Effects of Different Buffer Species on Partition Coefficients of Drugs Used in Quantitative Structure–Activity Relationships

POU-HSIUNG WANG and ERIC J. LIEN ˣ

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Abstract □ A study was conducted in which the same organic solvent, 1-octanol, and different buffer systems were used to obtain intercorrelations among log P_{corr} values given by log P (octanol–water) = a log P (octanol–buffer) + b, where a and b are different constants for the different systems used. The range of a was 0.418–1.156 and that of b was −1.962–1.176 for the four buffer systems examined. Only with neutral drugs was the slope (a) close to 1.0 and the intercept (b) close to 0.0 for the three buffer systems studied. For acidic drugs, only 1-octanol–phosphate buffer gave a 1:1 correlation with 1-octanol–water partition coefficients. Acetate and bicarbonate buffer systems gave different correlations. For basic drugs, none of the three systems examined gave a 1:1 correlation with 1-octanol–water partition coefficients.

Keyphrases □ Partition coefficients—acidic, basic, and neutral drugs, effects of various octanol–buffer systems □ Solvents—various octanol–buffer systems, effects on partitioning of acidic, basic, and neutral drugs □ Buffers—various octanol–buffer systems, effects on partitioning of acidic, basic, and neutral drugs

The distribution of a drug or chemical compound between two phases in which it is soluble is an important topic. Nernst (1) emphasized that the partition coefficient is constant only if a single molecular species is distributed between two immiscible phases. Therefore, partitioning can be considered as classical thermodynamics in an equilibrium process. Pharmacologists became interested in the partition coefficient following the work of Meyer (2) and Overton (3), who first showed the parallel relationship between the nonspecific narcotic activity of drugs and their oil–water partition coeffients. In biochemical and pharmacological systems, the partition coefficient is an extrathermodynamic property and is a useful parameter for hydrophobic interactions with macromolecules, membranes, enzymes, and drug receptors.

BACKGROUND

There has been a recent rapid development in correlation studies of molecular structure with biological activity based on a model that utilizes the partition coefficient of the active compound in a reference partition system as a major parameter in the correlation equation. Hansch and coworkers (4–9) successfully used the linear free energy approach to establish quantitative structure–activity relationships for many biological systems. Lien and coworkers (10–18) studied the quantitative structure–activity relationships of various derivatives of drugs having a high correlation of biological activity with lipophilicity. The linear free energy relationship approach still has limitations due to the nonadditivity of the partition coefficients of some organic compounds (19, 20).

Among the many solvent systems studied, 1-octanol–water has been used most for quantitative structure–activity relationship studies (21, 22). The possible intermolecular interactions of solute molecules have not been studied systematically. Purcell *et al.* (23) stated that the behavior of aliphatic acids can be complex but can be controlled by proper buffering. Several investigators (24–26) recently used phosphate buffer systems in which the ionic strength was adjusted by inorganic compounds (*i.e.*, sodium chloride, potassium chloride, *etc.*); the pKa values of the drug were determined using the Debye–Hückel equation. This factor then was used to calculate the degree of ionization (α) in the measurement of the partition coefficient.

Ballard (27), who has done extensive work in this field, recently studied the carbonic acid system. He suggested that the pKa value of carbonic acid is a mixed constant involving the pKa of carbonic acid, the hydration of carbon dioxide in solution to form carbonic acid, and the activity and degree of dissociation of bicarbonate ion. He concluded that the thermodynamic pKa value of carbonic acid probably is not as affected by pH as is pKa′. However, both pKa and pKa′ are affected by temperature.

Other investigators (27–31) used phosphate buffers without adjusting the ionic strength. Rouot *et al.* (32) used a combined buffer system such as 0.067 M KH_2PO_4 mixed with 0.067 M Na_2HPO_4, while Moore and Koreeda (33) used citric acid (pH 1.8) and sodium bicarbonate (pH 9.0) in addition to phosphate buffer (pH 7.00). Marco *et al.* (34) used a pH 7.4 tris(hydroxymethyl)aminomethane hydrochloride buffer system. The pKa′ values were ignored in all of these investigations.

Each buffer species has its optimum pH range. It is questionable whether only one buffer can be utilized for a wide range of pH values. Moser *et al.* (35) suggested that the measurement of the partition coefficient of many weak acids is valid only within a pH range of about three units above the pKa. Above this range, they showed that such effects as ion-pair extraction of some species with buffer counterions can cause extreme deviations from the straight line described by the log P *versus* pH plot.

Dearden and George (36) criticized the assumption that the ionized fractions of an ionizable drug are insoluble in the lipid phases. It generally is assumed that the partition coefficient of the unionized species may be measured from the experimental apparent distribution coefficient at a pH giving a significant degree of ionization and the pKa of the solute. However, when octanol is saturated with water, the small amount of water in the octanol layer may be expected to dissolve some ionized species. It was concluded that differences in log P values between unionized and ionized species decreased steadily as log P was increased due to ion-pair formation with buffer counterions (36). Thus, the extraction of such ion-pairs into the nonaqueous phase should be related directly to the lipophilicity of the drug. The buffer system still retains a certain complexity when it comes to the measurement of the partition coefficient of a drug. There are some advantages in using a particular buffer system since it may increase the solubility of a drug, keep the pH of the medium constant, and/or retain the neutral form of the drug to avoid intermolecular interaction.

