدم والإدرار بطريقة البولوغرافي النبضي المشتق

الملخص

تم دراسة الخواص البولاروكرافية لدواء الدوكسي سايكلين في محلول منظم الفوسفيت عند مدى واسع من الرقم الهيدروجيني pH 2-9. ولوحظ ظهور اربع موجات اختزال عند pH 2-4 , وثلاث موجات اختزال عند pH 5 , 6 , وموجتين فقط عند الارقام الهيدروجيني 7-9, ان احسن واوضح موجتي اختـزال تـم الحصول عليها عند pH 7 (ظهرت عند الجهد -1.09 فولت و - فولت1.26 ضد قطب الفضة / كلوريد الفضة) والتي تم استخدامها في هذه الدراسة لتقدير دواء الدوكسي سايكلين, وتم تطبيق الطريقـة بنجـاح كبير في تقدير وتتبع تركيز هذا الدواء في مصل الدم والادرار.

INTRODUCTION

Doxycycline is one of the broad spectrum tetracycline antibiotics.It is extracted from a type of fungi in the soil called (soil steptomyces) and used to stop the bacterial growth (Laurence and Bemmett 1980). These compounds are commonly used in veterinary medicine to prevent disease and as additive in animal food to promote growth (Schenck and Callery 1998). Several analytical methods have been reported for the determination of tetracycline (including doxycycline) in various samples such as pharmaceutical formulations, milk, honey and urine based on thin-layer chromatography (Weng et al., 2003), high performance liquid chromatography (HPLC) (Furusawa, 2003)

(Hisoa et al., 2000), spectrophotometry (Sokol and Matisova, 1994) and spectrofluorometry (Jiang and Zhang, 2004) and electrochemistry.Electrochemical methods are more interesting than other methods due to simplicity, no more reagent for derivation, low cost and somes time less analysis time, (Kasemifard and Moore, 1997).
Recently, we have applied the differential pulse polarographic method for the determination of some antibiotics like chloramphenicol (Sulaiman and Abdul Razzak, 2000) and nitro-furantoin ((Sulaiman and Abdul Razzak, 2002) in human serum and urine.

This paper reported a differential pulse polarographic study of doxycycline in aqueous phosphate buffer and its determination in human serum and urine (in vitro and vivo).

EXPERIMENTAL

Apparatus

Differential- pulse polarograms were recorded with a Metrohm E 506 polarographic analyser as described previously (Sulaiman and Abdul Razzak, 2002). A Three – electrode system was used, The working electrode was a dropping mercury electrode (DME); the reference electrode was an Ag/AgCl,KCl (sat.); and the counter electrode was a Pt wire (Ghatten et al., 1975).

The pH measurements are made using a Philips PW 9420 pH-meter. All polarograpgic measurement are carried out at room temp. (25 C°) .The solution is deaerated by passing through it a slow stream of purified N_2 gas for (10-15) min . and over the solution during measurements.

Reagents

All the chemical used are analytical reagent grade except doxycycline which as provided by the state company for drug industries –samarra. All solutions were prepared with deionized water.
The stock solution of 10^{-3} M doxycycline was prepared by dissolving appropriate amount of the drug in 25 ml of pH 7.0 phosphate buffer solution.

Procedure

The differential pulse mode was used with a 100 mV pulse, a 2s drop time and 3 mV s^{-1} scan rate.
For polarographic measurements appropriate amount of the drug stock solution was added to the pH 7.0 phosphate buffer (20 ml) solution to yield the desired concentration (calibration curve was then constructed). The same procedure was also followed in serum-phosphate and urine-phosphate media. In the case of serum phosphate, 0.5 ml of normal serum was added to the polarographic cell containing the phosphate buffer at pH 7.0 . In urine-phosphate media, 2 ml of normal urine was added to the polarographic cell together with phosphate buffer of pH 7.0.

Sample preparation

A 200 mg of doxycycline capsule was taken by the worker, then after 2 hrs a blood sample was taken, the serum separated by the well known method (Abdul Razzak, 1990). A 0.5 ml serum sample was then transferred to the polarographic cell containing the

phosphate buffer at pH 7.0 and the polarogram was then recorded after 2-5 minutes, degassing with N_2 gas. At the same time ,urine sample was also collected after 6 hrs and a 2 ml of urine was transferred to the polarographic cell containing the phosphate buffer at pH 7.0. The polarogram was then recorded. The amount of drug absorbed in blood and exerted in urine was then obtained from the corresponding calibration graphs constructed as described above.

