pH–Partition Behavior of Amino Acid-Like β-Lactam Antibiotics

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Abstract □ The pH–partition behavior of the ampholyte antibiotics ampicillin, cephalexin, and cephaloglycin, using *n*-butanol or *n*-octanol as the lipid phase and aqueous phosphate buffers, was determined at 37° over a pH range of 4.9–7.9. By comparing the pH–partition behavior of these drugs with computer-generated plots of the concentration of their various ionic species as a function of pH, the anion was found to be the major partitioning species. Minimum partitioning occurred in the isoelectric region, suggesting that the zwitterion did not partition to a significant extent. The pH–partition behavior of L-phenylalanine, as a model ampholyte, was studied over a pH range of 2.0–8.9; although the L-phenylalanine cation partitioned into the lipid phase, the anion partitioned to a significantly greater extent, while the minimum partitioning in the isoelectric region indicated that the zwitterion was a poor partitioning species. Studies of surface activity as a function of antibiotic concentration in phosphate buffers showed that there is a relationship between the partitioning behavior of these antibiotics and either the reduction in surface tension or the resulting surface excess at the lipid–water interface. To account for the difference in the pH–partition behavior of the compounds employed in this study and the previously reported data for the tetracyclines, two general types of ampholytes capable of forming zwitterions should be considered, depending on whether or not significant amounts of uncharged species are present in the isoelectric region. Maximum partitioning into the lipid phase should occur if uncharged species is present in significant concentrations (*e.g.*, tetracycline); the compounds in this study were of the other type and thus showed reduced partitioning in the isoelectric pH region.

Keyphrases □ pH–partition behavior—amino acid-like β-lactam antibiotics (ampicillin, cephalexin, cephaloglycin), relationships among surface tension, drug concentration, and pH □ Antibiotics, amino acid-like β-lactam—pH–partition behavior, relationships among surface tension, drug concentration, and pH □ Ampholyte antibiotics (ampicillin, cephalexin, cephaloglycin)—pH–partition behavior

The drug absorption mechanism by which organic ions are absorbed from the GI tract is difficult to explain solely on the basis of the pH–partition hypothesis (1, 2), which states that a drug is absorbed in its unionized form. However, drugs like tetracycline (3), ampicillin (4), cephaloglycin (5), and cephalexin (6) are always ionized and yet are sufficiently absorbed from the GI tract to produce therapeutic effects. Fiese and Perrin (7), noting that dextromethorphan is absorbed from the stomach even though it is completely ionized, found that absorption of this drug was related to its surface activity. Higuchi suggested the formation of lipophilic ion-pairs as one possible mechanism for the absorption of organic ions (8).

Colaizzi and Klink (9) studied the pH–partition behavior of tetracyclines in *n*-octanol–aqueous buffer systems and found that there was maximum partitioning into the lipid phase at the pH corresponding to maximum zwitterion concentration. To account for these results, they suggested that a relatively more lipophilic intramolecular ion-pair was formed, resulting from partial charge cancellation which produced more partitioning into the lipid phase. The tetracyclines, as noted in their paper (9), have a relatively complex dissociation pattern. A simpler model such as ampicillin, cephalexin, or cephaloglycin, each of which exhibits a diprotic dissociation pattern with the single amino and carboxylic acid group, might better illustrate the pH–partition behavior of ampholyte drugs.

Thus, the purpose of this study was to investigate the pH–partitioning behavior of several amino acid-like β-lactam antibiotics. Due to the potential relationship between surface activity of these drugs and their pH–partition behavior, the relationships between surface tension and drug concentration and pH were also studied.

