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Analysis Of Mixtures Of Demeclocycline, Minocycline And Tetracycline Utilizing Differential Pulse Polarography I

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ABSTRACT

A modified differential pulse polarographic technique was utilized to analyze tetracycline hydrochloride, minocyline hydrochloride and demeclocycline hydrochloride and two mixtures of these compounds. The results indicate that the method may be a useful technique for the analysis of the hydrochloride salts of tetracycline derivatives. A pH of 4.3 was found to be optimum for the polarographic peak separation and quantitative analysis.

The use of polarography in the analysis of tetracycline and its analogues is well studied (1-5). In 1972 the use of differential pulse

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polarography to analyze tetracycline was reported (6). In this study a modified differential pulse polarographic technique is employed in the in vitro analysis of the hydrochloride salts of demeclocycline, minocycline and tetracycline alone and two mixtures of the compounds.

Experimental

Apparatus - All polarograms were recorded on a polarograph[a] equipped with a three electrode system and an X-Y recorder[b]. The working electrode consisted of a dropping mercury electrode (DME) with a drop time of one second, which was controlled by a mechanical drop knocker. A commercial calomel electrode was used as the reference electrode and a platinum wire as the auxiliary electrode. The electrolysis cell[c] utilized had a total volume of 25 ml and the concentration of the tetracyclines derivatives studied were between 10^{-4} and 10^{-5} M. Potential sweeps of 1 or 2 mV/sec and pulse modulations of 10 or 25 mV were employed for the determinations.

Deaeration was performed by passing a stream of highly purified nitrogen through the solutions for a minimum of ten minutes. All experiments were carried out at room temperature and potentials are reported with respect to the saturated calomel electrode (SCE).

Reagents - Samples[d] of demeclocycline hydrochloride, minocycline hydrochloride and tetracycline hydrochloride of compendial grade were utilized without further purification. Acetate buffers were freshly prepared from reagent grade materials.

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[a] Princeton Applied Research Model 174 Polarographic Analyzer

[b] Princeton Applied Research Model 9002A X-Y Recorder

[c] Princeton Applied Research Cell Model 9301

[d] Provided by Lederle Laboratories, Pearl River, New York

Procedure - Analyses were performed by a standard addition technique. Known concentrations (~2mg/ml) of various tetracycline derivatives were added in small increments, of the order of 5-20 µl, using microliter pipettes[e] (tolerance ±0.5%) to exactly 25ml of acetate buffer, acetate buffers containing a single tetracycline derivative or acetate buffers containing a binary mixture of tetracycline derivatives. Volume changes were therefore negligible. The solutions were deaerated and current-voltage curves were recorded between -0.8 volts and 1.9 volts. Calibration curves were established for each tetracycline derivative alone and in binary mixtures by measuring peak heights at appropriate potentials.

Results and Discussion

The variation of peak current with concentration for demeclocycline hydrochloride, tetracycline hydrochloride and minocycline hydrochloride as given in (Table 1) demonstrates the direct proportionality between peak current and concentration. The peak potentials at which these currents were measured are the major reduction potentials in the polarographic spectrum for the individual tetracyclines (Figure 1). The excellent correlation of this data indicates that differential pulse polarography is a highly satisfactory technique for analyzing the three tetracyclines studied. (correlation coefficients for demeclocycline and minocycline were 0.999 and for tetracycline 0.997).

Polarograms of binary mixtures of minocycline hydrochloride with tetracycline hydrochloride and of demeclocycline hydrochloride with tetracycline hydrochloride demonstrate clearly separable peaks in acetate buffer. This was not the case for mixtures of minocycline hydrochloride and demeclocycline hydrochloride (Figure 2). Therefore

e. Fischer Lambda Pipettes

TABLE I. VARIATION OF PEAK CURRENT WITH CONCENTRATION OF VARIOUS TETRACYLCINE DERIVATIVES

(Acetate Buffer - pH = 4.3)

A. Tetracycline Hydrochloride

[Tetracycline HCl] x 10^6M	Peak Current (μA at -1.35 volts)
0	0
3.12	0.06
6.24	0.138
9.36	0.304
12.48	0.404
15.60	0.494
18.72	0.580
21.84	0.670
24.96	0.720
28.08	0.842

B. Minocycline Hydrochloride

[Minocycline HCl] x 10^6M	Peak Current (μA at -1.24 volts)
19.52	0.830
22.54	0.956
25.57	1.104
28.59	1.246
31.61	1.382

C. Demeclocycline Hydrochloride

[Demeclocycline HCl] x 10^6M	Peak Current (μA at -1.28 volts)
17.30	0.720
20.22	0.856
23.14	0.990
26.06	1.126
28.98	1.262

