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RESEARCH ARTICLES

pH-Partition Behavior of Tetracyclines

JOHN L. COLAIZZI and PAUL R. KLINK*

Abstract □ The apparent partition coefficients between *n*-octyl alcohol and aqueous buffers (ranging from pH 2.1 to 8.5) were determined for several tetracyclines. Using the previously suggested microscopic dissociation constants for tetracycline, the relative amounts of each microscopic ionic form of tetracycline theoretically present at each pH were calculated. The zwitterionic form, −0+ (tricarbonyl methane system ionized, phenolic diketone moiety unionized, dimethylammonium cation postively charged), which was present in highest concentration in the pH range from 4 to 7, appeared to be the most lipid soluble form, its reduced polarity possibly resulting from an intramolecular type of ion-pair formation. Possible relationships between the biological activity of the various tetracycline analogs and their pH-octanol solubility profiles have been discussed.

Keyphrases □ Tetracyclines—pH-partition behavior □ Apparent partition coefficients—tetracyclines between *n*-octanol, aqueous buffers □ Biological activity relationship, tetracyclines—pH-octanol solubility profiles □ UV spectrophotometry—analysis

The work of Pindell *et al.* (1) has demonstrated that tetracycline is fairly rapidly absorbed from the duodenum of the dog and that some absorption can also occur from the stomach when gastric emptying is delayed. However, only a surprisingly small amount (3.1%) of the total administered dose was actually absorbed in 1.5 hr. The amounts of tetracycline absorbed were directly proportional to the dose over a tenfold range. These factors have suggested that tetracycline absorption is a passive diffusion phenomenon.

Structural modifications have been shown to alter the gastrointestinal absorption of the tetracyclines. For example, one recent study showed that the extent of minocycline absorption from the gastrointestinal tract of dogs was two to three times greater than with tetracycline (2). Doxycycline has been shown to produce nearly identical initial plasma levels as demethylchlortetracycline upon oral administration to fasting humans, even though the dose of the latter was three times as great (3). Structural modifications can likewise influence the renal clearance of tetracyclines. Although they are bound to protein to nearly the same extent, doxycycline has been shown to have a 12% of creatinine clearance in man while demethylchlortetracycline was shown to have a 27% of creatinine clearance in the same study (3). The general structural formula for the tetracycline analogs is shown as Fig. 1, and the structures of the analogs discussed in this paper are listed in Table I and explained in terms of the general structure in Fig. 1.

The tetracyclines are ionized throughout the physiological pH range, existing in cationic form at more acidic pH values, in anionic form at more alkaline pH values,

Table I—Structure of Tetracycline Analogs Employed in This Study

Analog	R^1	R^2	R^3	R^4
Tetracycline	H	CH_3	OH	H
Oxytetracycline	H	CH_3	OH	OH
Chlortetracycline	Cl	CH_3	OH	H
Demethylchlortetracycline	Cl	H	OH	H
Methacycline	H	$=CH_2$		OH
Doxycycline	H	CH_3	H	OH
Minocycline	$-N(CH_3)_2$	H	H	H
6-Demethyl-6-deoxy-tetracycline	H	H	H	H

and in zwitterionic form at relatively neutral pH values. As several review articles on drug absorption mechanisms have noted (4, 5), it is difficult to explain the gastrointestinal absorption of organic ions in general and tetracyclines in particular on the basis of passive diffusion and the pH-partition hypothesis. Doluisio and Swintosky (6) showed that tetracycline was too polar to be transferred through a model lipid phase of cyclohexane. When the more polar lipoidal barrier of *n*-octyl alcohol was employed, tetracycline transfer occurred at pH 5.2 and 7.4, but not at pH 2.0. This led the authors to suggest that some ionized drugs may show sufficient lipid solubility to be absorbed passively *in vivo*.

Even in the absence of a definite mechanism explaining the absorption of organic ions like the tetracyclines, nevertheless, evidence does suggest that lipid solubility of such compounds influences gastrointestinal absorption and renal clearance. The objective of this investigation was to study the pH-partition behavior of various tetracyclines in an attempt to determine the relative lipophilicity of the zwitterionic, cationic, or anionic forms, and thus to determine the probable form or forms in which these antibiotics are predominantly absorbed.