In measuring the partition coefficient, the ratio of the concentrations of the solute in two immiscible solvents is determined after equilibrium is reached. Mathematically, it may be defined as $C_{organic}/C_{aqueous}$. The 1-octanol–water system is a system of two immiscible solvents that has been used extensively. In the Pomona College data bank, Hansch and coworkers collected several thousand compounds for which the partition coefficients were measured in 1-octanol–water. Therefore, 1-octanol–water is the most frequently used system for comparative correlation studies. In addition, many diverse immiscible systems have been employed to avoid hydrogen bonding, solubility problems, *etc.* These systems have parallel relationships with the 1-octanol–water system and with each other. Examples of other organic solvents are chloroform, heptane, cyclohexane, butanol, and oil.

In the measurement of the partition coefficient of a drug, one considers the species that distributes to the binary phases, a water-saturated organic solvent phase and an organic solvent-saturated water phase; the partition coefficient should not be mistaken as arising from a system composed of simply one pure solvent phase and one pure water phase. The true (corrected) partition coefficient, P_{corr}, should characterize the transfer of only an undissociated molecular species. It generally is assumed that the true or corrected partition coefficient of a drug is independent of the pH and the buffer species used. In other words, one can use different buffer systems and a given organic solvent (*e.g.*, 1-octanol) in measuring the partition coefficient of an acidic or basic drug as long

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as one considers the degree of ionization. The true partition coefficient of the undissociated form then is obtained from $P_{corr} = P_{app}/(1 - \alpha)$, where α is the degree of ionization. The α value can be readily calculated from $\alpha = 1/[1 + \text{antilog (pKa} - \text{pH)}]$ for acids and $\alpha = 1/[1 + \text{antilog (pH} - \text{pKa)}]$ for bases; these expressions are obtained from buffer equations.

The validity of the assumption that only the pH of the buffer system will affect the apparent partition coefficient, but not the pH of the buffer species, has been questioned but has never been proven or disproven. The major objectives of this study were to explore whether this assumption is valid and to ascertain how the results obtained may affect quantitative structure–activity relationship studies.

EXPERIMENTAL[1]

pKa Values—The pKa values of the drugs studied were obtained from the literature (38, 39). The pKa values of several drugs were estimated from homologs or analogs (Table I).

Purification of 1-Octanol—Recent grade 1-octanol was purified by shaking with 1 *N* NaOH, shaking with 1 *N* H_2SO_4, shaking with 5% $NaHCO_3$, drying with excess anhydrous magnesium sulfate, and fractional distillation with collection of the fraction at the boiling range of 192–194° (23, 40). The purified solvent did not show any UV-absorbing contaminant.

Buffer Preparation—Buffer systems such as acetate, phosphate, tris(hydroxymethyl)aminomethane, and bicarbonate were prepared as stock solutions according to a literature procedure (41) and then were diluted to 0.01 *M*. The pH values were adjusted by adding 0.1 *N* HCl or NaOH and were checked with a digital pH meter[2]. Each buffer was within the appropriate pH range for its own optimum buffer capacity.

The acetate buffer pH was adjusted to 4.00. Both the phosphate buffer and the tris(hydroxymethyl)aminomethane buffer were adjusted to pH 7.40. The pH value of the bicarbonate buffer was 9.20. The pH value of the deionized, distilled water was 6.90 ± 0.05. The pH value always changed when acidic or basic drugs were dissolved in water saturated with 1-octanol, so the final pH value of the water layer was measured after partitioning.

Determination of Apparent and True (or Corrected) Partition Coefficients—The drugs were partitioned between 1-octanol saturated with buffer (or distilled water) and the same aqueous phase saturated with 1-octanol. In most cases, a 1:1 (v/v) ratio (*i.e.*, 25 ml:25 ml) of 1-octanol to buffer (or water) was used. The drug was dissolved first in the aqueous phase and then equilibrated with gentle shaking for 3–4 hr at room temperature (25 ± 1°) followed by centrifugation for 30 min. A standard curve was obtained from six or eight points of the drug solution by the UV or visible spectrophotometric method[3] to give an absorbance of 0.2–0.9 in a 3-ml cell. The concentration of the sample in the aqueous phase after equilibrium and separation was determined from the absorbance and the standard curve. The absorbance of the sample was adjusted to 0.2–0.9 by dilution.

The apparent partition coefficient was calculated from $P_{app} = C_{1\text{-octanol}}/C_{aqueous}$. Each determination was done at least in quadruplicate using different amounts of the sample, and the mean and standard deviation were determined ($SD \leq 0.03$). Values with a deviation of >0.03 were remeasured. The true or corrected partition coefficient (log P_{corr} value) was calculated from $P_{corr} = P_{app}/(1 - \alpha)$, where α is the degree of drug ionization at the given pH. The problem of the low solubility of benzoic acid, hydrocortisone, and 5-methyl-5-phenylhydantoin was circumvented by using <2% absolute methanol.

Regression Analysis—For the regression analysis, a least-squares program was used to derive the equations listed in Table II and run on a computer through a remote terminal[4].

RESULTS AND DISCUSSION

The apparent and corrected partition coefficients of various drugs measured in this study are summarized in Tables I, III, and IV.

Bonner and Woolsey (42) showed that different solutes and temperature may affect the structure of water (*i.e.*, the percentage of unbonded water molecules and the number of water molecules on different solutes), which undoubtedly affects the solubility of a drug in the aqueous phase and the organic–aqueous partition coefficient. The buffer and water may form a complex structure, and since the buffer has a counterion function (ion-pair function), it may affect the physicochemical properties of the drug. The pH value of distilled water changes by ~2.5 when a certain amount of acidic drug is dissolved in the water layer. Therefore, after partitioning, the final pH value of the water layer has to be determined by a pH meter. Equations correlating the log P_{corr} values obtained from different systems with those from 1-octanol–water are summarized in Tables II and V and Figs. 1–3.