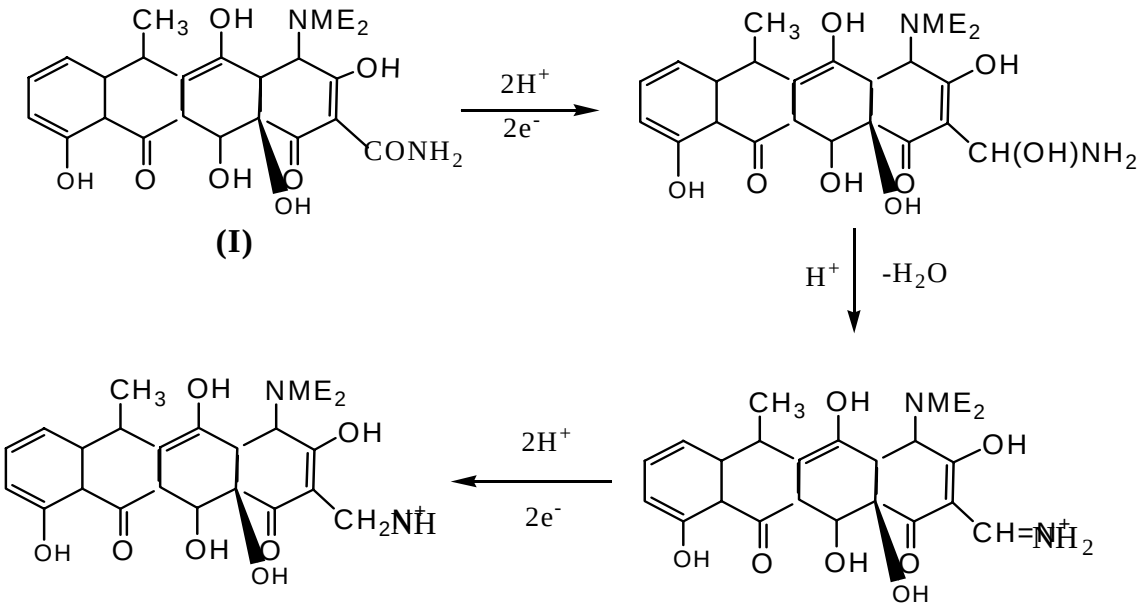
RESULTS AND DISCUSSION

Polarographic behavior of Doxycycline

The DC polarography of doxycycline has been previously studied by many workers. Silvestr et al (Silvestr et al., 1976) reported the appearance of two peaks at – 1.09 and -1.26 V

On other hand, Cedric et al (Cedric and Ghatten, 1977) used AC polarography for analysis of tetracycline derivative, two peaks were observed in acetate buffer at pH 4.0 appeared at -1.11 V and -1.42 V.

In the present work, the differential-pulse polarographic behavior of doxycycline was studied in different media containing phosphate – aqueous solution, phosphate-serum and phosphate-urine solution. Typical differential-pulse polarogram of 9.6 x 10^{-6} M doxycycline recorded in phosphate aqueous solution at pH 7.0 is shown in Fig. 1 . Two well defined reduction peaks observed at -1.09 V and -1.26 V vs Ag/AgCl. electrode, which belong to the reduction of the amide group of the drug as follows :



The effect of pH on the reduction of doxycycline

The differential-pulse polarogram of 9.6 x 10^{-6} M of doxycycline is investigated at different pH values (pH 2-9) using phosphate buffer. The peak potential (Ep) and peak current (Ip) at different pH are summarized in Table 1. The result indicate that the reduction process (the number of peaks observed) are greatly dependent on pH; at pH 2-4, four reduction peaks are observed, at pH 5-6, three reduction peaks are observed while at pH 7-9 only two reduction peaks are observed.