EXPERIMENTAL AND RESULTS

Materials—Samples of the various tetracycline analogs were obtained from the pharmaceutical manufacturers.[1] Melting point determinations and UV and IR spectra indicated that the compounds were of a high degree of purity. All of the phosphate buffers used had an ionic strength 0.1. The buffers of pH 2.1, pH 3.0, and pH 3.9 were prepared with sodium phosphate monobasic[2] ($NaH_2PO_4 \cdot H_2O$), and phosphoric acid[2] (H_3PO_4). The total molar buffer concentration was 0.05, and sodium chloride[2] (NaCl) was used to adjust the ionic strength. The buffers of pH 5.6 and 6.6 were prepared with anhydrous sodium phosphate dibasic[2] (Na_2HPO_4) and sodium phosphate monobasic. The total molar buffer concentration was also 0.05, and sodium chloride was used to adjust the ionic strength. For the buffers of pH 7.5 and 8.5, it was not necessary to add sodium chloride to adjust the ionic strength to 0.1, because using anhydrous sodium phosphate dibasic and sodium phosphate monobasic in the ratios required, provided buffers of 0.1 ionic strength at total molar buffer concentration of 0.0367 and 0.0337 for pH 7.5 and 8.5, respectively. All pH values were checked at 25° using a suitably standardized pH meter.[3]

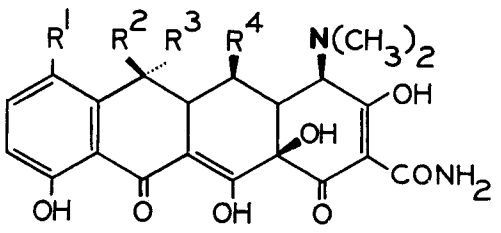

Figure 1—*General structural formula for the tetracycline antibiotics.*

The water used in these experiments was prepared by passing distilled water through two glass percolator columns containing a mixture of cationic and anionic exchange resins.[4] The ion content of the water was such that conductivity was below 0.1 p.p.m. (as sodium chloride) as determined with a purity meter.[5]

Procedure for Determination of Apparent Partition Coefficients of the Tetracycline Analogs—Exactly 5.203×10^{-5} moles of the particular tetracycline analog under study was dissolved in sufficient buffer solution to give a total volume of 100 ml. and thus provide a stock solution which was 5.203×10^{-4} *M* in tetracycline analog. Exactly 10 ml. of *n*-octyl alcohol[6] was then placed into each of four 50-ml. conical flasks. Exactly 10 ml. of the previously prepared 5.203×10^{-4} *M* tetracycline analog solution was added to each. The flasks were stoppered and placed in a metabolic shaking incubator,[7] regulated to 25° (±0.1°), and set to maximum shaking speed. Ten minutes after the samples were placed in the constant temperature shaker, they were vigorously shaken by hand to insure rapid distribution between the two solvent phases. The flasks were then immediately placed back in the constant temperature shaker.

One hour after the flasks were originally placed in the shaker, a 2-ml. sample of the aqueous phase was withdrawn from each flask with a suitable pipet, care being taken to maintain the flask in the shaker at 25° during the withdrawal of sample. The 2-ml. sample of equilibrated aqueous phase was then transferred into a 25-ml. volumetric flask, and buffer solution (which had been previously saturated with *n*-octyl alcohol) was added to volume. The absorbance of this solution was determined with a spectrophotometer[8] against a saturated buffer blank. This procedure was carried out for each of the four test flasks. It had been previously determined that the period of time the flasks remained in the shaker was sufficient for achieving equilibration.

The absorbance readings for each analog at each pH were determined at the wavelength of maximum absorbance that occurred within the range 345 to 380 mμ. (The only exception to this occurred with isotetracycline hydrochloride which gave absorption maxima within the range 275 to 286 mμ.)

A standard tetracycline solution was prepared under the same experimental conditions as described for the sample flasks. The standard solutions consisted of 10 ml. of a 5.203×10^{-4} *M* solution in octanol-saturated buffer. The standard solutions were maintained in the shaker for the same time period as the corresponding flasks containing the two immiscible phases. At the appropriate time, a 2-ml. sample of solution was withdrawn and appropriately diluted, and an absorbance value obtained on the spectrophotometer.

Based on the absorbance values obtained for each sample solution and its corresponding standard, it was possible to determine the concentration of tetracycline analog in the octanol phase and in the aqueous phase at each pH. The octanol/aqueous buffer apparent partition coefficients determined in this manner are summarized in Table II. The results are expressed graphically, in a manner that facilitates comparisons among certain analogs, in Figs. 2 through 6.

