Differential Pulse Polarography of Tetracycline: Determination of Complexing Tendencies of Tetracycline Analogs in the Presence of Cations

T. JOCHSBERGER ˣ, A. CUTIE, and J. MILLS

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Abstract □ The complexation tendencies, stoichiometries, and stability constants for tetracycline, minocycline, and demeclocycline with the metallic ions calcium(II), magnesium(II), zinc(II), aluminum(III), iron(II), and iron(III) were evaluated using a polarographic technique. Changes in pulse peak heights for each tetracycline derivative were measured as a function of cation concentration. The method provides an *in vitro* method of evaluating the selectivity of particular metal ions for different tetracycline analogs.

Keyphrases □ Tetracycline analogs—complexation, effect of cations, differential pulse polarography □ Tetracycline—complexation, effect of cations, differential pulse polarography □ Minocycline—complexation, effect of cations, differential pulse polarography □ Demeclocycline—complexation, effect of cations, differential pulse polarography □ Differential pulse polarography—tetracycline analogs, complexation, effect of cations

The phenomenon of complex formation between tetracycline analogs and various cations is well documented (1–3). This study reports the application of differential pulse polarography in determining the apparent complexation parameters for tetracycline, minocycline, and demeclocycline with Fe(II), Fe(III), and Al(III). The effect of other cations on the polarograms of the various tetracyclines was also examined.

EXPERIMENTAL

Apparatus—All polarograms were recorded on a polarograph[1] equipped with a three-electrode system and an *x–y* recorder[2]. The working electrode consisted of a dropping mercury electrode with a 1-sec drop time, which was controlled by a mechanical drop knocker. A commercial calomel electrode was used as the reference electrode, and a platinum wire was used as the auxiliary electrode. The electrolysis cell[3] had a 25-ml total volume, and the tetracycline derivative concentrations were 10^{-4}–10^{-5} *M*. Potential sweeps of 1 or 2 mv/sec and pulse modulations of 10 or 25 mv were employed.

Deaeration was performed by passing a stream of highly purified nitrogen through the solutions for ≥10 min. All experiments were carried out at room temperature, and potentials are reported with respect to the saturated calomel electrode.

Reagents—All salts (ferrous sulfate heptahydrate, ferric nitrate monohydrate, aluminum chloride hexahydrate, magnesium sulfate heptahydrate, calcium nitrate tetrahydrate, and potassium chloride) were reagent grade and used without purification. Compendial grade samples[4] of demeclocycline hydrochloride, minocycline hydrochloride, and tetracycline hydrochloride were utilized without further purification. Acetate buffers were freshly prepared from reagent grade materials.

Procedure—Complexation parameters were determined by measuring the polarograms of the cations in 0.1 *N* KCl in the absence and in the presence of added tetracycline derivative. The shifts in peak potential were determined as a function of concentration (Table I), and the complexation parameters (Table II) were calculated as described later.

In another set of experiments, the effect of successive additions of a given cation on the polarograms of the various tetracycline analogs was determined. In both sets of experiments, the standard addition technique was employed.

[1] Princeton Applied Research model 174 polarographic analyzer.
[2] Princeton Applied Research model 9002A.
[3] Princeton Applied Research model 9301.
[4] Provided by Lederle Laboratories, Pearl River, N.Y.

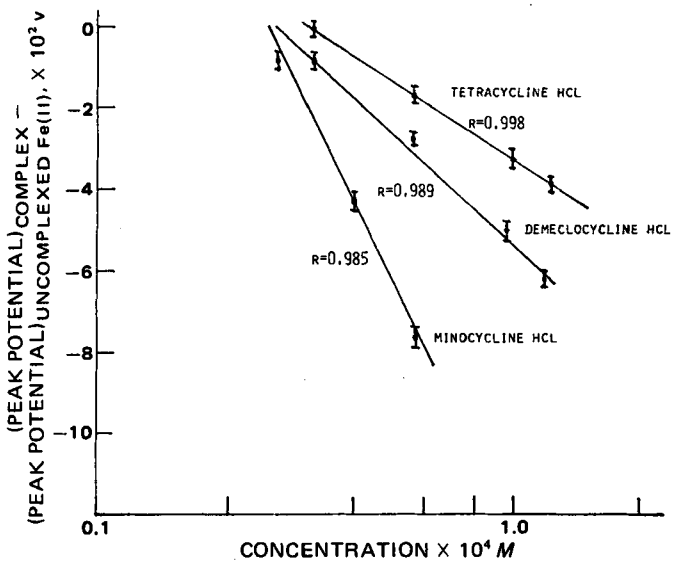

Figure 1—*Effect of tetracycline derivative concentration on Fe(II) peak potential (in 0.1 N KCl).*