EXPERIMENTAL

Materials—L-Phenylalanine[1] and samples of ampicillin, cephaloglycin, and cephalexin were used as supplied from their manufacturers[2]. Phosphate buffers of pH 4.9, 5.7, 6.4 ,7.2, and 7.9, which were employed in the partition studies with the antibiotics, had a total molar buffer concentration of 0.035 and an ionic strength of 0.10; phosphate buffers of pH 2.0, 4.7, 5.2, 6.6, and 8.9, with a total molar buffer concentration of 0.030 and an ionic strength of 0.14, were utilized for similar studies with L-phenylalanine. Sodium chloride[3] was added to the buffers whenever necessary to adjust the ionic strength. The buffer of pH 2.0 was prepared with phosphoric acid[3] and sodium phosphate monobasic[3]. The buffers for pH 4.7, 4.9, 5.2, 5.7, 6.4, 6.6, 7.2, and 7.9 were prepared with anhydrous dibasic sodium phosphate[3] and sodium phosphate monobasic, while the pH 8.9 buffer was prepared with anhydrous dibasic sodium phosphate and tribasic sodium phosphate[3]. All buffer pH's were measured at 25° using a suitably standardized pH meter[4].

The water for these experiments was prepared by passing distilled water through two glass percolator columns containing a mixture of anionic and cationic resins[5]. The conductivity, as determined with a conductivity meter[6], was maintained below 0.1 p.p.m. (as sodium chloride).

Procedure for Determination of Apparent Partition Coefficients of L-Phenylalanine and of Penicillin and Cephalosporin Antibiotics—Exactly 8.84×10^{-5} mole of L-phenylalanine, 5.72×10^{-4} mole of

[1] Eastman Kodak Co., Rochester, N. Y.

[2] The authors are grateful to Eli Lilly and Co. for supplying the cephalexin monohydrate and cephaloglycin dihydrate. They are also grateful to Wyeth Laboratories for supplying the anhydrous ampicillin.

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

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

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

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

Table I—Apparent Partition Coefficients (Organic Phase–Aqueous Buffer) for Ampicillin, Cephalexin, and Cephaloglycin at 37°

Compound	Organic Phase	Partition Coefficient at pH 4.9	5.7	6.4	7.2	7.9
Ampicillin	Butanol	0.14	0.16	0.19	0.38	0.48
Ampicillin	Octanol	0.18	0.20	0.21	0.23	0.31
Cephalexin	Butanol	0.08	0.12	0.13	0.30	0.87
Cephalexin	Octanol	0.04	0.13	0.11	0.14	0.26
Cephaloglycin	Butanol	0.06	0.11	0.24	0.41	0.93
Cephaloglycin	Octanol	0.04	0.08	0.13	0.26	0.24

Table II—Apparent Partition Coefficients (Organic Phase–Aqueous Buffer) for L-Phenylalanine at 37°

Compound	Organic Phase	Partition Coefficient at pH 2.0	4.7	5.2	6.6	8.9
L-Phenylalanine	Butanol	0.18	0.16	0.14	0.14	0.26

ampicillin, or 3.82×10^{-6} mole[7] of the particular cephalosporin was dissolved in sufficient buffer solution to give a total volume of 100 ml. Exactly 15 ml. of *n*-octanol[8] or *n*-butanol[8] was then placed into each of three 50-ml. glass-stoppered conical flasks, and exactly 15 ml. of the previously prepared drug-buffer solution was added to each of the three flasks; 15 ml. of the drug-buffer solution was placed into a fourth flask as a control. The flasks were stoppered and placed into a metabolic shaking incubator[9], regulated at 37 ± 0.1°, and set to maximum shaking speed. Every 15 min. each flask was individually handshaken to ensure rapid distribution between the two solvent phases and then immediately returned to the metabolic shaker.

When equilibrium was achieved[10], a 10-ml. sample of the aqueous phase was withdrawn from each flask. Its absorbance was determined spectrophotometrically[11] at 260 nm. (257 nm. for L-phenylalanine) using a water blank, and its concentration was determined from the appropriate Beer's law plot. By using the concentration of the drug in the fourth flask and the concentration of drug in the aqueous buffer phase, the concentration of drug in the lipid phase was calculated. The concentration of drug in the lipid phase and that remaining in the aqueous phase were utilized to calculate the *n*-octanol–aqueous buffer apparent partition coefficients (or the *n*-butanol–aqueous buffer apparent partition coefficients). The results of the partitioning studies are summarized in Tables I and II. UV spectral measurements revealed no evidence of degradation of the compounds studied during the partitioning experiments.