Isotetracycline hydrochloride showed no transfer from the aqueous phase to the organic phase at any pH. Tetracycline methiodide exhibited no transfer at any pH except at pH 6.6, at which it resulted in an octanol/aqueous buffer apparent partition coefficient of 0.010.

[1] The authors are especially grateful to Dr. James H. Boothe of Lederle Laboratories, Pearl River, N. Y., who supplied samples of tetracycline HCl, chlortetracycline HCl, demethylchlortetracycline HCl, 4-dedimethylaminotetracycline, 6-demethyl-6-deoxytetracycline, tetracycline methiodide, minocycline, and 9-dimethylamino-6-demethyl-6-deoxytetracycline. They also thank Charles Pfizer Laboratories, Groton, Conn., for providing samples of oxytetracycline HCl, methacycline HCl, doxycycline hyclate, and isotetracycline hydrochloride.

[2] Analytical reagent grade, Mallinckrodt Chemical Works, St. Louis, Mo.

[3] Model DR, E. H. Sargent and Co., Chicago, Ill.

[4] Rexyn AG 501, Fisher Scientific Co., Pittsburgh, Pa.

[5] Barnstead Still and Sterilizer Co., Boston, Mass.

[6] Certified Reagent Grade, Fisher Scientific Co., Pittsburgh, Pa.

[7] Dubnoff, Precision Scientific Co., Chicago, Ill.

[8] Model DB-G, Beckman Instruments, Inc., Fullerton, Calif.

Table II—Apparent Partition Coefficients (Octanol/Aqueous Buffer) of Tetracycline Analogs

Analog	pH 2.1	pH 3.0	pH 3.9	pH 5.6	pH 6.6	pH 7.5	pH 8.5
Tetracycline hydrochloride	0.014	0.0069	0.044	0.056	0.052	0.036	0.010
Oxytetracycline hydrochloride	0.0035	0.018	9.078	0.075	0.087	0.025	0.0086
Chlortetracycline hydrochloride	0.15	0.18	0.27	0.41	0.32	0.13	0.071
Demethylchlortetracycline hydrochloride	0.13	0.10	0.19	0.25	0.19	0.050	0.021
Methacylcine hydrochloride	0.69	0.72	0.82	0.91	0.82	0.43	0.16
Doxycycline hyclate	0.52	0.70	0.85	0.95	0.92	0.60	0.32
6-Demethyl-6-deoxytetracycline	0.82	0.73	0.76	0.83	0.96	0.83	0.45
Minocycline hydrochloride	0		0.051	1.11	1.48		0.36
9-Dimethylamino-6-demethyl-6-deoxytetracycline	0		0.034	0.40	0.77		0.36
4-Dedimethylamino-tetracycline	10.6	10.0	8.48	14.33	5.79	0.64	0.17

DISCUSSION

Ionization Pattern of Tetracyclines—Employing tetracycline as an example of the class, Fig. 7 describes the complete ionization scheme for the tetracycline antibiotics as described in the paper by Leeson *et al.* (7). The upper portion of the figure indicates the three macroscopic dissociation constants (expressed in terms of the pKa's) which may be considered as localized in various portions of the tetracycline molecule. As indicated, the first macroscopic dissociation constant is associated with the tricarbonylmethane system, the second with the phenolic diketone moiety, and the third with the dimethylammonium cation. However, the unqualified assignment of a macroionization constant to any specific functional group may not provide a complete description of what is really occurring in the ionization scheme. In order to completely characterize the ionization scheme for dibasic, tribasic, or polybasic acids in general, it is necessary to take into account all of the microscopic forms and microscopic ionization constants that make up the macroscopic constants (8). The microscopic forms and microscopic constants associated with the ionization of tetracycline are shown in the lower portion of Fig. 7. The most protonated species of tetracycline, as it exists in acidic media, is represented as 00+. If the first ionization resulted in the loss of a proton from the tricarbonyl methane system, the resultant microscopic form could be represented as −0+. If, on the other hand, the proton had been lost from the phenolic diketone moiety, the resultant form would have been 0− +. Initial proton loss from the dimethylammonium cation would have resulted in the microscopic form 000. It should be noted that each of these different microscopic forms has a net change of zero. Each of the respective microscopic ionizations is characterized by the microscopic constants k_1, k_2, or k_3. Together, these constants make up the macroscopic ionization constant, K_1, which may be expressed in terms of concentration expressions for the microscopic constants as indicated by the following equations

$$k_1 = \frac{[H^+][-0+]}{[00+]} \quad \text{(Eq. 1)}$$

$$k_2 = \frac{[H^+][O-+]}{[00+]} \quad \text{(Eq. 2)}$$

$$k_3 = \frac{[H^+]\,[000]}{[00+]} \quad \text{(Eq. 3)}$$

$$K_1 = k_1 + k_2 + k_3 = \frac{[H^+]\,([-0+] + [0-+] + [000])}{[00+]} \quad \text{(Eq. 4)}$$