RESULTS AND DISCUSSION

The polarography of complexed ions is well documented (4). The basic relationship between potential shifts and concentrations may be expressed by Lingane's equation (5):

$$E^{c}_{1/2} - E_{1/2} = \frac{-0.0591}{n} \log K_f - \frac{0.0591p}{n} \log M_L \qquad \text{(Eq. 1)}$$

where $E^{c}_{1/2}$ is the half-wave potential of a metal ion in the presence of M_L moles per liter of ligand, $E_{1/2}$ is the half-wave potential in the absence of ligand, n is the number of electrons gained in the reduction, K_f is the

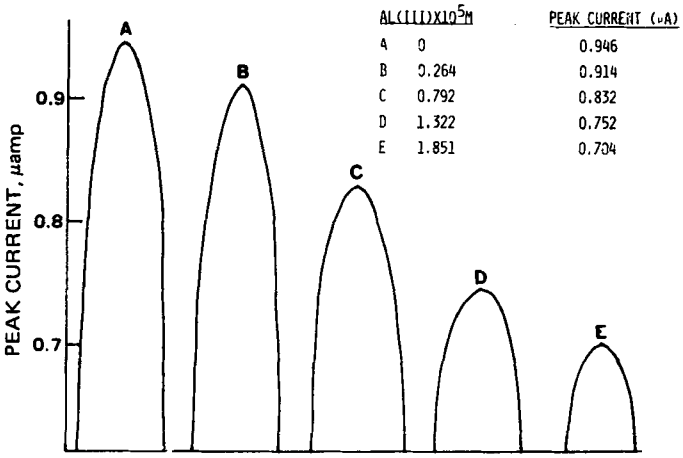

Figure 2—*Effect of aluminum ion on tetracycline hydrochloride polarograms (1.872×10^{-5} M) at a peak potential of −1.342 v.*

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Table I—Peak Potentials of Al(III), Fe(II), and Fe(III) as a Function of Tetracycline Derivative Concentration

Tetracycline Hydrochloride, $\times 10^4$ M	E_p, v	Minocycline Hydrochloride, $\times 10^4$ M	E_p, v	Demeclocycline Hydrochloride, $\times 10^4$ M	E_p, v
		Al(III)			
0	−1.788	0	−1.784	0	−1.786
0.661	−1.762	0.157	−1.660	0.322	−1.764
1.32	−1.726	0.314	−1.606	0.643	−1.746
1.98	−1.702	0.471	−1.560	0.965	−1.736
2.31	−1.696			1.29	−1.722
		Fe(II)			
0	−1.366	0	−1.366	0	−1.364
0.326	−1.368	0.265	−1.376	0.329	−1.374
0.651	−1.386	0.398	−1.404	0.657	−1.396
0.977	−1.400	0.530	−1.440	0.986	−1.414
1.30	−1.406			1.31	−1.432
		Fe(III)			
0	−1.614	0	−1.610	0	−1.606
0.648	−1.636	0.278	−1.576	0.629	−1.636
0.972	−1.652	0.417	−1.560	0.943	−1.672
1.30	−1.662	0.556	−1.546	1.26	−1.684

Table II—Complexation Parameters for Cation-Tetracycline Derivative Complexes

Cation	Derivative	Log K_f	Apparent Coordination Number of Cation
Al(III)	Tetracycline	14.5	3
Al(III)	Minocycline	34.5	6
Al(III)	Demeclocycline	10.8	2
Fe(II)	Tetracycline	6.9	2
Fe(II)	Minocycline	17.8	4
Fe(II)	Demeclocycline	10.2	3
Fe(III)	Tetracycline	14.0	4
Fe(III)	Minocycline	20.2	4
Fe(III)	Demeclocycline	20.6	6

apparent formation constant, and p is the complex coordination number. There have been few attempts to apply differential pulse polarography to the study of complexation. Since the peak potential observed with the technique is identical to the half-wave potential (6), Eq. 1 can be modified to:

$$E_p^c - E_p = \frac{-0.0591}{n} \log K_f - \frac{0.0591p}{n} \log M_L \qquad \text{(Eq. 2)}$$

where E_p^c is the peak potential of the ion in the presence of M_L moles per liter of ligand and E_p is the peak potential in the absence of ligand. The other symbols have the same meaning as in Eq. 1.