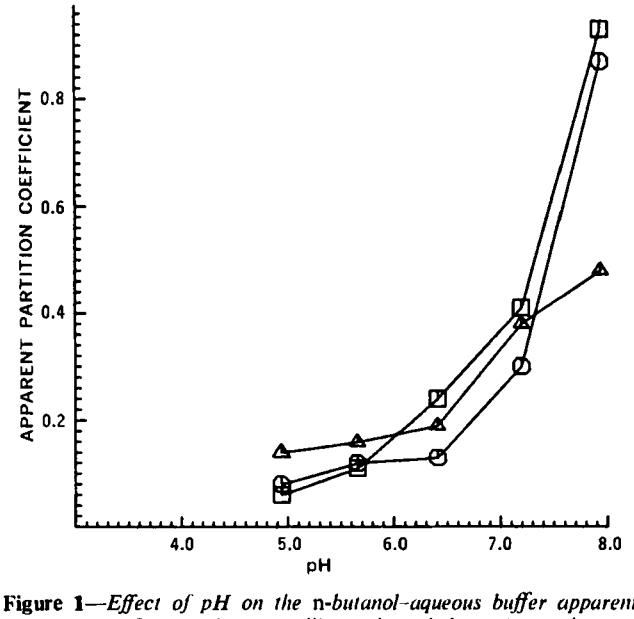

Figure 1—*Effect of pH on the* n-*butanol–aqueous buffer apparent partition coefficients for penicillin and cephalosporin analogs at 37°. Key:* □, *cephaloglycin;* ○, *cephalexin; and* △, *ampicillin.*

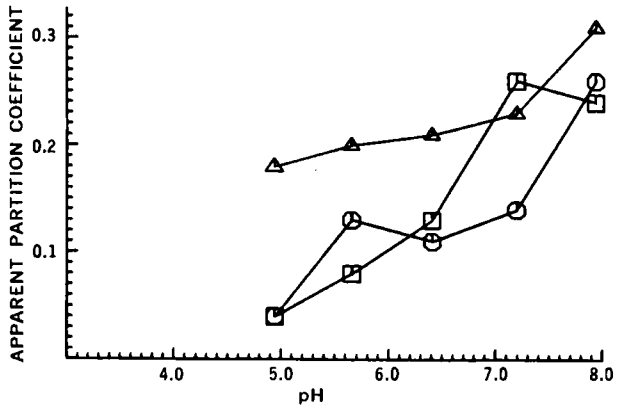

Figure 2—*Effect of pH on the* n-*octanol–aqueous buffer apparent partition coefficients for penicillin and cephalosporin analogs at 37°. Key:* □, *cephaloglycin;* ○, *cephalexin; and* △, *ampicillin.*

Procedure for Determination of Reduction of Surface Tension of Buffered Solutions of Ampicillin, Cephalexin, and Cephaloglycin—Buffered solutions of the drugs were prepared using the phosphate buffers previously prepared for the partition studies in concentrations ranging from 0.25 to 7 mg./ml., depending on the drug[12]. The surface tension of the resulting solutions was determined at 25° using a previously calibrated tensiometer[13] equipped with a platinum-iridium ring with a mean circumference of 5.985 cm.