Lesson *et al.* (7) indicate, however, that for tetracycline, K_1 may be considered as essentially equal to k_1. Therefore, k_2 and k_3 may be omitted from consideration, and the apparent first macroscopic dissociation constant for tetracycline may be taken as

$$K_1 = k_1 = \frac{[H^+]\,[-0+]}{[00+]} \quad \text{(Eq. 5)}$$

As can be observed from an examination of the microscopic ionization scheme in Fig. 7, the microscopic constants k_{12} and k_{13} will be the only microscopic constants contributing to the K_2 dissociation.

$$k_{12} = \frac{[H^+]\,[--+]}{[-0+]} = 10^{-7.80} \quad \text{(Eq. 6)}$$

$$k_{13} = \frac{[H^+]\,[-00]}{[-0+]} = 10^{-8.80} \quad \text{(Eq. 7)}$$

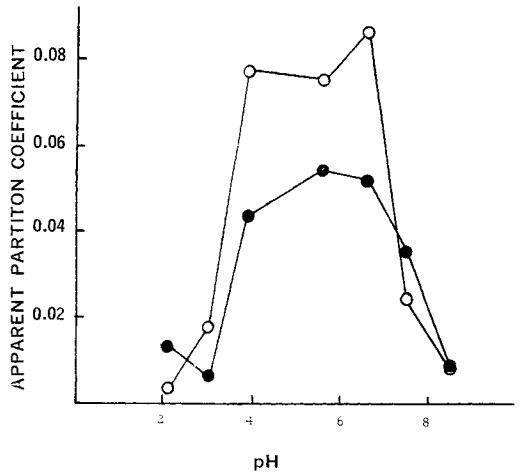

Figure 2—*Plot showing effect of pH on the* n-*octyl alcohol/aqueous buffer apparent partition coefficients for 5.203 × 10⁻⁴ tetracycline analog at 25°. Key: ●, tetracycline; ○ oxytetracycline.*

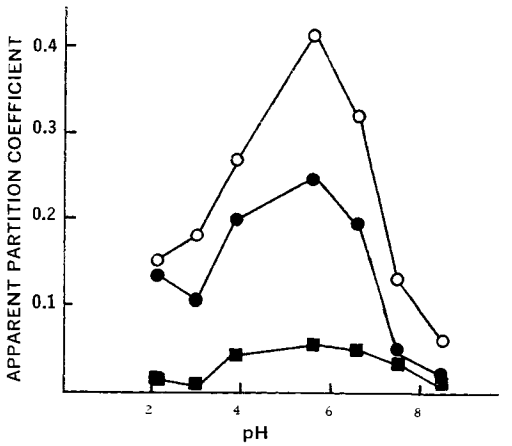

Figure 3—*Plot showing effect of pH on the* n-*octyl alcohol/aqueous buffer apparent partition coefficients for 5.203 × 10⁻⁴ tetracycline analog at 25°. Key: ■, tetracycline; ●, demethylchlortetracycline; ○, chlortetracycline.*

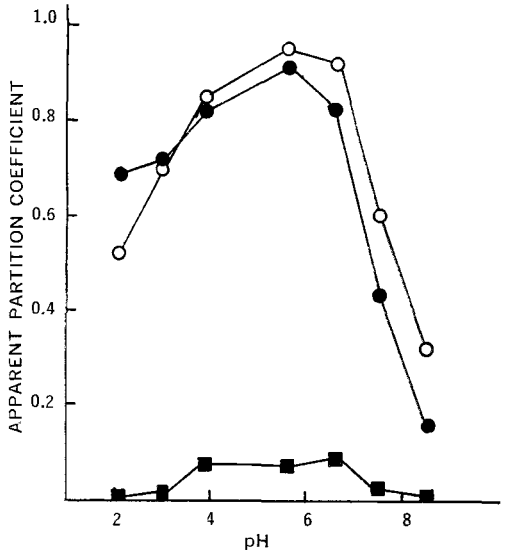

Figure 4—*Plot showing effect of pH on the* n-*octyl alcohol/aqueous buffer apparent partition coefficients for 5.203 × 10^{-4} tetracycline analog at 25°. Key:* ■, *oxytetracycline;* ●, *methacycline;* ○, *doxycycline.*

