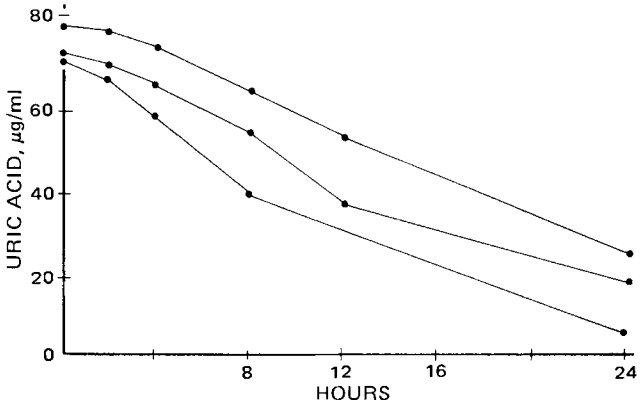

Figure 7—*Photodecomposition of uric acid in the presence of riboflavin in human plasma. Each line represents a different human plasma. Each point represents the average of triplicate determinations.*

The plasma curves (Fig. 7) indicate that the photodecomposition occurred in plasma *in vitro*. Uric acid levels at the termination of each experiment were lower than endogenous levels. Plasma containing uric acid in the absence of riboflavin showed no significant loss of uric acid after exposure to light for 24 hr. During HPLC analysis, the uric acid peak appeared 2.75 min after sample injection. A minor peak, which was not present in the aqueous solutions, was observed 1.75 min after sample injection and may be attributed to a lower molecular weight compound which was not retained by the membrane filter. The nonlinearity of the plasma curves may be due to the settling out of less translucent plasma components during irradiation. Also, a more complex reaction may occur in plasma than in the aqueous solutions.

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ac Polarography for Tetracycline Analysis

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Abstract □ The electrode processes for the reduction of several tetracyclines by ac polarography were examined. In pH 4.0 Walpole acetic acid–acetate buffer, two main waves occurred; the first was quasireversible and the second was reversible. Results showed that the first wave can readily be used for quantitative work. The second wave would also be suitable provided that there was no interference from other electroreducible substances.

Keyphrases □ Tetracyclines, various—ac polarographic analysis, electrode processes identified □ Polarography, ac—analysis, various tetracyclines, electrode processes identified □ Antibacterials—various tetracyclines, ac polarographic analysis, electrode processes identified

Many methods have been reported for the quantitative analysis of the tetracycline antibacterials (1), including a range of electroanalytical methods (2). One method utilized ac polarography for the assay of tetracycline, chlortetracycline, and oxytetracycline with a mercury pool reference electrode (3). This method also was used to analyze doxycycline capsules (4). Studies on minocycline, lymecycline, and demeclocycline have not been reported. Therefore, it was of interest to see whether these antibiotics behaved similarly and to extend the examination, using phase-sensitive ac polarography, to some pharmaceutical preparations on the British market.

Previous workers (3, 4) who investigated the application of ac polarography to tetracycline analysis made no attempt to determine whether the wave was the result of a reversible, a quasireversible, or an irreversible electrode process. Bond (5) stated that a knowledge of the reversibility of the electrode reaction involved is very important when using ac polarography for quantitative analysis but that the analytical use of quasireversible ac electrode processes can be extremely unreliable. Moreover, irreversible electrode processes may be a source of interference (5).

EXPERIMENTAL

Apparatus—All polarograms were obtained on a polarograph[1] equipped with a saturated calomel electrode (SCE), a dropping mercury electrode (DME), a mercury pool counter electrode, and a drop timer.

[1] Cambridge polarographic analyzer model 82P.

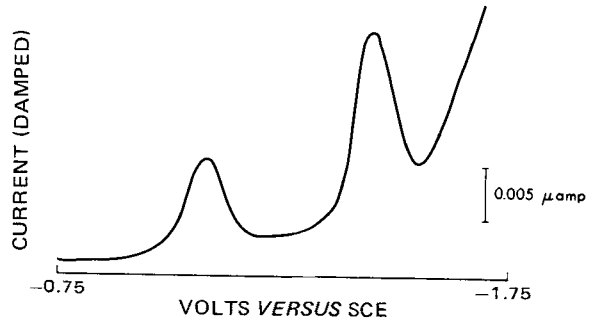

Figure 1—*The ac polarogram for 5 × 10⁻⁵ M tetracycline hydrochloride in pH 4.0 acetate buffer.*

A recorder[2] was attached to the polarograph. A pH meter[3] was fitted with a glass–calomel electrode system.