In Table II, according to the slopes and intercepts in the equations, acidic, basic, and neutral drugs should not be grouped together in a single equation in most cases. The correlation coefficients and standard deviations of separate equations are much better than those of the composite equation (compare Eqs. 1–3 with Eqs. 5–7, 8–10, and 11–13). Several basic drugs such as propanolol and labetalol were not soluble enough in an alkaline pH (9.2). Hence, the partition coefficients could not be determined by the conventional methodology; consequently, Eq. 7 has only six data points. For a basic drug with Eqs. 5, 6, and 7, the slopes are 0.870, 0.421, and 0.496, respectively; they are all less then one, and the intercepts are −1.253, 1.143, and 1.137, which do not approach zero. If the slope of an equation is approximately one and the intercept approaches zero, it may be interpreted that the ratio of the true partition coefficient of the drug in octanol–water and in octanol–buffer is 1:1. In this case, the true partition coefficient, which was measured in octanol–buffer, is equivalent to that obtained in octanol–water. These values could be utilized in quantitative structure–activity relationship studies without correction.

It may be seen from Eqs. 5–7 that no 1:1 ratio exists between the three systems examined. Equation 5 has a higher slope and a lower intercept compared to Eqs. 6 and 7. It is suggested that the acetate buffer interacts with the protonated form of basic drug molecules and increases their lipophilicity. On the other hand, phosphate buffer and bicarbonate buffer attract basic drug molecules in the aqueous layer. For acidic drugs, according to the slope (0.984) and the intercept (0.081) from Eq. 9, the measured partition coefficients of acidic drugs in phosphate buffer as well as in octanol–water can be combined in the quantitative structure–activity relationship work. The partition coefficient of the acidic drug measured in acetate buffer or in bicarbonate buffer must be corrected with the necessary equations before extrapolation to octanol–water.

For neutral drugs, the slopes of Eqs. 11, 12, and 13 are 0.988, 0.992, and 0.997, respectively, all of which are very close to one, and the intercepts are −0.020, −0.045, and −0.025, respectively, which are nearly zero. The measured partition coefficients of neutral drugs in any of the three buffer systems will give similar results to the octanol–water system. The slope of Eq. 4 is 0.985, which is close to unity, and the intercept is 0.015, which approaches zero. Therefore, the partitioning behavior of the drugs analyzed in octanol–tris(hydroxymethyl)aminomethane is the same as in octanol–phosphate.

The ability of counterions in different buffers to affect the mechanism of intermolecular or intramolecular bonding forces in the partitioning behavior presents a complex problem. In the measurement of the partition coefficients of drugs, many different solvent systems have been used to ensure proper solubility of the drug. Equations for various organic solvent systems were published by Hansch and Leo (21, 22). It is proposed that the equations obtained from this study, as outlined in Table II, be used to correct for different buffer systems. These equations should be important in quantitative structure–activity relationship studies since no previous systematic study with different buffers has been reported.

Salicylic acid may be used to illustrate the complex phenomenon of molecular interaction in partitioning. According to the Debye–Hückel equation (43), the errors in the measurement of the partition coefficient of salicylic acid and phenformin might be attributed to the difference between pKa and pKa′ due to the ionic strength of the electrolytes.

[1] Phenolsulfonphthalein, 1-octanol (reagent grade), and salicylic acid were purchased from Fisher Scientific Co. Atropine sulfate and hydrocortisone were purchased from Sigma Chemical Co. Benzoic acid was purchased from Pfizer, Inc. 3,5-Diamino-1,2,4-triazole was purchased from Aldrich Chemical Co. Thiosemicarbazide was purchased from Eastman Organic Chemicals. Phthalimide potassium was purchased from Matheson, Coleman & Bell. Theobromine and ephedrine hydrochloride were purchased from Merck & Co. Thiourea and sulfanilamide were purchased from Mallinckrodt Chemical Works. Procainamide hydrochloride was purchased from Pfaltz & Bauer. Sulfadiazine sodium was purchased from City Chemical Corp. Diphenhydramine, propranolol, labetalol, atenolol, metoprolol, and methapyrilene hydrochloride were kindly provided by Dr. S. H. Wan, School of Pharmacy, University of Southern California. 5-Methyl-5-phenylhydantoin was kindly provided by Dr. J. Cohen, School of Pharmacy, University of Southern California. Trimethylene thiourea, *N,N′*-diethylethylene thiourea, butylene thiourea, and ethylene thiourea were synthesized by Lien (37).

[2] Beckman 3500.

[3] Perkin-Elmer double-beam spectrophotometer or Coleman 124.

[4] IBM 370/155 computer *via* an IBM 4012 RJE terminal.

Table I—log P_{app} and log P_{corr} of Various Drugs in Octanol–Phosphate and in Octanol–Bicarbonate