$$K_2 = \frac{[H^+]([--+]+[-00])}{[-0+]} = 10^{-7.75} \qquad \text{(Eq. 8)}$$

Thus the two microscopic molecular forms resulting from the second ionization are $--+$ (both the tricarbonyl methane and the phenolic diketone groups ionized) and -00 (both the tricarbonyl methane and the dimethylammonium groups ionized), each of which has one net unit of negative charge. The numerical values for the microscopic constants shown in Eqs. 6 and 7 are those indicated by Leeson *et al.*, and they indicate that k_{12} contributes about 10 times as much to the overall K_2 as does k_{13}. The numerical value for K_2 given in Eq. 8 is the potentiometric value given by Leeson *et al.*

Similarly, the third step in the ionization scheme of tetracycline is made up of two microionization steps as explained by the following equations.

$$k_{123} = k_{213} = \frac{[H^+][--0]}{[--+]} = 10^{-9.56} \qquad \text{(Eq. 9)}$$

$$k_{132} = k_{312} = \frac{[H^+][--0]}{[-00]} = 10^{-8.56} \qquad \text{(Eq. 10)}$$

$$K_3 = \frac{[H^+][--0]}{[--+]+[-00]} = 10^{-9.61} \qquad \text{(Eq. 11)}$$

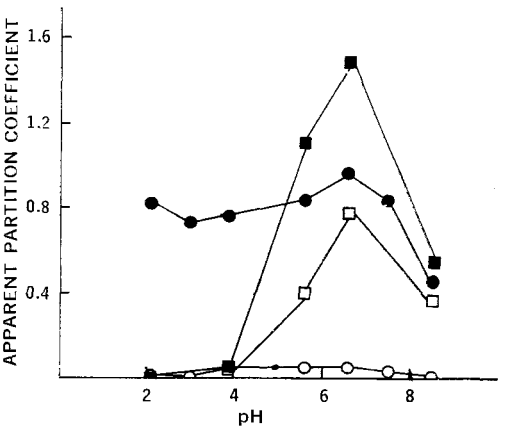

Figure 5—*Plot showing effect of pH on the* n-*octyl alcohol/aqueous buffer apparent partition coefficients for 5.203 × 10^{-4} tetracycline analog at 25°. Key:* ○, *tetracycline;* ●, *6-demethyl-6-deoxytetracycline;* ■, *minocycline;* □, *9-dimethylamino-6-demethyl-6-deoxytetracycline.*

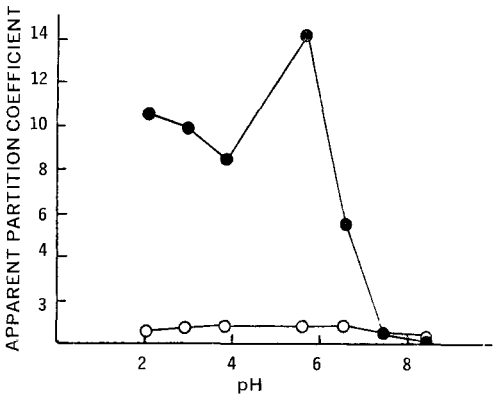

Figure 6—*Plot showing effect of pH on the* n-*octyl alcohol/aqueous buffer apparent partition coefficients for 5.203 × 10^{-4} tetracycline analog at 25°. Key:* ○, *doxycycline;* ●, *4-dedimethylaminotetracycline.*

Converting Eqs. 1, 6, 7, 9, and 10, into logarithmic form the authors obtain

$$\text{pH} = pk_1 + \log \frac{[-0+]}{[00+]} \qquad \text{(Eq. 12)}$$

$$\text{pH} = pk_{12} + \log \frac{[--+]}{[-0+]} \qquad \text{(Eq. 13)}$$

$$\text{pH} = pk_{13} + \log \frac{[-00]}{[-0+]} \qquad \text{(Eq. 14)}$$

$$\text{pH} = pk_{12} + \log \frac{[--0]}{[--+]} \qquad \text{(Eq. 15)}$$

$$\text{pH} = pk_{132} + \log \frac{[--0]}{[-00]} \qquad \text{(Eq. 16)}$$

Additional relationships involving the microscopic constants which become apparent are

