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[x] To whom inquiries should be directed.

# Oil–Water Distribution of *p*-Alkylpyridines

**KUANG C. YEH * and WILLIAM I. HIGUCHI [x]**

**Abstract** □ The distribution of a homologous series of *p*-alkylpyridines between water and six organic solvents with varying degrees of polarity was investigated. The distribution coefficients were considered as reflections of the strength of net interactions involved in the solvents. The order was chloroform > octanol > carbon tetrachloride > butyl ether > hexadecane > octane. The effect of the methylene group upon the distribution coefficients differed little among the six solvents. The relative constancy was attributed to the predominance of the dispersion forces in the incremental effect.

**Keyphrases** □ Alkylpyridines—distribution between water and six organic solvents, distribution and partition coefficients □ Distribution coefficients—*p*-alkylpyridines, water and six organic solvents, effect of methylene group □ Partition coefficients—*p*-alkylpyridines, water and six organic solvents □ Pyridines, *p*-alkyl—distribution and partition coefficients, water and six organic solvents

The distribution behavior of solutes between two immiscible phases is a phenomenon important to numerous pharmaceutical and biological situations. The partition principle has been utilized in various chromatographic and extraction techniques common to many analytical pharmaceutical chemistry procedures. In biopharmaceutics, the pH–partition principle is basic to the theories and concepts of drug absorption, tissue distribution, renal reabsorption, and other membrane transport situations important to bioavailability and therapeutics. The partitioning of organic compounds between water and various oil phases has been studied to correlate biological activities (1–10).

Earlier systematic investigations of partition coefficients employed several alcohols and ethyl ether as the oil phases (11, 12); in general, the partition coefficients of different solutes differed considerably less in the butanol–water system than in the ether–water system. Depending on the nature of the solute, the following linear relationship was observed between the partition coefficient obtained from the ether system and that obtained from the butanol system (11, 12):

$$\log (k_{\text{butanol}}) = A \log (k_{\text{ether}}) + B \qquad \text{(Eq. 1)}$$

where the $k$'s are the partition coefficients, and $A$ and $B$ are constants. Later, this relationship was shown to hold between other solvent pairs (13, 14) when the partition coefficients of a number of barbiturates between water and various organic solvents were studied.

The partitioning of compounds in several alkyl homologous series between several organic solvents and semiaqueous solutions has been studied (15). A linear relationship was found between the logarithm of the partition coefficient and the number of carbons in the chain:

$$\log (k) = A + Bn \qquad \text{(Eq. 2)}$$

where $A$ and $B$ are constants, and $n$ equals the number of carbons. The equation did not hold for the lower terms of the homologous series ($n < 3$ or 4). The deviation was ascribed to the inductive effect in which the interactions between the parent group and the adjacent $CH_2$ groups changed the physical properties of these groups.

Equation 2 implies that $\log (k)$ is additive; *i.e.*, $\log (k)$ is equal to the sums of the contribution from each group in the molecule. Using octanol and water as the partitioning phases, Hansch and Anderson (16, 17) defined a substituent constant ($\pi$) as follows:

$$\pi_x = \log (k_x) - \log (k_o) \qquad \text{(Eq. 3)}$$

where $k_o$ is the partition coefficient of a parent compound between octanol and water, and $k_x$ is that for a derivative. Tables of $\pi$ values for various functional groups have been compiled (16–19). The $\pi_x$ for the $CH_2$ group has a value of 0.5.