Drug	pKa	1-Octanol–Phosphate Buffer (pH 7.4)			1-Octanol–Bicarbonate Buffer (pH 9.2)		
		$1-\alpha$	log P_{app}	log P_{corr} ($\pm SD$)	$1-\alpha$	log P_{app}	log P_{corr} ($\pm SD$)
Diphenhydramine	8.98	0.025640	1.60	3.20 (0.02)	—	—[a]	—[a]
Propranolol	9.45	0.008836	1.24	3.29 (0.01)	—	—[a]	—[a]
Phenformin	11.80	0.000040	−1.47	2.94 (0.02)	0.002562	−0.96	1.64 (0.02)
Labetalol	9.45	0.008836	1.13	3.18 (0.01)	—	—[a]	—[a]
Atropine sulfate	9.25	0.013933	−0.06	1.24 (0.01)	0.471500	0.08	1.14 (0.02)
Metoprolol	9.68	0.005223	−0.25	2.04 (0.02)	0.248700	1.62	2.22 (0.02)
Atenolol	9.45	0.008836	−1.62	0.43 (0.01)	0.360205	−0.37	0.08 (0.01)
Methapyrilene	8.90	0.030679	1.29	2.81 (0.02)	—	—[a]	—[a]
Procainamide	9.24	0.014254	−1.33	0.51 (0.01)	0.476985	0.37	0.65 (0.01)
Ephedrine	9.63	0.005989	−1.35	0.87 (0.02)	0.271191	0.46	1.02 (0.02)
Phenolsulfonphthalein	7.90	0.759927	−1.60	−1.45 (0.03)	0.047727	−2.15	−0.83 (0.03)
Salicylic acid	2.75	0.000447	−1.62	1.73 (0.03)	0.00000036	−1.27	(5.18)[b] (0.02)
Sulfanilamide	10.43	0.999068	−0.73	−0.73 (0.03)	0.944439	−0.74	−0.71 (0.01)
Benzoic acid	4.34	0.000863	−1.34	1.72 (0.03)	0.000014	−2.46	2.43 (0.03)
Theobromine	10.05	0.997741	−0.72	−0.72 (0.01)	0.875001	−0.81	−0.75 (0.03)
Phthalimide	8.30	0.984078	−1.47	−1.47 (0.02)	0.112045	−2.57	−1.60 (0.01)
5-Methyl-5-phenylhydantoin	8.33	0.999226	1.01	1.01 (0.01)	0.119040	0.51	1.44 (0.03)
Sulfadiazine sodium	6.48	0.107342	−1.00	−0.03 (0.02)	0.001902	−2.12	0.06 (0.02)
N,N'-Trimethylene thiourea	—	1.00	−0.65	−0.66 (0.02)	1.00	−0.71	−0.72 (0.02)
N,N'-Diethylethylene thiourea	—	1.00	1.16	1.17 (0.01)	1.00	1.11	1.11 (0.01)
N,N'-Ethylene thiourea	—	1.00	−0.65	−0.65 (0.01)	1.00	−0.65	−0.65 (0.02)
Thiourea	—	1.00	−0.97	−0.97 (0.02)	1.00	−0.98	−0.98 (0.02)
Thiosemicarbazide	—	1.00	−1.26	−1.26 (0.02)	1.00	−1.25	−1.25 (0.01)
N,N'-Butylene thiourea	—	1.00	−0.09	−0.09 (0.01)	1.00	−0.10	−0.10 (0.01)
Hydrocortisone	—	1.00	1.53	1.53 (0.01)	1.00	1.51	1.51 (0.01)

[a] Not determined due to limited solubility. [b] This was probably due to the error in high α and low $1-\alpha$; see *Discussion*.

Table II—Equations Correlating log P_{corr} in 1-Octanol–Water with that in 1-Octanol–Buffer

Equation	n[a]	r[b]	s[c]
All Drugs Pooled Together			
(1) log P (octanol–water) = −0.260 + 0.685 log P (octanol–acetate buffer, pH 4.0)	23	0.941	0.541
(2) log P (octanol–water) = 0.122 + 0.892 log P (octanol–phosphate buffer, pH 7.4)	24	0.947	0.504
(3) log P (octanol–water) = 0.037 + 1.029 log P (octanol–bicarbonate buffer, pH 9.2)	20	0.939	0.480
(4) log P (octanol–phosphate buffer, pH 7.4) = 0.015 + 0.985 log P [octanol–tris(hydroxymethyl)aminomethane buffer, pH 7.4]	12	0.997	0.109
For Basic Drugs			
(5) log P (octanol–water) = −1.253 + 0.870 log P (octanol–acetate buffer, pH 4.0)	9	0.901	0.257
(6) log P (octanol–water) = 1.143 + 0.421 log P (octanol–phosphate buffer, pH 7.4)	10	0.954	0.167
(7) log P (octanol–water) = 1.137 + 0.496 log P (octanol–bicarbonate buffer, pH 9.2)	6	0.870	0.235
For Acidic Drugs			
(8) log P (octanol–water) = −0.217 + 1.156 log P (octanol–acetate buffer, pH 4.0)	7	0.989	0.292
(9) log P (octanol–water) = 0.081 + 0.984 log P (octanol–phosphate buffer, pH 7.4)	7	0.980	0.291
(10) log P (octanol–water) = −0.304 + 0.895 log P (octanol–bicarbonate buffer, pH 9.2)	7	0.967	0.375
For Neutral Drugs			
(11) log P (octanol–water) = −0.020 + 0.988 log P (octanol–acetate buffer, pH 4.0)	7	0.998	0.070
(12) log P (octanol–water) = −0.045 + 0.992 log P (octanol–phosphate buffer, pH 7.4)	7	0.999	0.061
(13) log P (octanol–water) = −0.025 + 0.997 log P (octanol–bicarbonate buffer, pH 9.2)	7	0.999	0.052

[a] Number of data points used in the regression analysis. [b] Correlation coefficient. [c] Standard deviation of regression (standard error of estimate).