RESULTS AND DISCUSSION

The apparent partition coefficients for ampicillin, cephaloglycin, cephalexin, and L-phenylalanine as a function of pH can be found in Figs. 1–3. To determine the influence of the different ionic species (cation, zwitterion, and anion) on the apparent partition coefficient, the percent of total concentration of each ionic species was calculated as a function of pH. These calculations were performed by a computer program utilizing Eqs. 1–3 for a diprotic acid, where K_1 and K_2 are the acidity constants (10):

$$\% \text{ cation} = (H_3O^+)^2\, 100/[(H_3O^+)^2 + K_1(H_3O^+) + K_1K_2] \quad \text{(Eq. 1)}$$

$$\% \text{ zwitterion} = K_1(H_3O^+)100/[(H_3O^+)^2 + K_1(H_3O^+) + K_1K_2] \quad \text{(Eq. 2)}$$

$$\% \text{ anion} = K_1K_2\, 100/[(H_3O^+)^2 + K_1(H_3O^+) + K_1K_2] \quad \text{(Eq. 3)}$$

The calculated concentrations were then used to prepare Calcomp[14] plots for each compound (Figs. 4–7).

The apparent partition coefficients for ampicillin, cephalexin, and cephaloglycin with *n*-butanol as the lipid phase, plotted as a function of the pH of the aqueous phase, are found in Fig. 1. When the percent of total concentration of each of the drug's ionic species is compared with Fig. 1 from pH 4.9 to 8.0, the apparent partition coefficients are found to increase as the concentration of anion increases.

For ampicillin the percent of total concentration as zwitterion decreases from 99% at pH 4.9 to 12% at pH 7.9, while the percent of total concentration as anion increases from 0 to about 90% over the same pH range. After noting the variation in the apparent partition

[7] The amount of drug was chosen to be the minimum amount that could be accurately assayed.
[8] Certified reagent grade, Fisher Scientific Co., Pittsburgh, Pa.
[9] Dubnoff Precision Scientific Co., Chicago, Ill.
[10] To achieve equilibrium between the phases, the flasks were shaken for 1 hr. for the antibiotics and for 3 hr. for L-phenylalanine.
[11] Model DB-G, Beckman Instruments, Inc., Fullerton, Calif.
[12] Four different concentrations of drug were used, none of which exceeded 50% of the solubility.
[13] Fisher Surface Tensiomat, Fisher Scientific Co., Pittsburgh, Pa.
[14] IBM 360/50 interfaced digital plotter.

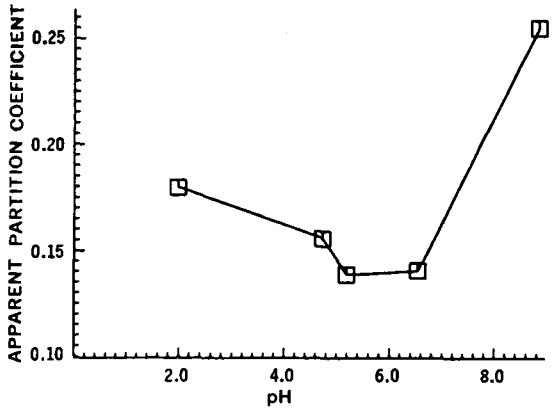

Figure 3—*Effect of pH on the* n-*butanol–aqueous apparent partition coefficients for* L-*phenylalanine* (□) *at 37°.*

coefficients over the same pH range, the anion appears to be the partitioning species. Similar results occurred for cephaloglycin and cephalexin, but, in addition, the cation species (not present in significant concentration over the pH range employed in the partitioning studies for ampicillin) appears to be inhibiting the partitioning into the lipid phase for these drugs.

For cephalexin the percent of total concentration as cation is approximately 60% at pH 4.9 and 0% at around pH 7.5. If it is assumed that the cephalexin anion should partition better than ampicillin anion[15], then the smaller apparent partition coefficients for cephalexin in the pH range from 4.9 to 7.2 may be attributed to the cation forming an ion-pair with the anion and thus reducing the effective concentration of anion that may partition. At pH 4.9 and 5.7, the apparent partition coefficients for cephaloglycin are smaller than for ampicillin; at all other higher pH values, the apparent partition coefficients of cephaloglycin are greater than for ampicillin. The smaller apparent partition coefficients may also be due to the significant concentration of cation reducing the amount of anion available for partitioning.