Reagents and Solutions—All chemicals were reagent grade. The tetracyclines were used as received. The potency of the reference tetracyclines was taken as the value provided by the manufacturer's microbiological assay. This value was employed in calculating the weight of material required to prepare 10^{-3} *M* stock solutions in pH 4.0 Walpole acetic acid–acetate buffer (6).

The concentration range (1×10^{-6}–5×10^{-4} *M*), the pH range of the buffer (3.7–5.8), the drop time, and the damping were varied as required to determine the relationship between these variables and the ac waves. The linearity range for six tetracyclines was determined using the given concentration range and the acetate buffer, scanning over a voltage range of from −0.75 to −1.75 v *versus* a saturated calomel electrode with a scan time of 200 sec, a drop time of 0.5 sec, a damping of 6, and an applied ac voltage of 10 mv, root mean square (rms) at 38 Hz.

Working standards contained either 5×10^{-4} *M* or 25 mg of tetracycline/100 ml in the pH 4 acetate buffer.

Tablets—Depending upon the number available, either 10 or 20 tablets were weighed and finely ground. According to the weight per tablet and the labeled potency, suitable samples were weighed to give 100 ml of solution in the pH 4.0 acetate buffer of theoretically either 5×10^{-4} *M* or 25 mg of the tetracycline salt. This solution was filtered, and 1 ml was diluted to 10 ml with the same buffer to give a theoretical concentration of 5×10^{-5} *M* or 2.5 mg/100 ml. Five samples were prepared from each batch of tablets.

Capsules—The contents of 10 capsules were emptied and weighed, and a suitable sample was taken as for the tablets. In certain cases, 3 min of sonication[4] of the solution was necessary.

Syrups—Appropriately sized samples were weighed and diluted with buffer to give a theoretical 5×10^{-4} *M* solution after determination of the density of the syrup. The rest of the procedure was identical to that for tablets.

Polarographic Analysis of Samples—All solutions were deoxygenated with oxygen-free nitrogen for 10 min. In all instances, the polarographic analyzer was set with the same conditions as for the linearity range experiments and with a suitable current sensitivity. Working standards were run alternately with the samples. Triplicate runs were carried out on each sample solution. All assays were performed at room temperature.

The standard addition technique also was used. In these instances, four 10-ml samples containing a theoretical tetracycline content of 1.25 mg were measured. Three samples received an accurately measured aliquot of the standard solution, *i.e.*, 0.5, 1, or 1.5 ml. The usual calculations were performed to compensate for dilution of the sample and the standard.

RESULTS AND DISCUSSION

Two well-defined ac polarographic waves resulted at pH 4 for all tetracyclines studied when a damping of 6 was used (Fig. 1). With zero damping, other waves became apparent (Fig. 2), the number and position depending upon the solution pH. The half-widths for the two main waves for tetracycline are given in Table I. The logarithmic analysis of the first of these waves at pH 4 gave two intersecting lines with slopes of 60 ± 4 and 108 ± 3 mv; the second main wave gave a value of 120 ± 2 mv. Variation in the drop time had no effect on the peak potential, E_p, of the second wave, but an increased drop time caused a cathodic shift in the first wave. Concentration changes appeared to have no effect on the E_p values of either main wave. At pH 4 and damping 6, the values for the first and second waves were −1.11 and −1.42 v, respectively.

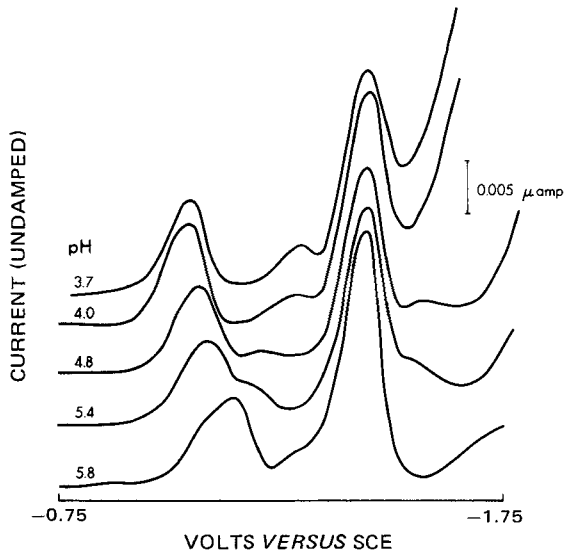

Figure 2—*Effect of pH on ac polarogram of tetracycline hydrochloride, 5×10^{-5} M in acetate buffer.*