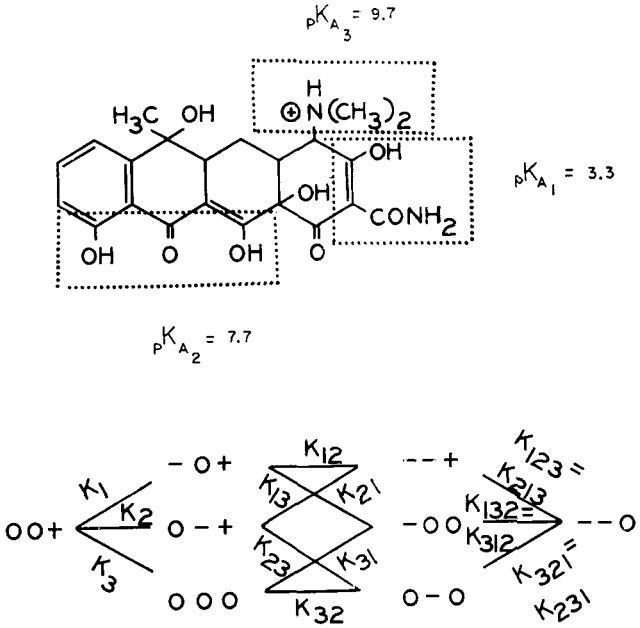

Figure 7—*Top portion of figure shows the three functional groups associated with each of the macroscopic dissociation constants of tetracycline. Bottom portion of figure gives the complete ionization scheme for tetracycline with the microscopic dissociation constants indciated.*

Table III—Ratios of Concentrations of Various Microscopic Forms of Tetracycline at Different pH Values at 25°

pH	$\frac{[-0+]}{[00+]}$	$\frac{[--+]}{[00+]}$	$\frac{[--+]}{[-0+]}$	$\frac{[-00]}{[00+]}$	$\frac{[-00]}{[-0+]}$	$\frac{[--0]}{[00+]}$	$\frac{[--0]}{[-0+]}$	$\frac{[--0]}{[--+]}$	$\frac{[--0]}{[-00]}$
2.1	0.0589	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
3.0	0.468	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
3.9	3.72	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
5.6	186	1.15	0.00631	0.100	[a]	[a]	[a]	[a]	[a]
6.6	1860	115	0.0631	10.0	[a]	0.126	[a]	[a]	[a]
7.5	14,800	7250	0.501	760	0.0501	63.1	[a]	[a]	0.0871
8.5	148,000	72,5000	5.01	76,000	0.501	63,100	0.440	0.0871	0.871

[a] Ratio is small enough to be considered insignificant.

$$k_1k_{12} = \frac{[H^+]^2[--+]}{[00+]} \quad \text{(Eq. 17)}$$

$$k_1k_{13} = \frac{[H^+]^2[-00]}{[00+]} \quad \text{(Eq. 18)}$$

$$k_{12}k_{123} = \frac{[H^+]^2[--0]}{[-0+]} \quad \text{(Eq. 19)}$$

$$k_1k_{12}k_{123} = \frac{[H^+]^3[--0]}{[00+]} \quad \text{(Eq. 20)}$$

Expressing Eqs. 17–20 in logarithmic form we obtain

$$\text{pH} = {}^1/_2\,pk_1 + {}^1/_2\,pk_{12} + {}^1/_2 \log \frac{[--+]}{[00+]} \quad \text{(Eq. 21)}$$

$$\text{pH} = {}^1/_2\,pk_1 + {}^1/_2\,pk_{13} + {}^1/_2 \log \frac{[-00]}{[00+]} \quad \text{(Eq. 22)}$$

$$\text{pH} = {}^1/_2\,pk_{12} + {}^1/_2\,pk_{123} + {}^1/_2 \log \frac{[--0]}{[-0+]} \quad \text{(Eq. 23)}$$

$$\text{pH} = {}^1/_3\,(pk_1 + pk_{12} + pk_{123}) + {}^1/_3 \log \frac{[--0]}{[00+]} \quad \text{(Eq. 24)}$$

Based on the nine different ratios of one microscopic species to another which are expressed in Eqs. 12–16 and 21–24, and on the numerical values for the microscopic k values taken from the work of Leeson *et al.*, it is a simple matter to calculate the ratios for each of the experimental pH values employed in this study. These ratios are summarized in Table III. All the values are for 25°.