The apparent partition coefficients for the antibiotics as a function of pH, using *n*-octanol as the lipid phase, are shown in Fig. 2.

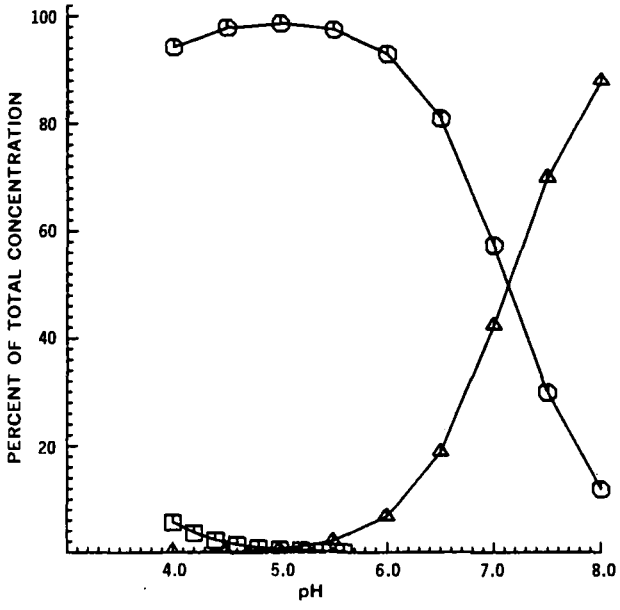

Figure 4—*Percent of total concentration as a particular ionic species of ampicillin in aqueous solution as a function of pH at 25°. Key:* □, *ampicillin cation;* ○, *ampicillin zwitterion; and* △, *ampicillin anion.*

[15] This would be a reasonable assumption since at pH 7.9, where essentially only anion is present for both drugs, the apparent partition coefficient for cephalexin is almost double that of ampicillin.

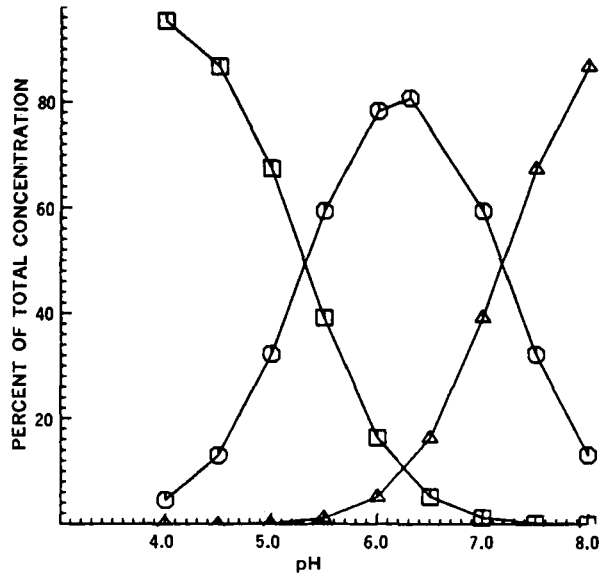

Figure 5—*Percent of total concentration as a particular ionic species of cephalexin in aqueous solution as a function of pH at 25°. Key:* □, *cephalexin cation;* ○, *cephalexin zwitterion; and* △, *cephalexin anion.*

In this system, which is more lipophilic than the *n*-butanol–aqueous buffer system, ampicillin partitions best but the apparent partition coefficients are substantially less than when *n*-butanol is used as the lipid phase. However, the anion appears to be the partitioning species since the apparent partition coefficients appear to be related to the anion concentration. In this system the effect of cation on the partitioning of anion for cephalexin and cephaloglycin is not clear, but it may account for the larger apparent partition coefficients with cephaloglycin than with cephalexin at pH 6.4 and 7.2, since cephalexin has a larger apparent partition coefficient at pH 7.9.