On the basis of the logarithmic analysis, the variation of E_p with drop time, and its asymmetry, the first main wave would appear to be complex, involving quasireversible or irreversible electrode processes. The second wave had a slope of 120 mv for the logarithmic analysis and a half-width of 90 mv. These parameters indicate that it is the result of a one-electron reversible process (5). Both waves gave linear relationships over the range from 2×10^{-6} to 1×10^{-4} *M* for all tetracyclines tested including methacycline. The correlation coefficients for all plots were greater than 0.9997 for the first wave and 0.998 for the second wave. Deviations from linearity occurred with both waves when the concentration of the solutions exceeded 1×10^{-4} *M*.

With controlled conditions, the first wave was reproducible, as indicated by the correlation coefficient and the results in Table II. In the present investigation, conditions were established so that only one buffer system was required for all seven tetracyclines. With the acetate buffer, a pH of 4 was suitable for the first wave in all instances. For the second wave, no single pH value was suitable for all tetracyclines. As shown in Fig. 2, the curved baseline demonstrates the influence of the background current for the second wave at pH 4.0. This curvature could account for the slightly lower correlation coefficient (0.998) obtained with the second wave compared to that for the first wave (0.9997).

The results obtained using the ac polarographic procedure for the analysis of tablets, capsules, and a syrup, together with the microbiological assay results supplied by the manufacturers, are summarized in Table II. The first wave was employed in all instances, and generally good agreement was obtained between the values obtained by the proposed method and those supplied by the manufacturers. In most instances, the calibration method gave answers similar to those obtained with the standard addition technique.

For the first four products in Table II, however, the standard addition method gave results that agreed well with those of manufacturer but the calibration method did not. The significant differences between the values obtained by the proposed method and those of the manufacturer for the last entry in Table II can be explained on the basis of the rather lengthy lapse between the time of manufacture and its analysis in this investigation. Where appreciable differences occurred between the two techniques, the standard addition method gave a nonlinear relationship between peak current, I_p, and the concentrations of tetracycline added.

Table I—Half-Width Values for the Two Main ac Waves for a 5 × 10^{-5} *M* Tetracycline Solution at Various pH Values

	pH					
Wave	3.7	4.0	4.8	5.2	5.4	5.8
First, mv ± 2	75	80	88	98	100	103
Second, mv ± 2	88	90	87	91	88	86

[2] Telsec model 700.
[3] Vibret.
[4] Elliott Accoustica ESC520.

Table II—Results for Tetracycline Content of Commercial Preparations Using the First ac Polarographic Wave

Antibiotic	Dosage Form	Mean of Stated Potency ± *SD*, %		Manufacturer's Results
		Calibration Method	Standard Addition Method	
Tetracycline hydrochloride	250-mg capsule	98.1 ± 1.0	102.8 ± 2.8	104.1[a]
	250-mg capsule	101.1 ± 0.3	108.4 ± 2.2	106[b]
	250-mg tablet	98.8 ± 0.5	106.4 ± 3.0	108[b]
	250-mg tablet	102.1 ± 0.4	109.2 ± 2.5	109[b]
	250-mg tablet	100.1 ± 0.6	105.2 ± 3.1	101.4[b]
	100-mg tablet	100.9 ± 0.8	102.1 ± 2.3	104[b]
	250-mg tablet	97.9 ± 0.3	99.5 ± 2.0	98.8[b]
Chlortetracycline hydrochloride	250-mg capsule	99.2 ± 0.6	98.9 ± 1.9	101.1[b]
Oxytetracycline hydrochloride	250-mg tablet	98.9 ± 0.7	100.3 ± 2.5	102.1[b]
Demeclocycline hydrochloride	300-mg tablet	103.8 ± 0.8	102.6 ± 1.8	101.8[b]
	150-mg capsule	100.5 ± 0.6	101.9 ± 2.1	101.0[b]
Minocycline hydrochloride	100-mg tablet	107.8 ± 0.4 103.3 ± 0.4	110.2 ± 2.3 103.9 ± 2.5	106.3[b] 102.0[b]
Lymecycline	204-mg capsule	104.9 ± 0.4	105.5 ± 2.0	103.8[b]
Doxycycline hyclate	100-mg capsule	100.6 ± 0.3	106.4 ± 2.9	101.5[b] 104[a]
	10 mg/ml syrup	99.2 ± 1.2	98.8 ± 3.2	110[b] 115.4[a]

[a] UV absorption method of manufacturer. [b] Microbiological assay (BP 1973).