From the values for the ratios which are given in Table III, it is possible to calculate amounts of each microscopic species present at each pH. For the first three pH values this can be calculated directly from Eq. 12. The amounts for the other pH values may be obtained by using simultaneous equations as is illustrated for pH 6.6. At pH 6.6, some of the calculated ratios from Table III may be expressed as

$$[-0+] = 1860\,[00+] \quad \text{(Eq. 25)}$$

$$[--+] = 115\,[00+] \quad \text{(Eq. 26)}$$

$$[-00] = 10\,[00+] \quad \text{(Eq. 27)}$$

$$[--0] = 0.126\,[00+] \quad \text{(Eq. 28)}$$

Since all of the significant microscopic forms at pH 6.6 must add up to the number of moles of tetracycline present in the experimental solubility flask (5.2×10^{-6} moles), then

$$\begin{aligned} &[00+] + [-0+] + [--+] + [-00] + [--0] = 5.2 \times 10^{-6} \text{ moles} \\ &-([-0+] = 1860\,[00+]) \\ &-([--+] = 115\,[00+]) \\ &-([-00] = 10\,[00+]) \\ &-([--0] = 0.126\,[00+]) \end{aligned} \quad \text{(Eq. 29)}$$

When Eqs. 25–28 are subtracted from Eq. 29, as indicated above, we obtain

$$1986.13\,[00+] = 5.2 \times 10^{-6} \quad \text{(Eq. 30)}$$

$$[00+] = 2.61 \times 10^{-9} \text{ moles} \quad \text{(Eq. 31)}$$

and the number of moles of the other contributing microscopic forms can be calculated from Eqs. 25–28.

Similar calculations were made for each of the experimental pH values and the percentage concentrations of each of the microscopic forms of tetracycline at each pH are listed in Table IV.[9] A plot of the percent of each microscopic species as a function of pH is shown in Fig. 8.

Partitioning Behavior of the Microscopic Forms of Tetracycline—Although the specific mechanism by which tetracycline is absorbed is not known, some evidence suggests that its absorption pattern resembles a passive diffusion process and thus might be explained on the basis of the pH-partition hypothesis (1, 6). The pH-partition hypothesis maintains that pH influences absorption because it determines the fraction of drug present in the unionized, lipid-soluble form. As the preceding discussion has shown, tetracycline is ionized at all pH values, so it is obviously not absorbed in an unionized form. Schanker (5) has suggested several possible ways in which organic ions might penetrate the gastrointestinal-blood barrier. He indicates that they might be slowly diffused through the lipid areas of the barrier through a limited number of large pores; or that they might penetrate the barrier in the form of a less polar complex formed with some material present in the lumen.

Comparison of Figs. 2 and 8 reveals that tetracycline transfer into *n*-octyl alcohol occurs for the most part when the drug is present in the zwitterionic form, −0+. This indicates the more lipophilic nature of the zwitterionic form. It is interesting to note that tetracycline antibiotics exhibit their optimum antimicrobial activity at between pH 5.5 and 6 (9). Thus it would appear that optimum antimicrobial activity occurs within a pH range providing for the maximum concentration of zwitterion, −0+, as Fig. 8 indicates. This would also be (as Fig. 2 shows) the pH range of the maximum lipid solubility of tetracycline. Pindell *et al.* (1) showed that rates of tetracycline absorption were markedly greater from the ileum and duodenum of the dog as compared with the stomach or colon. It is interesting to note that the pH of the ileum and duodenum would generally coincide with the pH range of greatest tetracycline solubility in *n*-octyl alcohol, and would, of course, also coincide with the pH range of greatest zwitterion concentration.

It is possible that in the zwitterionic form of tetracycline, the positively charged dimethylammonium group "interacts" with the negative charge associated with the tricarbonylmethane system to produce an effective cancellation of charge within the molecule. This would account for the obviously greater lipid solubility of the zwitterionic form and might also facilitate absorption. This possibility is in some way related to the suggestion that the absorption of ionic compounds might be explained on the basis of the formation of less polar complexes with other substances. Recently, Irwin *et al.*

[9] The authors are indebted to Dr. Paul J. Niebergall of the Philadelphia College of Pharmacy and Science, Philadelphia, Pa., who designed a computer program to check the values which appear in Table IV. His work showed that the peak percentage value for the zwitterionic form, −0+, occurs at pH 5.5.