The differences between the apparent partition coefficients in the *n*-butanol–buffer system as compared with those in the *n*-octanol–buffer system are probably due to the large amount of water present in the buffer-saturated *n*-butanol phase, which would tend to increase the amount of ions in the *n*-butanol phase by increasing the dielectric constant of the *n*-butanol phase.

Since the acid instability of the β-lactam antibiotics would cast doubt on the determination of the apparent partition coefficients at a pH where only cation was in high concentration, a model com-

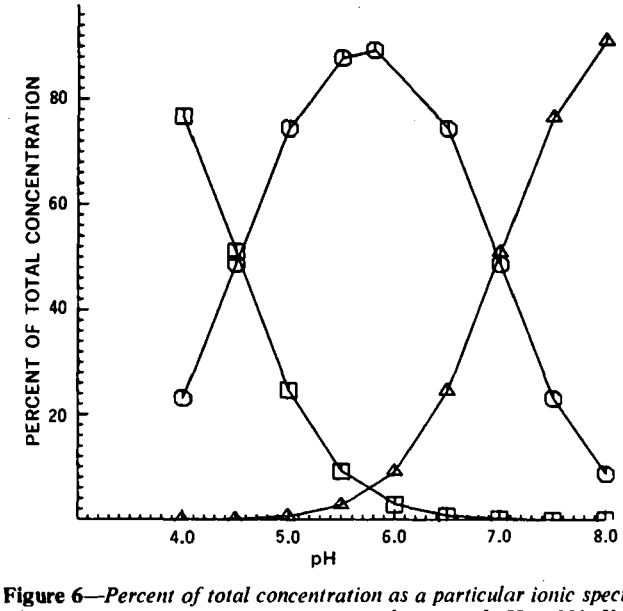

Figure 6—*Percent of total concentration as a particular ionic species of cephaloglycin in aqueous solution as a function of pH at 25°. Key:* □, *cephaloglycin cation;* ○, *cephaloglycin zwitterion, and* △, *cephaloglycin anion.*

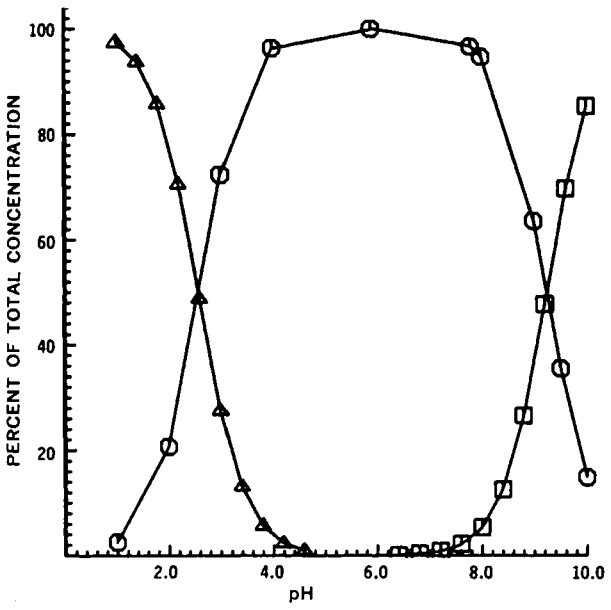

Figure 7—*Percent of total concentration as a particular ionic species of* L-*phenylalanine in aqueous solution as a function of pH at 25°. Key:* △, *phenylalanine cation;* ○, *phenylalanine zwitterion; and* □, *phenylalanine anion.*

pound without such a limitation was desired. Through the use of L-phenylalanine, a relative comparison of the effects of cation, zwitterion, and anion on the apparent partition coefficients in Fig. 3 with the concentration of the ionic species in Fig. 7 shows that the minimum values for the apparent partition coefficients occurred at the maximum zwitterion concentration. Comparing the apparent partition coefficient at pH 2.0, where the cation species is 80% of the total concentration, to pH 8.9, where the anion concentration is about 50% of the total concentration, suggests that the anion partitions better than the cation even though the cation concentration is 1.6 times greater than the anion.