The primary purpose of this research was to con-

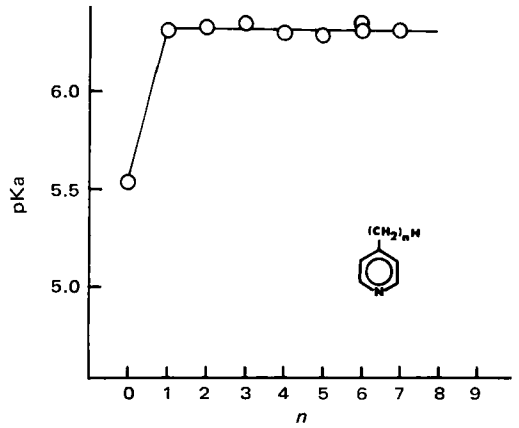


**Figure 1**—*Apparent pKa of 4-alkylpyridines in 1* M *NaCl at 25°.*

sider the oil–water partitioning of an organic alkyl series. Emphasis was to be placed upon developing useful theoretical concepts and methods jointly with meaningful and accurate experimental procedures. The basic objectives included obtaining experimental data of high accuracy and analyzing them by the appropriate concepts and theoretical relationships in terms of the different solute–solute, solvent–solvent, and solute–solvent interactions. Of special interest was the incremental effects of the $CH_2$ group upon the distribution coefficient as a function of the nature of the organic solvent.

For these purposes, the *p*-alkylpyridine homologous series ranging from pyridine to decylpyridine was chosen as solutes, and six organic solvents with varying degrees of complexity were selected: octane, hexadecane, butyl ether, carbon tetrachloride, chloroform, and octanol. The present article describes the experimental methods and the results obtained. The employment of Scatchard–Hildebrand-type theory (20) and some suggested modifications (21) will be reported[1].

## EXPERIMENTAL

**Materials**—Reagent grade pyridine[2], 4-picoline[3], 4-ethylpyridine[3], and 4-propylpyridine[3] were obtained commercially. The higher homologs (4-butyl- to 4-decylpyridine) were synthesized according to the method of Wilbaut and Hey (22).

Pyridine and picoline were purified by recrystallizing the corresponding hydrochloride salts twice in absolute alcohol (23). Ethyl- and propylpyridines were purified by single distillation. The higher homologs were purified through partition chromatographic columns, using deactivated silica gel[4] (100–200 mesh) as the stationary phase. The silica gel was saturated with 0.6 *M* aqueous sodium citrate buffer (approximately 370 ml/kg of the powder). The pH of the buffer had been preadjusted to produce suitable $R_f$ values. Normal heptane was used as the mobile phase.

The elutions were made on an automatic fraction collector[5], and the eluents were scanned in the range of 230–300 nm[6]. The impurities were detected by the shape of their UV spectra and were separated from the desired fraction (24).

**Table I—Some Experimental Constants of Alkylpyridines**

| Chain Length | pKa[a] | CMC, m*M*[b] |
|---|---|---|
| Pyridine | 5.539 | — |
| C-1 | 6.323 | — |
| C-2 | 6.338 | — |
| C-3 | 6.357 | — |
| C-4 | 6.314 | — |
| C-5 | 6.304 | — |
| C-6 | 6.345 | — |
| C-7 | 6.331 | 50 |
| C-8 | — | 17 |
| C-9 | — | 5 |
| C-10 | — | 1.5 |

[a] For alkylated derivatives, the average value was taken to be 6.332 in 1 *M* NaCl. [b] In 1 *M* NaCl.

**Solvents**—Six organic solvents were used as the nonaqueous phase in the partitioning measurements. The aqueous phase contained 1 *M* NaCl as the swamping electrolyte together with 0.01 *M* each of the citrate and phosphate buffers. The six solvents were *n*-hexadecane, *n*-octane, carbon tetrachloride, *n*-butyl ether, chloroform, and 1-octanol[7]. They were chosen because of their low mutual solubility with water. Octane and hexadecane were further purified by shaking with chromic–sulfuric acid solution[8] until the extract did not show any green color and then washing three times with double-distilled water. All other solvents were used without further treatment.

**Determination of pKa**—The apparent dissociation constant, $K_a$, for each alkylpyridine conjugate acid in 1 *M* NaCl was determined at 25° by a spectrophotometric method (25) (Table I and Fig. 1). Measurements of pH were made[9].

**Critical Micelle Concentration (CMC) Determinations**—In measuring the apparent partition coefficients, the solute concentrations in the aqueous phase were always kept below the CMC of the corresponding conjugate acid. The CMC of each protonated alkylpyridine in the aqueous phase was determined at room temperature using a modified drop volume method.