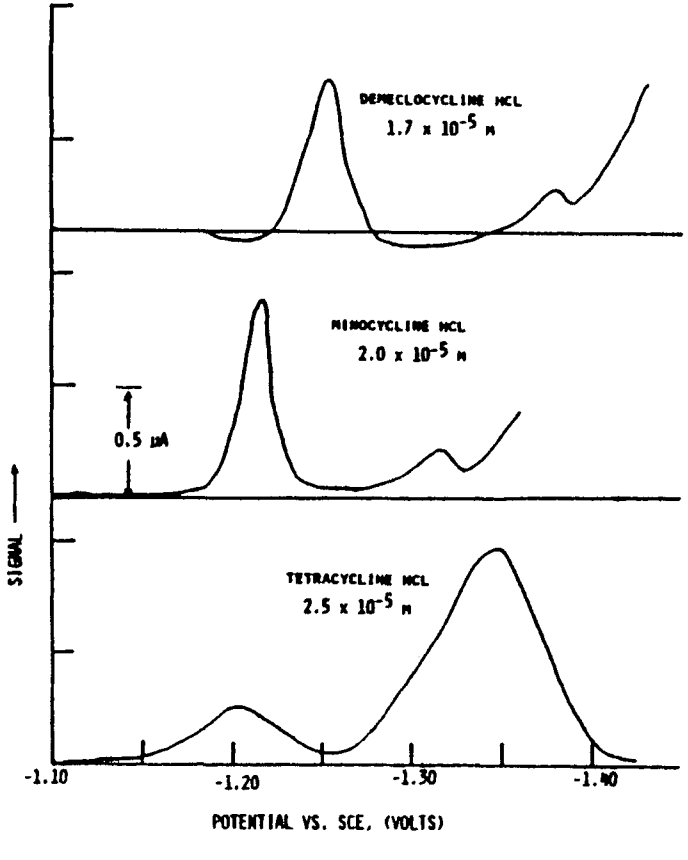

FIGURE 1 DIFFERENTIAL PULSE POLAROGRAMS FOR VARIOUS TETRACYCLINE DERIVATIVES (ACETATE BUFFER - pH = 4.3)

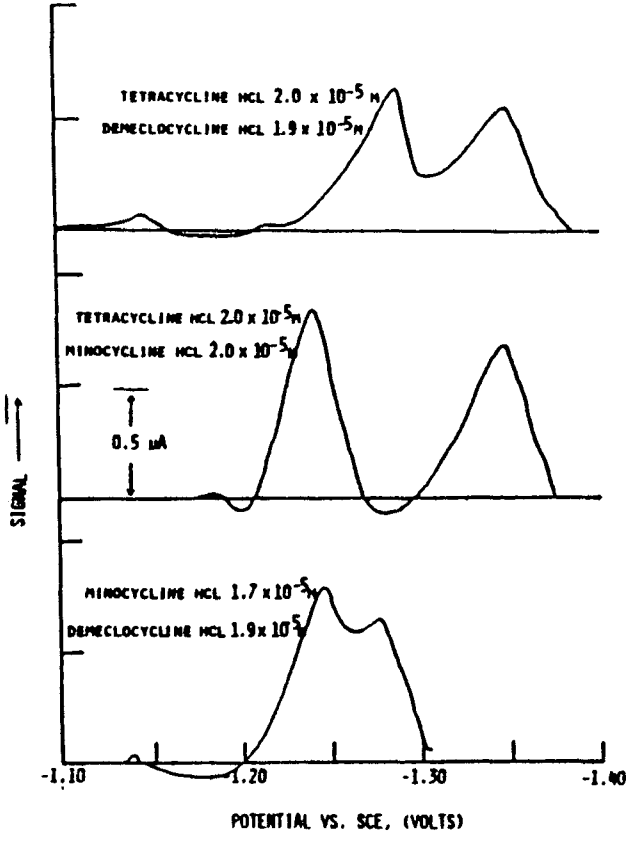

FIGURE 2 DIFFERENTIAL PULSE POLAROGRAMS OF MIXTURES OF TETRACYCLINE DERIVATIVES (ACETATE BUFFER - pH = 4.3)

in the former two cases it is possible to analyze for one derivative in the presence of another. The separation of the polarographic peaks was found to be clearly pH dependent. This is shown for tetracycline hydrochloride and minocycline hydrochloride in Table II. Optimum separation occurred at a pH of 4.3.

While both minocycline and tetracycline exhibit positive potential shifts with decreasing pH (Table II), the effect is more pronounced with minocycline hydrochloride, leading to the observed increase in peak separation at low pH values. Peak separation for mixtures of minocycline hydrochloride and demeclocycline hydrochloride was not appreciably improved by pH changes in the range studied.