The partitioning results indicate that the zwitterion is not the dominant partitioning ionic species. This would seem to be contrary to the fact that these compounds exhibit minimal water solubility at the isoelectric point (11). However, this decrease in water solubility is a reflection of the high crystal lattice energy of the zwitterion species as compared to its heat of solution in water and not of an increase in lipid solubility (12). The oil–water partition coefficient would be expected to decrease to a minimum at the isoelectric point since the lipid layer, with its low dielectric constant, would have less tendency to solvate the dipolar ion (zwitterion) than water.

Colaizzi and Klink (9) reported maximum partitioning into the *n*-octanol phase in the pH range where the zwitterion concentration of tetracycline and its derivatives would be at its maximum. These results, also seemingly contradictory to the findings reported here, are probably a result of the apparent fact that for tetracycline a significant concentration of the uncharged molecular species exists in equilibrium with the zwitterion present (Fig. 7 of *Reference 9*). This uncharged species would be expected to be highly lipid soluble and thus partition into the *n*-octanol phase. Leeson *et al.* (13) reported the K_{a1} for tetracycline hydrochloride and tetracycline methiodide to be 4.68×10^{-4} and 2.76×10^{-4}, respectively. As stated in their paper (13), the quaternization of the dimethylamino group of tetracycline hydrochloride to form tetracycline methiodide destroys the dimethylamino acidic center of tetracycline hydrochloride but should not affect other acidic centers since the dimethylammonium group should be electronically similar to the quaternary ammonium group. Therefore, the K_{a1} of tetracycline methiodide should represent the sum of the microionization constants ($k_1 + k_2$) associated with the equilibrium of the cation species with the zwitterion species of tetracycline hydrochloride. The difference in acidity between K_{a1} of tetracycline hydrochloride and tetracycline methiodide (1.92×10^{-4}) should approximate the contribution of the proton associated with the dimethylamino group in tetracycline hydrochloride, *i.e.*, the microionization constant k_3 associated with the equilibrium of the cation species with the uncharged species of tetracycline hydrochloride. Since at any pH, each of the isoelectric forms of tetracycline hydrochloride is in equilibrium with the cation species, they must also be in equilibrium with each other.

Table III—Surface Excess as a Function of pH for Ampicillin, Cephalexin, and Cephaloglycin

Compound	pH	Surface Excess, moles/cm.^{2}, $\times 10^{-11}$
Ampicillin	4.94	30.5
Ampicillin	6.49	16.6
Ampicillin	7.20	9.4
Ampicillin	7.94	8.4
Cephalexin	4.94	5.27
Cephalexin	6.49	5.70
Cephalexin	7.20	5.79
Cephalexin	7.94	6.49
Cephaloglycin	4.94	6.43
Cephaloglycin	6.49	9.11
Cephaloglycin	7.20	9.52
Cephaloglycin	7.94	9.67

The ratio between ($k_1 + k_2$) and k_3, which is independent of pH, should represent the tautomeric equilibrium constant (K_z) between the zwitterion species and uncharged species of tetracycline hydrochloride (14).

The value of K_z calculated[16] for tetracycline is 1.44. From this value for K_z, it is a simple matter to calculate[17] that 41% of the isoelectric species will be in the uncharged form. Thus the enhanced partitioning into the lipid phase when the isoelectric forms are in maximum concentration may be due to the uncharged tetracycline molecules. Colaizzi and Klink (9) also reported that the tetracycline methiodide did not partition into the *n*-octanol phase. This would be expected on the basis of the explanation just put forth, since the drug cannot form an uncharged species (although it does form a zwitterion).