This deviation could be due to adsorbable species (5) such as methylcellulose, polysorbate, gelatin, and povidone, which may be present in pharmaceutical preparations.

Where nonlinearity occurs in the assay of dosage forms and there is linearity between log I_p and log concentration for the pure drug, the method of Beukelman and Lord (7) may be applicable for determining the tetracycline concentration in pharmaceutical products. The results obtained by this method gave reasonable agreement between the values calculated from the three additions. The standard deviation was approximately ±2.5%. Although a filtration step was always carried out, in many instances this step was unnecessary because the solution contained no undissolved material.

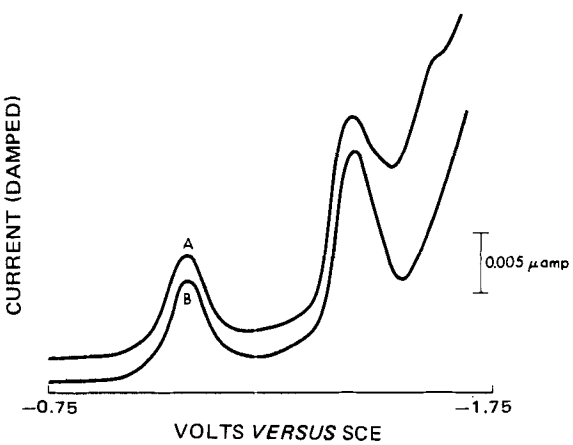

Figure 3—*Effect of nystatin, 10 mg/ml, on the ac polarogram of tetracycline hydrochloride, 5 × 10⁻⁵ M, in pH 4 acetate buffer. Key: A, tetracycline hydrochloride plus nystatin; and B, tetracycline hydrochloride.*

In some instances, erratic results occurred; they were attributed to incomplete dissolution of the tetracycline, even after continuous shaking for several hours. A few minutes of sonication overcame this problem. If the sonication was prolonged for 10 min or more, however, low results occurred for which no satisfactory explanation can be offered.

The assay exhibits a high degree of precision and accuracy if standard addition techniques do not have to be employed. It is also rapid when compared to many chemical methods, and the sensitivity is sufficiently high to permit single tablet or capsule assays.

The second wave is reversible and appears to be ideal for use in analytical ac polarography (5). At pH 4, however, the precision, as shown by the correlation coefficient for the second wave, is less than that for the first wave. Linear relationships were also obtained at pH 5.8 for tetracycline over the concentration range of 2×10^{-6}–1×10^{-4} *M* with the first wave and of 5×10^{-7}–1×10^{-4} *M* with the second wave. The correlation coefficients were 0.996 and 0.999, respectively. The baseline for the second wave was almost parallel to the axis at pH 5.8 (Fig. 2), which may account for the increased sensitivity when compared with the results at pH 4.

An assay carried out at pH 5.8 for a batch of 250-mg tetracycline hydrochloride capsules by the standard addition technique gave a mean percentage of the stated potency of 102.0 ± 3.2 and 103.9 ± 1.0% for the first and second waves, respectively. These values compared favorably with the manufacturer's value of 104.1%. Thus, for tetracycline, it may be preferable to use pH 5.8, provided no other substance in the preparation is electroreducible at a potential of −1.42 v *versus* SCE. This value is considerably more negative than that of the first wave (E_p = −1.22 v *versus* SCE); therefore, there is an increased possibility of interfering reductions overlapping with the second wave. For example, nystatin at a concentration greater than 1 mg/ml in the final solution will interfere with the results obtained with the second wave but have no effect on the first wave (Fig. 3). Substances such as amphotericin and common excipients such as lactose and magnesium stearate have no effect on tetracycline reduction at either the first or second wave. Thus, for certain tetracyclines, a pH value other than 4 may be preferred and, in some instances, it may be advantageous to use the second wave.

More detailed studies are being carried out on the mechanism of the electrode processes.

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