Table III—log P_{app} and log P_{corr} of Various Drugs in Octanol–Acetate and in Octanol–Water

Drug	pKa	1-Octanol–Acetate Buffer (pH 4.0)			1-Octanol–Water		
		$1-\alpha$	log P_{app}	log P_{corr} ($\pm SD$)	$1-\alpha$	log P_{app}	log P_{corr} ($\pm SD$)
Diphenhydramine	8.98	0.000010	−0.61	4.37 (0.01)	0.000926	−0.27	2.76 (0.02)
Phenformin	11.80	0.00000008	−0.95	(6.12)[a] (0.01)	0.000140	0.03	2.43 (0.02)
Atenolol	9.45	0.000035	−1.84	2.62 (0.02)	0.002812	−0.05	1.21 (0.03)
Atropine sulfate	9.25	0.000006	−1.83	3.42 (0.02)	0.003970	−0.59	1.81 (0.02)
Propranolol	9.45	0.000035	−0.23	4.22 (0.01)	0.002812	−1.40	2.41 (0.03)
Metoprolol	9.68	0.000002	−1.72	3.96 (0.02)	0.001857	−0.71	2.01 (0.02)
Labetalol	9.45	0.000035	−0.42	4.03 (0.01)	0.002812	−1.27	2.51 (0.02)
Procainamide	9.24	0.000006	−2.18	3.06 (0.02)	0.005104	−1.11	1.19 (0.01)
Methapyriline	8.90	0.000013	−1.10	3.80 (0.01)	0.011115	0.22	2.17 (0.01)
Ephedrine	9.63	0.000002	−1.71	3.93 (0.01)	0.000229	−1.96	1.70 (0.02)
Salicylic acid	2.75	0.053240	1.60	2.87 (0.01)	0.025626	1.10	3.10 (0.02)
Phenolsulfonphthalein	7.90	—	—	−1.16[b] (0.04)	0.788070	−1.77	−1.66 (0.03)
Sulfadiazine sodium	6.48	—	—[c]	—[c]	0.705874	−0.27	−0.08 (0.01)
Sulfanilamide	10.43	0.999963	−0.70	−0.70 (0.02)	0.999898	−0.75	−0.75 (0.02)
Benzoic acid	4.34	0.685211	1.82	1.98 (0.02)	0.369592	1.61	2.04 (0.02)
5-Methyl-5-phenylhydantoin	8.33	0.999953	1.03	1.03 (0.03)	0.999226	1.01	1.01 (0.01)
Theobromine	10.05	0.9999991	−0.68	−0.68 (0.01)	0.999985	−0.69	−0.69 (0.02)
Phthalimide	8.30	0.999950	−0.66	−0.66 (0.03)	0.984078	−1.47	−1.47 (0.02)
N,N′-Trimethylene thiourea	—	1.00	−0.74	−0.74 (0.02)	1.00	−0.76	−0.76 (0.02)
Thiourea	—	1.00	−0.96	−0.96 (0.02)	1.00	−1.09	−1.09 (0.01)
N,N′-Diethylethylene thiourea	—	1.00	1.14	1.14 (0.01)	1.00	1.11	1.11 (0.01)
N,N′-Ethylene thiourea	—	1.00	−0.65	−0.65 (0.02)	1.00	−0.65	−0.65 (0.01)
Hydrocortisone	—	1.00	1.52	1.52 (0.02)	1.00	1.48	1.48 (0.01)
N,N′-Butylene thiourea	—	1.00	−0.09	−0.09 (0.01)	1.00	−0.09	−0.09 (0.01)
Thiosemicarbazide	—	1.00	−1.31	−1.31 (0.01)	1.00	−1.22	−1.22 (0.01)

[a] This was probably due to the error in high α and low $1-\alpha$; see *Discussion*. [b] E. J. Lien, *Drug Intell.*, **4**, 7 (1970). [c] Not determined due to limited solubility.

Table IV—log P_{app} and log P_{corr} of Various Drugs in Octanol–Tris(hydroxymethyl)aminomethane

Drug	pKa	1-Octanol–Tris(hydroxymethyl)aminomethane (pH 7.4)		
		$1-\alpha$	log P_{app}	log $P_{corr} \pm SD$
Propranolol	9.45	0.008836	1.33	3.39 ± 0.01
Atropine sulfate	9.25	0.013933	−0.05	1.31 ± 0.01
Labetalol	9.45	0.008836	1.08	3.13 ± 0.01
Diphenhydramine	8.98	0.025640	1.61	3.20 ± 0.02
Metoprolol	9.68	0.005223	−0.25	2.04 ± 0.01
Atenolol	9.45	0.008836	−1.78	0.27 ± 0.03
Methapyrilene	8.90	0.030679	1.35	2.87 ± 0.02
Procainamide	9.24	0.014254	−1.09	0.76 ± 0.01
Ephedrine	9.63	0.005989	−1.48	0.75 ± 0.02
Sulfadiazine sodium	6.48	0.107352	−1.00	−0.03 ± 0.02
N,N′-Trimethylene thiourea	—	1.00	−0.68	−0.68 ± 0.02
N,N′-Diethylethylene thiourea	—	1.00	1.13	1.13 ± 0.01

Therefore, pKa′ is introduced to correct for the ionic strength of electrolytes according to:

$$pKa' = pKa + \frac{0.51(2Z-1)\sqrt{\mu}}{1+\sqrt{\mu}}$$

where μ is the ionic strength of the electrolytes. In this study, the concentration of the buffer solution was 0.01 *M*. The second term in the equation contributed to the increase in the pKa′ value by ~0.05. However, the corrected value of α was not altered substantially. The value of pKa′ did not affect log P_{corr} of the drug in this study. The salicylic acid was dissolved in water, which resulted in a pH change of the solution from 6.8 to 4.33. According to $\alpha = 1/[1 + \text{antilog}(pKa - pH)]$, α is 0.9744. Therefore, it may be assumed that most of the drug molecules have ionized and subsequently are concentrated in the aqueous layer. This effect