By using the standard addition technique, peak current versus concentration curves were constructed and are given in Figure 3 for tetracycline hydrochloride (peak potential = -1.35 volts) in the presence of demeclocycline hydrochloride and minocycline hydrochloride (peak potential = -1.24 volts) in the presence of tetracycline hydrochloride. In the former

TABLE II. EFFECT OF pH ON THE PEAK POTENTIAL OF TETRACYCLINE HYDROCHLORIDE AND MINOCYCLINE HYDROCHLORIDE

pH	Ep (TETRACYCLINE HCl) (volts)	Ep (MINOCYCLINE HCl (volts)	Δ Ep (millivolts)
7.0	-1.420	-1.400	20
6.5	-1.440	-1.390	50
5.5	-1.402	-1.318	84
5.0	-1.374	-1.290	84
4.5	-1.354	-1.264	90
4.0	-1.330	-1.240	90
3.5	-1.312	-1.222	90
3.0	-1.295	-1.208	87

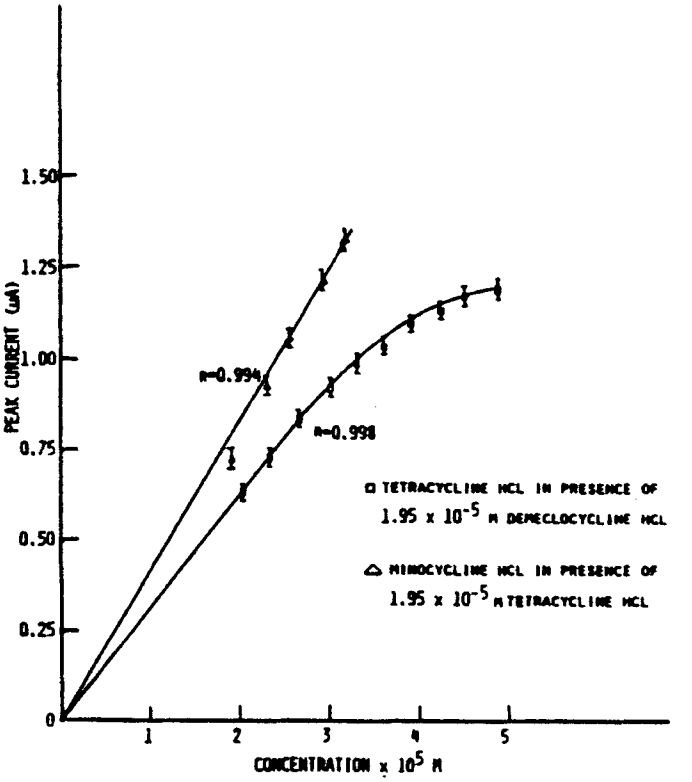

FIGURE 3 ANALYSIS OF MIXTURES OF TETRACYCLINE DERIVATIVES USING DIFFERENTIAL PULSE POLAROGRAPHY (ACETATE BUFFER - pH = 4.3)

case the linearity of the current - concentration relationship falls off at higher demeclocycline/tetracycline ratios (Figure 3).

These curves were used to analyze various mixtures of tetracycline analogues. The data are presented in Table III. The correlation between observed and actual concentrations in ±0.58 to ±7.92%. The percent error for tetracycline/minocycline is consistently lower than for tetracycline/ demeclocycline as would be expected in view of the better peak separation.

Table III ANALYSIS OF MIXTURES OF

TETRACYCLINE DERIVATIVES USING DIFFERENTIAL PULSE POLAROGRAPHY

TETRACYCLINE HCl IN PRESENCE OF DEMECLOCYCLINE HCl

AMOUNT FOUND ($M \times 10^5$)	ACTUAL AMOUNT ($M \times 10^5$)		% ERROR
1.64	1.70		3.53
1.77	1.66		6.63
1.89	1.87		1.07
2.16	2.04		5.88
2.18	2.02		7.92
		Average	5.01

MINOCYCLINE HCl PRESENCE OF TETRACYCLINE HCl

AMOUNT FOUND ($M \times 10^5$)	ACTUAL AMOUNT ($M \times 10^5$)		% ERROR
1.58	1.63		3.07
1.70	1.71		0.58
1.88	1.93		2.59
1.90	1.82		4.40
2.06	2.02		1.98
		Average	2.52

Conclusion

Differential pulse polarography can be used to analyze mixtures of tetracycline with a good degree of accuracy. The technique is pH dependent and, under the conditions utilized, a pH of 4.3 proved to be optimum for both peak separation and drug quantification. The authors feel the technique may be applicable to (1) the study of mixtures of tetracycline in the

presence of other antibiotics, and (2) the study of mixtures of tetracyclines in the presence of their metabolites and degradation products, both *in vitro* and *in vivo*. Work in these areas is currently under study.

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