In the CMC determinations, a 2-ml pipet, graduated to 0.01 ml and with ground tip, was attached vertically to an automatic titrator[10]. The pipet was filled with the desired test solution, and the solution was delivered at a constant rate of 0.18 ml/min. At this slow speed, distinctive drops were formed at the tip. Results are shown in Table I.

**Measurement of Partition Coefficients**—Aliquots (about 0.01–0.1 ml) of alkylpyridine stock solutions (0.1 *M* as hydrochloride salt in 1 *M* NaCl) were added to 10-25-ml glass-stoppered

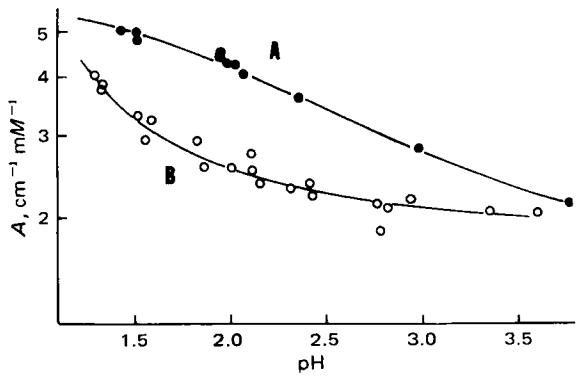


**Figure 2**—*Calibration curves for determining solute concentrations in the oil phase where significant amounts of ion-pairs are present. Key: A, chloroform phase; and B, octanol phase.*

[1] K. C. Yeh and W. I. Higuchi, *J. Pharm. Sci.*, in press.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] K & K Laboratories, Plainview, N.Y.
[4] Davison Chemical, Baltimore, Md.
[5] Gilson Medical Electronics, Middletown, Wis.
[6] Cary model 14 recording spectrophotometer, Cary Instruments, Monrovia, Calif.
[7] All from Matheson, Coleman & Bell, Norwood, Ohio. Hexadecane, carbon tetrachloride, and butyl ether were of spectrograde, octane was of chromatographic grade, and chloroform was of reagent grade.
[8] Fisher Scientific Co., Fair Lawn, N.J.
[9] Beckman model 1019 research pH meter, Beckman Instruments, Fullerton, Calif.
[10] Sargent-Welch Scientific Co., Skokie, Ill.

**Table II—Mutual Solubilities between Water and Six Organic Solvents**

| Oil Phase | Mutual Solubility, moles/liter | |
|---|---|---|
| | Oil in Water | Water in Oil |
| Hexadecane | — | 0.003[a] |
| Octane | — | 0.0047[b] |
| Butyl ether | 0.017[b] | 0.0795 |
| Octanol | 0.0041[c] | 0.758 |
| Carbon tetrachloride | 0.005[c] | 0.0087[e] |
| Chloroform | 0.069[d] | 0.072[f] |

[a] Reference 26. [b] Estimated. [c] Reference 27. [d] 20°, Ref. 27. [e] Reference 28. [f] Reference 29.

flasks containing 1–10 ml of the desired oil phase and 5–23 ml of the aqueous phase. The aqueous phase had been made alkaline or been preadjusted to the desired acid pH using the mixed buffers. The contents were shaken[11] for 24–48 hr at 25 ± 0.1°. For the octanol–aqueous system, the shaking speed was set at or below 100 rpm to avoid emulsion formation. The solute concentrations in both phases, usually in the range of 0.02–5 m*M*, were determined spectrophotometrically using diluted stock solutions as the reference.