Table V—Intercorrelation between log P_{corr} in Octanol–Water and log P_{corr} in Octanol–Buffer

			log P (octanol–water) = a log P (octanol–buffer) + b	
			a	b
Drug	Buffer	pH	(slope)	(intercept)
Basic	Acetate	4.0	0.870	−1.253
	Phosphate	7.4	0.421	1.143
	Bicarbonate	9.2	0.496	1.137
Acidic	Acetate	4.0	1.156	−0.217
	Phosphate	7.4	0.984	0.081
	Bicarbonate	9.2	0.895	−0.304
Neutral	Acetate	4.0	0.988	−0.020
	Phosphate	7.4	0.992	−0.045
	Bicarbonate	9.2	0.997	−0.025

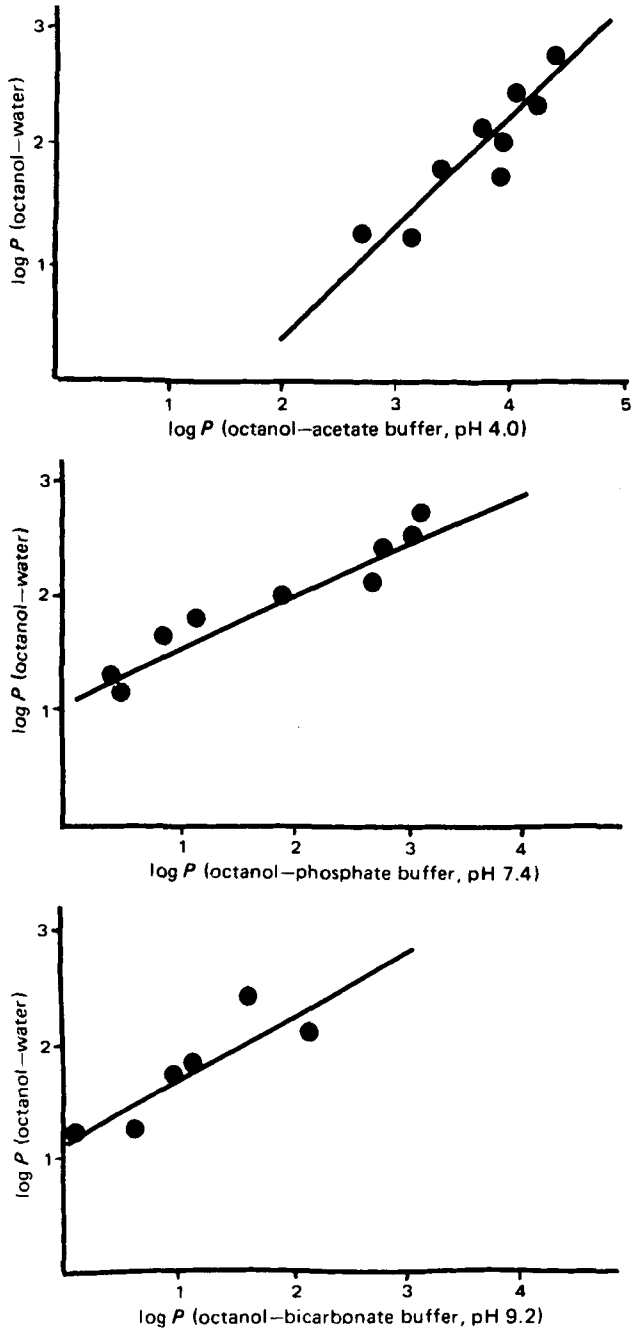

Figure 1—*Linear relationships between the corrected (true) partition coefficient in 1-octanol–water and that in 1-octanol–buffer for basic drugs.*