The peak potentials (Ep) for all these peaks were found to be highly pH dependent and as expected with increasing the pH, Ep move to more negative values.
Regression analysis on the standard curve indicated a linear relationship between peak potential (Ep) and pH for all four peaks and follows the following equations:

For first peak : $E_1 = 0.723 + 0.0530\ pH$
For second peak : $E_2 = 0.855 + 0.0521\ pH$
For third peak : $E_3 = 0.90 + 0.0615\ pH$
and For forth peak : $E_4 = 1.02 + 0.0620\ pH$

The slope of all these peaks (0.053-0.062 VpH^{-1}), which is infact very close to the theoretical value (0.060 VpH^{-1})
On the other hand, it is very clear from Table 1 that the degree of reduction and sensitivity of the differential pulse peak current (Ip) are dependent on pH. pH 7.0 was chosen for the present study which gives the best reduction peak and is quite similar to the blood pH value (pH 7.4).

Table 1: Effect of pH on differential pulse peak potential (Ep) and peak current (Ip) of 9.6 x 10^{-6} M Doxycycline

pH	**Ep_1(V)**	**Ip_1 x10^{-2} µA**	**Ep_2(V)**	**Ip_2 x10^{-2} µA**	**Ep_3(V)**	**Ip_3 x10^{-2} µA**	**Ep_4(V)**	**Ip_4 x10^{-2} µA**
2	-0.81	9.1	-0.97	3.7	-1.01	4.6	-1.150	7.6
3	-0.90	5.5	-0.98	2.8	-1.115	2.1	-1.215	6.4
4	-0.925	5.3	-1.07	3.8	-1.115	2.3	-1.275	
5	-1.020	5.1	-1.13	3.1	-1.230	4.0		
6	-1.040	4.9	-1.18	3.1	-1.260	1.8		
7	-1.10	4.3	-1.23	3.1				
8	-1.115	3.1	-1.26	2.1				
9	-1.215	1.5	-1.32	1.2				

Stability of doxycycline in aqueous phosphate buffer and serum- phosphate buffer at pH 7.0

The differential pulse polarograms of 9.6 x 10^{-6} M doxycycline were recorded at different time(Table2) at pH 7. In both aqueous and serum-phosphate solution, It can be seen from Table 2 that doxycycline is stable more than 160 min in aqueous phosphate buffer and about 60 min in serum-phosphate buffer. This period is quite enough to perform the polarographic measurements.

Analytical consideration

Using the optimum conditions (pulse amplitude 100 mV, drop time 2s scan rate 3 mV $s^{-1}$and pH 7.0) the calibration curves were constructed using serial dilution of a standard doxycycline in aqueous phosphate buffer, serum-phosphate buffer and urine-phosphate buffer. Some typical results are listed in Table 3.

Regression analysis on the standard curve indicated a linear relationship between peak current and concentration (for both peaks) for the drug in the three media studied.

The correlation coefficient (R) and the standard deviation of the plot are also shown in Table 3 .The lowest determine for the drug was found to be 1.9 x 10^{-7} M in both aqueous phosphate buffer and serum phosphate buffer and 6.9 x 10^{-7} M in urine phosphate buffer.