Equation 2 indicates that plots of peak potential shift *versus* the logarithm of the ligand concentration should be linear. The slopes of these plots can be used to determine the complex coordination number, and the intercepts yield values for the formation constants. Figure 1 shows representative plots for ferrous ion and the various tetracyclines analogs. Slopes and intercepts were computed by linear regression, and correlation coefficients ranged from ±0.982 to ±0.999. Apparent complexation parameters determined utilizing this technique are presented in Table II.

The data indicate that minocycline forms more stable complexes with Al(III), Fe(III), and Fe(II) than do the other analogs. Since the exact point of attachment between the cations and the tetracyclines has not been elucidated, it is difficult to assess the precise basis of this greater stability. However, previous investigators (1) suggested the dimethylamino group as one coordinating group of tetracycline; the existence of two such groups on minocycline could account for the increased stability of its complexes. The data indicate that in the presence of Fe(II) and Al(III) ions, the apparent coordination number toward minocycline is twice that of tetracycline; this finding further supports the idea that complexing is *via* the dimethylamino groups.

Apparently, Ca(II) and Mg(II) do not complex as strongly with minocycline as they do with tetracycline and demeclocycline. Due to the close proximity of their reduction potential to that of potassium ion, it was not possible to obtain quantitative data in the form of complexation parameters for Ca(II) and Mg(II). However, these two ions had no effect on the polarogram of minocycline (Table III). The lack of an apparent interaction between Ca(II) and minocycline is consistent with the low effect of dairy products on the *in vivo* availability of this analog compared to tetracycline and other derivatives (7). This effect may also be indicative of an interaction between Ca(II) and Mg(II) and the various tetracyclines that is different from that of other cations (1).

The general effect of cation addition on tetracycline, minocycline, and demeclocycline solutions was to decrease the peak current in the polarograms (Table III and Fig. 2). This effect was not uniform. Al(III), Fe(III), and Fe(II) had significant effects on all three derivatives studied. With Fe(III) and tetracycline, the effect was observable at a peak potential of −1.04 v but not at −1.35 v. Ca(II) had a significant effect on the tetracycline and demeclocycline polarograms but not, as noted, on those of minocycline. Again the Ca(II)-tetracycline interaction appeared at a peak potential of −1.04 v but not at −1.35 v.

The dependence of the observed effect on peak potential is possibly related to the point of attachment between the cation and the tetracycline molecule. The present data, however, do not permit any definitive conclusion about that relationship. Zn(II) had no effect on the −1.35-v peak of the tetracycline polarogram. It was not possible to determine if an interaction occurred in the −1.0-v region due to the reduction of the cation itself. The interaction between Al(III) and the three tetracycline derivatives appears to be somewhat different from that of Fe(II) and Fe(III). The tetracycline and demeclocycline complexes of both iron salts and the minocycline-Fe(II) complex exhibited more negative peak potentials than the uncomplexed species, which is the usual observation with complexed species (8). However, Al(III) complexes exhibited a positive shift in peak potential with increasing ligand concentration.

The relative magnitude of the formation constants (Table II) for the three cations studied is consistent with previous observations made under different experimental conditions (2). The values for the coordination numbers determined by this technique (Table II) are generally consistent with those reported for tetracycline derivative complexes (1, 2). However, both significant clinical effects (9) and spectrophotometric changes occur

Table III—Relative Effect [a] of Cations on Peak Currents in Tetracycline Derivative Polarograms

Derivative	Al(III)	Fe(III)	Fe(II)	Ca(II)	Mg(II)	Zn(II)
Tetracycline	++	++[b]	++	++[b]	++	0[b]
Minocycline	++	++	++	0	0	—[c]
Demeclocycline	++	++	++	++	+	—[c]

[a] 0 = no effect, + = moderate effect, and ++ = significant effect. [b] Effect depends on peak potential. [c] No data.

at equimolar ratios of tetracycline to cation[5] (3). Therefore, even partial complexing can have important effects on both *in vivo* and *in vitro* tetracycline interactions.