When significant amounts of ion-pairs were present in the octanol or chloroform phase, solute concentrations in the nonaqueous layer were determined by a modified mass balance computation. This procedure was necessary because the UV spectrum of the ion-pair differed from that of the free base. In this case, the solute concentrations in the aqueous phase were determined as usual. By using the mass balance relationship, the solute concentrations in the oil phase were estimated. The spectra of the corresponding oil layers were taken, and the apparent absorptivities at $\lambda_{max}$ were computed. These values were plotted against the aqueous pH on a semilog basis ($\lambda_{max}$ varied with pH). A smooth curve was constructed, and it provided the Beer's law coefficients for determining the solute concentrations in the oil phase. As can be seen in Fig. 2, this procedure reduces random errors when a mass balance method is used to calculate the partition coefficient.

**Solubility of Water**—The solubility of water in octanol and in butyl ether was determined by the Karl Fischer method[12] after the solvents were saturated with double-distilled water by shaking[11]. The results are shown in Table II together with other solubility data.

**Determination of Molar Volumes**—Apparent densities of the solutes were determined by weighing a 1-ml sample of each alkylpyridine at room temperature. The values were then normalized and smoothed to the corresponding values at 25°, using pyridine as the reference (0.978 g/ml) (Fig. 3 and Table III).

## RESULTS AND DISCUSSION

**Apparent Partition Coefficients**—Preliminary experiments showed that when the chain length of the alkyl group is greater than five or six, its intrinsic partition coefficient becomes so high that there will be significant error in measuring it directly. Instead, by determining the apparent partition coefficient ($k'$) in the acid region, its intrinsic partition coefficient ($k_t$) may be estimated more accurately by extrapolating to a high pH. The two quantities are defined as:

$$k' = [B]_o/([B]_w + [HB^+]_w) \quad \text{(Eq. 4)}$$

$$k_t = [B]_o/[B]_w \quad \text{(Eq. 5)}$$

$$K_a = [B]_w[H^+]_w/[HB^+]_w \quad \text{(Eq. 6)}$$

where $[B]_o$ and $[B]_w$ are the concentrations of the free solute base in the oil phase and in the water phase, respectively; $[HB^+]_w$ is the concentration of the protonated conjugate acid; $[H^+]_w$ is the hydrogen-ion concentration, and $K_a$ is the apparent dissociation constant of the conjugate acid in 1 *M* NaCl. All concentrations are expressed in units of moles per liter.

**Table III—Densities and Molar Volumes of Alkylpyridines at 25°**

| Chain Length | Molecular Weight | Density, g/ml[a] | Molar Volume, ml/mole |
|---|---|---|---|
| Pyridine | 79.10 | 0.978 (0.978) | 80.88 |
| C-1 | 93.13 | 0.961 (0.961) | 96.91 |
| C-2 | 107.15 | 0.949 (0.947) | 113.15 |
| C-3 | 121.18 | 0.936 (0.934) | 129.74 |
| C-4 | 135.21 | 0.923 (0.923) | 146.49 |
| C-5 | 149.23 | 0.909 (0.915) | 163.09 |
| C-6 | 163.26 | 0.908 (0.909) | 179.60 |
| C-7 | 179.29 | 0.907 (0.905) | 195.90 |
| C-8 | 191.32 | 0.902 (0.902) | 212.11 |
| C-9 | 205.34 | — (0.900) | 228.15 |
| C-10 | 219.37 | — (0.898) | 244.29 |

[a] Values in parentheses were estimated from Fig. 3.

The apparent partition coefficient may also be expressed as:

$$k' = k_t K_a/(K_a + [H^+]_w) \quad \text{(Eq. 4a)}$$

When the pH of the aqueous phase is in the acid region and is much below the apparent pKa of 6.332, Eq. 4*a* may be simplified to:

$$\log (k') = \log (k_t) - \text{pKa} + \text{pH} \quad \text{(Eq. 4b)}$$

Since $k_t$ and pKa are constant for any particular solute at constant temperature (25°), Eq. 4*b* predicts a linear relationship between log ($k'$) and pH with a slope of 1.0.

On the other hand, in the alkaline region where the pH is much above the pKa, the experimentally measured $k'$ is equal to the intrinsic $k_t$ values since Eq. 4*a* is reduced to:

$$\log (k') = \log (k_t) \quad \text{(Eq. 7)}$$

Figure 4 compares the experimental data of log ($k'$) in the carbon tetrachloride–aqueous phase pair and the theoretical curves generated from Eq. 4*a*.