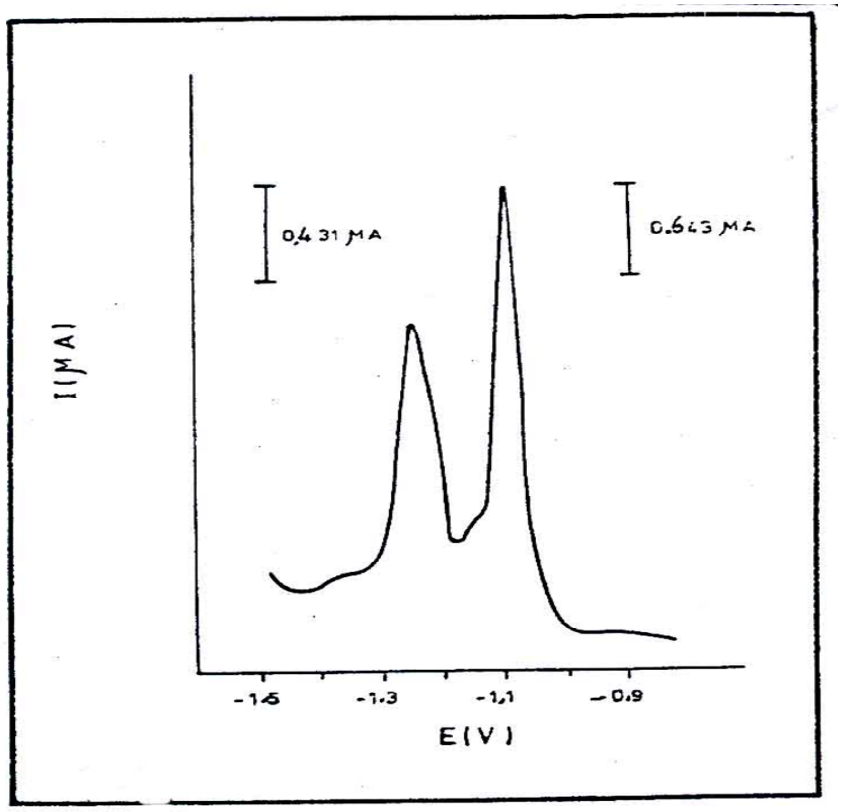


Fig. 1: Differential pulse polarogram of 9.6x 10^{-6} M doxycline in phosphate buffer at ph 7.0

Determination of doxycycline in Vivo

In Serum

The half life period of absorption of doxycycline in blood reported equal to 2 hrs. accordingly the blood sample was taken from the worker after 2 hrs .from orally taking the drug (200 mg) in capsule form.The serum was then separated and analysed polarographically The concentration was obtained from calibration graph constructed previously in the presence of serum found to be equal to (2.28 x 10^{-6} M) .

In Urine

It is well known that 90% of the drug is extracted via the urine (Moats, 1986). Accordingly a sample of urine from the above worker was taken after 6 hrs. and analyzed polarographically. The concentration was obtained from the calibration graph constructed previously in the presence of urine and found to be equal (7.5 x 10^{-6}M).

Table 2: Effect of time on the peak current (Ip) on 9.6 x 10^{-6} M doxycycline in aqueous phosphate solution and $9.3x10^{-6-}$ M in serum phosphate.

	In aqueous solution- phosphate				**In serum -phosphate**			
Time	**Ep_1(V)**	**Ip_1 x10^{-2} µA**	**Ep_2(V)**	**Ip_2 x10^{-2} µA**	**Ep_1(V)**	**Ip_1 x10^{-2} µA**	**Ep_2(V)**	**Ip_2 x10^{-2} µA**
30	-1.135	9.8	-1.24	8.5	-1.085	14.2	-1.25	9.8
45	-1.135	9.0	-1.24	8.5	-1.085	14.7	-1.25	9.8
60	-1.135	8.7	-1.24	8.1	-1.085	14.5	-1.25	9.8
75	-1.140	8.1	-1.24	7.9	-1.085	13.8	-1.25	9.4
90	-1.040	8.0	-1.24	7.8	-1.085	13.2	-1.25	9.0
100	-1.140	8.0	-1.24	7.8	-1.085	12.5	-1.25	8.5

Table 3: The variation of peak current (Ip)with the concentration of doxycycline

In aqueous phosphate			**In serum phosphate**			**In urine phosphate**		
Conc x 10^{-7}M	**Ip_1 x10^{-2} µA at Ep -1.095 V**	**Ip_2 x10^{-2} µA at Ep -1.260 V**	**Conc x 10^{-7}M**	**Ip_1 x10^{-2} µA at Ep -1.115 V**	**Ip_2 x10^{-2} µA at Ep -1.245 V**	**Conc x 10^{-7}M**	**Ip_1 x10^{-2} µA at Ep -1.085 V**	**Ip_2 x10^{-2} µA at Ep -1.23 V**
1.85	0.45	0.22	1.9	0.2		6.9	2.5	*
5.50	0.68	0.57	3.8	0.5		13.0	4.0	
9.10	0.91	0.90	7.5	0.8		33.0	8.8	
12.6	1.36	1.14	11.0	1.0		65.0	15	12.2
16.0	1.60	1.36	14.7	1.1		97.0	24	17.2
35.0	3.40	2.70	18.0	1.5		120.0	28	23.5
69.5	6.38	4.50	36.0	3.1		150.0	35	27.2
103.0	9.50	6.80	71.0	6.1	8.8			
136.0	12.5	9.10	105.0	8.6	11.4			
160.0	16.4	10.90	140.0	11.4	14.4			
R	0.996		**R**	0.990		**R**	0.04	
S.D	0.018		**S.D**	0.0130		**S.D**	0.998	

*The value of Ip in urine phosphate higher than that in aqueous solution may be due to the ionic media of urine

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