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Photolytic Decomposition of Indapamide

REG DAVIS *x, CLIFFORD H. J. WELLS *, and ALBERT R. TAYLOR ‡

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Abstract □ Photolytic decomposition of indapamide (I) in nitrogen-flushed methanol yields 3-sulfamoyl-4-chlorobenzamide (II), 2-methylindoline (III), semicarbazide (IV), and 1-(*N*-formamido)-2-methylindoline (V); in oxygen-flushed methanol, II–V, 1-aminocarboxymethyl-2-methylindoline (VI), 3-sulfamoyl-4-chlorobenzoic acid (VII), methyl-3-sulfamoyl-4-chlorobenzoate (VIII), and 2-(*N*-acetamido)-benzoic acid (IX) are formed. A comparison is made with thermal decomposition of I.

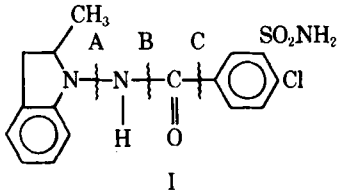
Keyphrases □ Indapamide—photolytic decomposition, thermal decomposition, decomposition products identified □ Photolysis—indapamide, products identified □ Thermolysis—indapamide, products identified □ Diuretics—indapamide, photolytic and thermal decomposition, products identified

There has recently been a growth in drug photosensitization studies. Numerous drugs have photosensitizing properties (1), and these properties appear to show a correlation with *in vivo* photosensitivity. In particular, the saluretic furosemide exhibits a high affinity for oxygen under UV irradiation, and this affinity may be related to the reported skin rash reaction among patients taking the drug (2).

As part of detailed studies of indapamide [*N*-(3-sulfamoyl-4-chlorobenzamido)-2-methylindole] (I), a compound with prolonged saluretic action (3), a study of the photolytic decomposition of this compound was conducted. In contrast to earlier studies (1), the products of *in vitro* photolytic decomposition were identified prior to *in vivo* experiments.

EXPERIMENTAL[1]

Indapamide (I) was supplied as a pure compound[2]. 2-Methylindoline (III), semicarbazide (IV), and 2-(*N*-acetamido)benzoic acid (IX) were supplied as pure reference compounds[3].



I

Photolytic decompositions were performed on 2.7×10^{-3} *M* I solutions in nitrogen- or oxygen-flushed methanol. The reactant solutions were contained in the inner well of a cylindrical quartz reaction vessel, which was mounted vertically before a 1000-w Hanovia medium-pressure mercury lamp. A filter solution of saturated aqueous copper sulfate was pumped through the annular space (4-mm i.d.) surrounding the inner well of the reaction vessel and also was pumped through a heat-exchanger to maintain the reactant solution at 293°K. The filter solution had a short wavelength cutoff at 300 nm. After photolysis for 12 hr, the solution was evaporated to dryness under reduced pressure. The residue was dissolved in a small volume of redistilled methanol prior to spotting on TLC plates.

Thermal decompositions were performed by heating samples in an automatic thermo-recording balance[4]. A 1-g sample of I was heated either at a constant rate of 5°K/min from ambient temperature to 453°K until the weight loss rate stabilized or at 453°K for 12 hr while the sample underwent a regular and continuous weight loss. The solids were dissolved in redistilled methanol prior to spotting on TLC plates.

Preparative TLC was performed using 20 × 20-cm plates coated with a 1-mm layer of silica gel[5]. Two solvent systems were used for plate development: 1, 100% dimethyl carbonate; and 2, the top layer obtained after vigorously shaking pentan-1-ol (85 cm^3) and ammonia (specific gravity 0.880, 15 cm^3) together and then allowing them to stand. All solvent systems were prepared fresh daily, and not more than two developments were carried out in any one preparation.

After development, reactant and product spots were visualized under UV light, scraped off the plates, and extracted into a small volume of redistilled methanol, which was evaporated to dryness under reduced pressure. The residual material was identified spectroscopically.

RESULTS AND DISCUSSION

The results relating to the identification of the products formed by I photolysis are summarized in Table I. These products arise principally as a result of bond cleavages in the N–NH–CO–C linkage.

[1] Mass spectra were recorded on an A.E.I. MS9 spectrometer, IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer, NMR spectra were recorded on a Perkin-Elmer R.10 spectrometer, and UV spectra were recorded on a Unicam SP 8000 spectrophotometer.
[2] Les Laboratories, Servicer, Gidy, France.
[3] Servier Laboratories, Greenford, Middlesex, England.
[4] Stanton.
[5] Merck GF_{254}.

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