One assumption in Eq. 4*a* is that the free base is always the only solute species in the oil phase throughout the pH range. Experimental results show that this is the case not only in carbon tetrachloride but also in hexadecane, octane, and butyl ether. By using

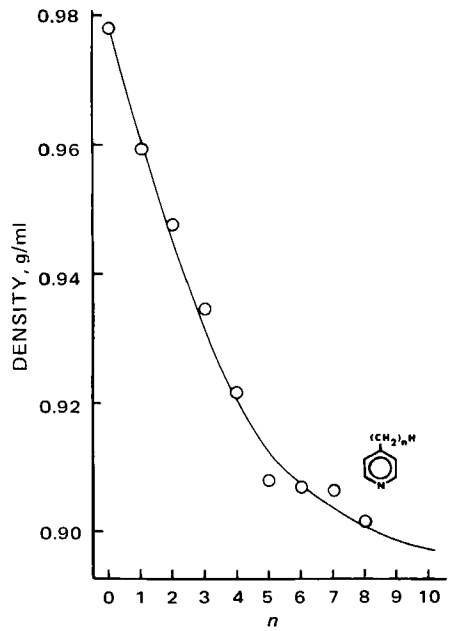


**Figure 3**—*Density of alkylpyridines as a function of the chain length at 25°.*

[11] Precision shaker bath, New Brunswick Scientific Co., New Brunswick, N.J.

[12] The determination was performed by Dr. Wenhai Hong, Pharmaceutical Development, Parke, Davis & Co., Detroit, MI 48232

Table IV—Logarithm of Partition Coefficients of Alkylpyridines between Various Organic Solvents and Aqueous 1 *M* NaCl at 25°

| Chain Length | Hexadecane | Octane | Butyl Ether | Octanol | Chloroform | Carbon Tetrachloride |
|---|---|---|---|---|---|---|
| Pyridine | −0.305 | −0.211 | 0.109 | 0.781 | 1.319 | 0.425 |
| C-1 | 0.126 | 0.213 | ).530 | 1.327 | 1.821 | 0.839 |
| C-2 | 0.741 | 0.829 | — | — | — | — |
| C-3 | — | — | — | — | — | — |
| C-4 | — | — | 2.373 | 3.128 | 3.737 | 2.812 |
| C-5 | 2.641 | 2.810 | — | 3.750 | 4.380 | 3.433 |
| C-6 | 3.274 | 3.448 | 3.617 | 4.348 | 5.006 | 4.114 |
| C-7 | 3.919 | 4.105 | — | 5.008 | 5.653 | — |
| C-8 | 4.569 | 4.794 | 4.894 | 5.542 | 6.331 | — |
| C-9 | 5.208 | 5.445 | 5.543 | 6.109 | — | 6.046 |
| C-10 | 5.839 | — | — | — | — | — |

this procedure, $k_t$ values for the higher alkylpyridine homologs were calculated (Table IV).

**Ion-Pair Effects**—When octanol and chloroform were used as the nonaqueous phases, different log ($k'$)–pH profiles were found. Figure 5 shows the results obtained in the octanol–water system. It differs from Fig. 4 in the low pH region where all curves are leveled off. This type of deviation from Eq. 4*b* has been attributed to the presence of ion-pairs in the oil phase (30).

**Intrinsic Distribution Coefficients**—The distribution coefficient $k$, defined as the ratio of the two mole fractions, is related to the experimentally determined partition coefficient $k_t$ (Eq. 5) as follows:

$$k = [X]_i/[X]_w = g_i k_t \qquad \text{(Eq. 8}a\text{)}$$

or:

$$\log (k) = \log (g_i) + \log (k_t) \qquad \text{(Eq. 8}b\text{)}$$

where:

$$g_i = V_i(1000d + M_w - M)/1000M_w \qquad \text{(Eq. 9)}$$

$[X]_i$ and $[X]_w$ are the mole fractions of the solute free base in the oil phase $i$ and in the aqueous phase $w$, respectively; $g_i$ is the ratio of the mole numbers per liter needed to convert $k_t$ into $k$; $M$ is the molecular weight of sodium chloride (58.45); $M_w$ is the molecular weight of water (18.01); $d$ is the density of the aqueous 1 *M* NaCl at 25° (1.037 g/ml); and $V_i$ is the molar volume of the organic phase. Values of $V_i$ and $g_i$ are shown in Table V.