Table IV—Calculated Percent of Each Microscopic Species of Tetracycline Present in Aqueous Solution at Each Experimental pH at 25°

pH	00+	−0+	−−+	−00	−−0
2.1	94.2	5.6	[a]	[a]	[a]
3.0	68.1	31.9	[a]	[a]	[a]
3.9	21.2	78.8	[a]	[a]	[a]
5.6	0.5	98.7	0.6	[a]	[a]
6.6	0.1	93.3	5.8	0.5	[a]
7.5	[a]	64.6	31.7	3.3	0.3
8.5	[a]	14.4	72.1	7.2	6.3

[a] Less than 0.1%.

(10) have shown that trichloroacetate can enhance both the lipid solubility and the absorption of the quaternary ammonium compound, isopropamide, presumably through ion-pair formation. Perhaps the observed lipophilicity of the zwitterionic form of tetracycline which has been observed in this study might be accounted for on the basis of an intramolecular type of ion-pair formation. Ion-pair formation could occur between tetracyclines and adjunctive substances such as glucosamine. It is interesting to note that although the ability of certain additives to clinically enhance the absorption of tetracyclines has been the subject of much controversy, nevertheless, glucosamine has been shown to enhance the gastrointestinal absorption of tetracycline in man even when the presence of excipients was carefully controlled and accounted for (11).

Effect of Structural Modifications on Tetracycline Lipophilicity—The ionization scheme described for tetracycline is generally applicable to other tetracycline group antibiotics (7) unless alteration of one of the acidic functions of the molecule had occurred as with 4-dedimethylaminotetracycline, isotetracycline, or tetracycline methiodide. Figs. 2 and 3 indicate that tetracycline, oxytetracycline, chlortetracycline, and demethylchlortetracycline all produce a similar pH-octanol solubility profile, but that chlortetracycline and demethylchlortetracycline are considerably more lipophilic. In general, demethylchlortetracycline is more active than tetracycline or oxytetracycline (9). It also produces higher plasma levels after oral administration. Chlortetracycline activity also appears to be generally more active against bacteria than tetracycline or oxytetracycline, but its marked chemical instability makes meaningful comparisons difficult (9). Fig. 4 shows that although methacycline and doxycycline both resemble the general pH-partition profile of the parent compound oxytetracycline, they differ in that they are nearly 10 times more lipid soluble. Both methacycline (12) and doxycycline (3) show enhanced absorption over tetracycline or demethylchlortetracycline, and are effective clinically in lower doses. Fig. 5 indicates that minocycline attains greater maximum lipid solubility than the structurally related compounds 9-dimethylamino-6-demethyl-6-deoxytetracycline or 6-demethyl-6-deoxytetracycline. As might be expected, the presence of an additional dimethylamino cationic group markedly decreases lipid solubility at low pH. Apparently, however, its presence at Position 7 enhances lipophilicity at neutral pH values. In this respect it might be interesting to compare the absorption of 6-demethyl-6-deoxytetracycline from the acidic environment of the stomach with other tetracyclines. The greater lipid solubility of minocycline at neutral pH values, which is evident in Fig. 5, is in agreement with the work of Kelly and Kanegis (13) which showed that minocycline was unique among the tetracycline family of antibiotics in that it showed therapeutically desirable increased tissue penetration rate in the brain, thyroid gland and fat. Figure 6 indicates that 4-dedimethylaminotetracycline, which has an altered acidity function in relation to the other tetracyclines, exhibits lipid solubility of a very different magnitude. Isotetracycline and tetracycline are likewise different from the other tetracycline analogs in regard to pH-partition profiles.

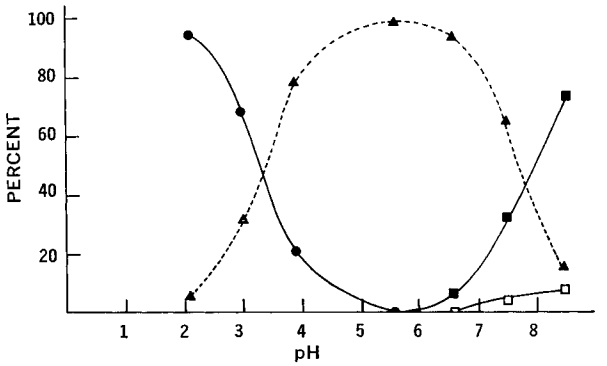

Figure 8—*Plot showing percent of various microscopic forms of tetracycline in aqueous solution as a function of pH at 25°. Key:* ●, *00+;* ▲, *−0+;* ■, *−−+;* □, *−00. The species −−0, which is present in significant amounts at about pH 7 and above, is not shown in this figure.*

In general, it appears that octanol solubility is a relevant consideration in understanding the structure-activity relationships of the tetracycline antibiotics.

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