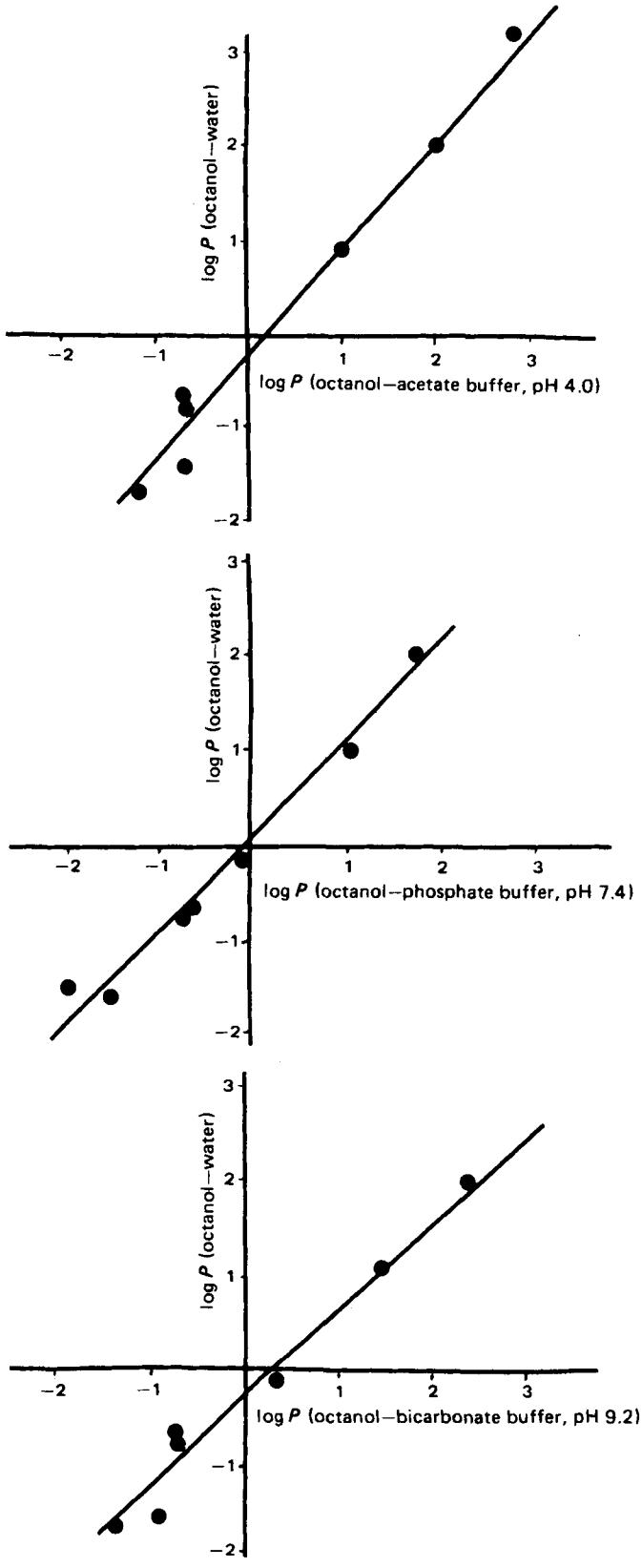

Figure 2—*Linear relationships between the corrected (true) partition coefficient in 1-octanol–water and that in 1-octanol–buffer for acidic drugs.*

would result in a very low value for the apparent partition coefficient. Consequently, the $1 - \alpha$ value is very small. A small error in $1 - \alpha$ will result in a large difference in log P_{corr}. If one utilizes π constants or fragment constants, f (44, 45), to calculate log P_{corr} of salicylic acid, the value appears to be much lower than that obtained in Table I. It appears

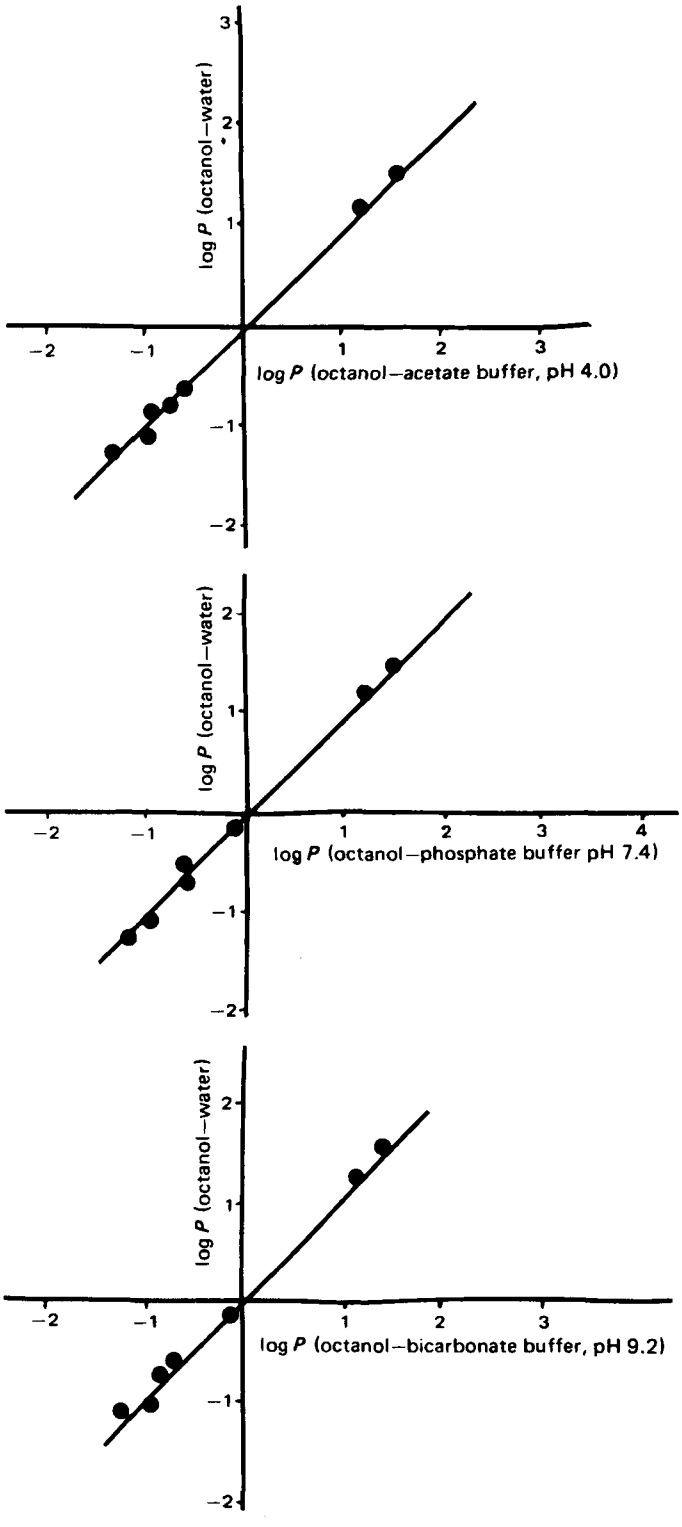

Figure 3—*Linear relationships between the corrected (true) partition coefficient in 1-octanol–water and that in 1-octanol–buffer for neutral drugs.*

to be an outlier on the statistical line. This result may be attributed to entrapment of the ionized species by the small amount of the buffer solution dissolved in the octanol phase. Phenformin, a basic drug highly ionized at low pH, presents the same problem. Consequently, both of these outliers were deleted from the appropriate equations.