Since $k$ also may be expressed as the reciprocal of the two activity coefficients:

$$k = \gamma_w/\gamma_i \qquad \text{(Eq. 10)}$$

the ratio of the two activity coefficients remains constant only when both mixtures are in the Henry's law region. Analysis of the data showed a constant log ($k$) in all six cases. Figure 6 gives a typical plot for the octane–aqueous phase system.

Figure 7 illustrates the relationship between log ($k$) and the chain length for the hexadecane–aqueous phase system. An examination of the other systems (Table VI) revealed that they all shared the same characteristic features: a linear limiting relationship between log ($k$) and chain length $n$ when $n$ is greater than 3 and small positive deviations when $n$ is less than 2.

Least-squares limiting slopes of each system are shown in the second column of Table VII. For convenience, these limiting slopes will be called the $\pi$ values of the $CH_2$ groups. As can be seen in Table VII, these $\pi$ values are all comparable. The constancy of the $\pi$ values suggests that the effect of the $CH_2$ group on the net interaction energies in these different nonaqueous solvents is approximately the same. This finding probably reflects the predominance of the dispersion forces in the net interaction between the $CH_2$ group and the solvent.

The activity coefficient terms in Eq. 10 are actual values when the solvents are mutually saturated. The effect of the organic solvents dissolved in the aqueous phase is likely to be negligible due to their low water solubility (Table II). This assumption is based on the reasoning that the effect is small compared to the large primary interactions between the solute and the water molecules. In addition, the solubility would be further reduced in the presence of 1 *M* NaCl. Therefore, the $\gamma_w$ may be taken as the value when there is no organic solvent in the aqueous phase.

Similarly, the solubility of water in these organic solvents, except octanol, is generally low. The effect of water in the organic solvent was studied by Johnson *et al.* (28). They found that, in the presence of water, pyridine may form a molecular complex with water whose equilibrium constant is in part a function of the intrinsic water solubility in that solvent.

The activity of water in 1 *M* NaCl at 25° is known to be 0.967 (33). If a linear relationship is assumed between its solubility and the activity, the hydration effect would not be significantly shifted by the small difference of 3.3% in the water solubility. A brief discussion of the hydration effect is presented in another paper[1].

The salt appears to have a strong effect on the distribution coefficient. Based on experimental data (28), the log ($k$) for pyridine in carbon tetrachloride–water has a value of 1.009. Our value of 1.155 represents an increase of 40% in the distribution coefficient or 0.146 on the log basis. The experimental log ($k$) for picoline in hexadecane–pure water was 1.158. A comparison with the corresponding value of 1.337 in 1 *M* NaCl shows an even greater change:

Table V—Conversion Factors and Molar Volumes of Six Organic Solvents

| Organic Solvent ($i$) | log ($g_i$) | $V_i$, ml |
|---|---|---|
| Hexadecane | 1.213 | 294.1 |
| Octane | 0.958 | 163.5 |
| Butyl ether | 0.974 | 170.3 |
| Octanol | 0.944 | 158.8 |
| Chloroform | 0.649 | 80.5 |
| Carbon tetrachloride | 0.730 | 96.8 |