In contrast to tetracycline hydrochloride, the pH–partition experiments of this study tend to indicate that very little uncharged species is present in the cases of ampicillin, cephalexin, cephaloglycin, and L-phenylalanine. This was borne out by Hou and Poole (12), who reported that ampicillin, like other amino acids in aqueous solutions at 25° (*e.g.*, L-phenylalanine), exists essentially as zwitterions within the isoelectric region. Similar behavior would be expected for cephalexin and cephaloglycin due to the similarity in structure with ampicillin. This lack of significant amounts of uncharged lipophilic species would also explain the significantly lower oral absorption observed with ampicillin as compared to its acyloxymethyl esters (15), which can exist as an uncharged molecule.

It can be rationalized that the surface activity of the various dissociative species of the drugs employed in this study may be influencing the apparent partition coefficient by changing the surface excess or reducing the interfacial tension at the interface between the lipid and the aqueous buffer phases. Because the concentrations of the various dissociative species change with pH, the surface activity may not remain constant throughout the pH range employed in the partition studies. To evaluate these affects on the pH–partition studies, the Gibbs adsorption equation (4), which defines surface excess as a function of concentration of drug and surface tension, was used (16):

$$s = (-c/RT)(dv/dc)_T \qquad \text{(Eq. 4)}$$

where s is the surface excess in moles/cm.^{2}, R is the universal gas law constant in ergs mole^{-1} deg.$^{-1}$, T is the absolute temperature, c is the concentration of drug in the bulk solution in moles/l., and v is the surface tension in dynes/cm.

The slopes of the regression lines of surface tension as a function of drug concentration at pH 4.9, 6.4, 7.2, and 7.9 were substituted into Eq. 4, along with appropriate values for R and T, to obtain the surface excess for each drug at a concentration of 1 mg./ml. The

[16] $K_z = (k_1 + k_2)/k_3 = 2.76 \times 10^{-4}/1.92 \times 10^{-4} = 1.44$.

[17] K_z = zwitterion species/uncharged species = 1.44. Let zwitterion species = x. Then the percent of total isoelectric concentration as uncharged species would be $(100 - x)$. $K_z = x/(100 - x) = 1.44$, where $x = 59\%$ of the isoelectric concentration as zwitterionic species. Then uncharged species = $(100 - 59) = 41\%$ of isoelectric concentration.

values for surface excess at each pH are summarized in Table III for each drug. The regression lines from which the slopes were calculated had linear correlation coefficients ranging from −0.879 to −0.994.

The surface excess increased for cephalexin and cephaloglycin as the buffer became more alkaline, while for ampicillin the opposite effect was observed. The molar surface tension reduction for most amino acids is at its minimum in the isoelectric region and increases when the pH is increased or decreased from the isoelectric point (17). The observed values for surface excess may account for some of the larger increases in the apparent partition coefficients for cephalexin and cephaloglycin as pH increases from 4.9 to 7.9, as compared to the similar changes observed for ampicillin. The apparent partition coefficients of cephalexin and cephaloglycin in *n*-butanol–aqueous buffer are, respectively, approximately 11 and 16 times greater at pH 7.9 as compared to pH 4.9. For ampicillin in the same partitioning system, only a threefold increase was observed. It may be that an increase in surface excess and a reduction in surface tension occurring in concert with the anion concentration approaching its maximum, as observed with cephalexin and cephaloglycin, enhance the partitioning of the anion into the lipid phase. However, with ampicillin, the largest surface excess (maximum reduction in surface tension) occurs when the zwitterion concentration is at its maximum and the anion concentration is at its minimum. This could explain the observation that there is much less of a difference in the partitioning coefficients of ampicillin over the pH range studied, as compared to the difference observed with cephalexin and cephaloglycin over the same pH range.

In summary, the partitioning results for ampicillin, cephalexin, and cephaloglycin indicate that the anion is the partitioning species. The cation appears to partition to some extent in the case of L-phenylalanine, but it tends to inhibit the partitioning of the anion for the cephalosporins. The zwitterion does not appear to partition to any extent for any of the compounds studied. The surface activity of the drugs appears to modify the partitioning results by increasing the surface excess of the drug and/or by reducing surface tension at the aqueous buffer–lipid interface.

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