Both water and buffer species have their own intrinsic capacity, which may affect the partitioning behavior of the drug. A comparison of acetate buffer, tris(hydroxymethyl)aminomethane buffer, phosphate buffer, and bicarbonate buffer reveals that the phosphate buffer is similar to water, according to Eqs. 6, 9, and 12. All of these equations have high correlation coefficients. It may be concluded that phosphate buffer (pH 7.4) is appropriate to keep the constant pH in the measurement of the partition coefficient of neutral and acidic drugs as long as they are soluble enough in the system.

CONCLUSION

These findings have significant bearing on quantitative structure–activity relationship studies; *i.e.*, the true partition coefficients (log P_{corr}) of acidic and basic drugs obtained from different buffer systems may not be suitable for regression analysis without correction. For acidic and neutral drugs, the phosphate buffer (pH 7.4) appears to give log P_{corr} values closer to those obtained from octanol–water than acetate and bicarbonate buffers. Therefore, the phosphate buffer system probably is the most suitable system for measuring true (or corrected) partition coefficients for quantitative structure–activity relationship work as long as the drug is soluble enough in this system. For basic drugs, any discrepancies must be corrected when different buffer systems are used in the measurement of partition coefficients.

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Effect of Polybasic Acids on Structure of Aluminum Hydroxycarbonate Gel

MING K. WANG *, JOE L. WHITE *, and STANLEY L. HEM ‡x

Received September 24, 1979, from the **Department of Agronomy and the* ‡*Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN 47907.* Accepted for publication January 11, 1980.

Abstract □ The effect of oxalic acid, citric acid, and their sodium salts on the structure of aluminum hydroxycarbonate gel was studied to illustrate the various mechanisms by which polybasic acids interact with aluminum hydroxycarbonate gel. Analysis of changes in the pH-stat titrigram, the carbonate to aluminum ratio, the aluminum content of the supernate following centrifugation, and the adsorption of the organic solute indicates that polybasic acids may: (*a*) partially neutralize the aluminum hydroxycarbonate gel by a general acid effect, (*b*) modify the aluminum hydroxycarbonate surface by adsorption of the anionic form of the polybasic acid, (*c*) extract aluminum by the formation of a soluble complex, and (*d*) lead to the precipitation of a new crystalline phase.

Keyphrases □ Polybasic acids—oxalic acid, citric acid, and their sodium salts, effect on aluminum hydroxycarbonate gel structure, complexation of polybasic acids with aluminum, adsorption of polybasic anion, acid neutralization □ Complexation—polybasic acids and their sodium salts with aluminum hydroxycarbonate gel, acid neutralization, structural changes in aluminum hydroxycarbonate gel □ Acid neutralization—complex formation with polybasic acids and aluminum hydroxycarbonate gel □ Aluminum hydroxycarbonate—complexation with polybasic acids and their sodium salts, structural changes, acid neutralization

Citric acid, oxalic acid, and other polybasic acids can inhibit the crystallization of aluminum hydroxide and form complexes with aluminum (1–3). Gibaldi and Mufson (4) found that the neutralization of dried aluminum hydroxide gel was inhibited when sodium citrate or sodium tartrate was added to the 0.1 *N* HCl used as the reaction medium. It was concluded that dried aluminum hydroxide gel reacts with sodium citrate or sodium tartrate to produce a water-soluble complex as well as to reduce surface area by flocculation.

Polybasic acids are important in soil-weathering reactions due to their role in complexing and solubilizing aluminum (5, 6). In addition, it was found that the edge faces of gibbsite (7), as well as synthetic goethite (8), can adsorb oxalate anion.

The nature of the interaction of polybasic acids with the surface of aluminum hydroxycarbonate has not been examined in detail. This study describes the mechanisms by which polybasic acids interact with aluminum hydroxycarbonate and the effect of these interactions on the acid reactivity of aluminum hydroxycarbonate gel.

EXPERIMENTAL

An aluminum hydroxycarbonate gel that exhibited a moderate rate of acid neutralization was selected so that any increase or decrease in the acid neutralization rate would be observed easily. The gel was amorphous to X-rays and had an IR spectrum characteristic of aluminum hydroxycarbonate gel (9).

Mixtures containing 0.2 *M* equivalent aluminum oxide and either 0.1 or 0.2 *M* polybasic acid or salt were prepared by dissolving the polybasic acid or salt in water and adding the solution to an appropriate quantity of aluminum hydroxycarbonate gel with mixing.

The acid neutralization reaction was monitored by an automated pH-stat titration at pH 3.0 and 25° (10).

The carbonate content was determined by gasometric displacement using the Chittick apparatus[1] (11) and is expressed as the molar ratio of carbonate to aluminum.

The aluminum content of the supernate was determined by centrifuging at 15,000 rpm (27,000×*g*) for 30 min and measuring the aluminum content of the supernate by atomic absorption spectroscopy. The result represents the aluminum-ion content as well as the aluminum content of hydroxyaluminum polymers that remain in the supernate after centrifugation. The aluminum content of the supernate depended on the conditions of centrifugation, and all results reported were obtained under carefully standardized conditions.

The distribution of the organic solute was monitored by analyzing both the supernate and the solid phase for organic carbon content by the Walkley–Black method (12). In every case, the sum of the organic carbon in solution and in the solid phase was 82–101% of the theoretical carbon content.

The solid phase that formed when 0.2 *M* citric acid was added to the aluminum hydroxycarbonate gel was analyzed by X-ray diffraction[2] and IR spectroscopy. A sample of the solid phase was vacuum dried over phosphorus pentoxide and ground to a fine powder with an agate mortar

[1] Sargent-Welch Scientific Co., Skokie, Ill.
[2] Siemens AG Kristalloflex 4 generator, type F diffractometer, Karlsruhe, West Germany.

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