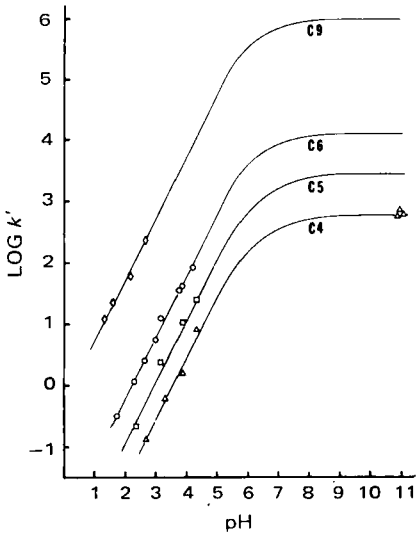


**Figure 4**—*Apparent partition coefficients of alkylpyridines between carbon tetrachloride and aqueous phase as a function of pH at 25°. Curves represent theory, and points represent experimental data.*

Table VI—Logarithm of Distribution Coefficients of Alkylpyridines between Six Organic Solvents and Aqueous 1 *M* NaCl[a]

| Chain Length | Hexadecane | Octane | Butyl Ether | Octanol | Chloroform | Carbon Tetrachloride |
|---|---|---|---|---|---|---|
| Pyridine | 0.906 | 0.745 | 1.083 | 1.724 | 1.975 | 1.155 |
| C-1 | 1.337 | 1.169 | 1.504 | 2.263 | 2.471 | 1.569 |
| C-2 | 1.940 | 1.792 | (2.118) | (2.865) | (3.100) | (2.227) |
| C-3 | (2.578) | (2.452) | (2.733) | (3.468) | (3.729) | (2.884) |
| C-4 | (3.216) | (3.113) | 3.347 | 4.071 | 4.357 | 3.542 |
| C-5 | 3.854 | 3.773 | (3.969) | 4.693 | 5.029 | 4.163 |
| C-6 | 4.487 | 4.414 | 4.591 | 5.291 | 5.655 | 4.844 |
| C-7 | 5.132 | 5.071 | (5.229) | 5.952 | 6.302 | (5.488) |
| C-8 | 5.782 | 5.752 | 5.868 | 6.485 | 6.980 | (6.132) |
| C-9 | 6.421 | 6.411 | 6.517 | 7.052 | — | 6.776 |
| C-10 | 7.052 | — | — | — | — | — |

[a] Values in parentheses were obtained by interpolation.

a 50% increase in $k$ or 0.179 on the log basis.

Recently, Davis *et al.* (32) compiled various literature $\pi$ values for the $CH_2$ group in a series of nonaqueous solvents. For comparison, some of their preferred values are reproduced in Table VII under the column headed "Water." The data from the present study are consistently higher, probably reflecting a significant effect of the electrolyte. Quantitative comparison of the salt effect may be made by using the empirical relation noted by Wilcox and Schrier (34). The effect of 1 *M* NaCl at 25° was found to increase the logarithm of the activity coefficient of ethanol by 0.1 unit. The corresponding value for 1-propanol was 0.15. If it is assumed that the salt effect is additive and composed of independent contributions from the constituent functional group, the difference of 0.05 may be regarded as the salting-out effect of 1 *M* NaCl on the $CH_2$ group. A comparison of this value with the differences in the data presented in Table VII indicates that these differences, although scattered and ranging from 0.03 to 0.11, are reasonable considering that they are taken from various studies.

The basis for the linear slope shown in Fig. 7 is well known. By definition:

$$RT \ln (\gamma_i) = \Delta G_i \qquad \text{(Eq. 11)}$$

where $\Delta G_i$ is the excess free energy of solution in the solvent $i$. That there are two steps in the dissolution of a solute molecule may be assumed. The first step is to remove the molecule from its own liquid. The second step is to place this molecule in the solvent after a cavity has been created in it. If the solute is increased by a $CH_2$ group, there is only a small difference in the two free energies of solution when the solvent is nonaqueous. However, there is a relatively large increase in the free energy of solution in the aqueous solution. This finding has been explained on the basis that, although the enthalpy of solution is favorably negative, it is more than counterbalanced by a very large negative entropy change on the immediately surrounding water molecules (35).

To compare the two activity coefficients shown in Eq. 10, it is only necessary to compare the free energy difference of the second step in the dissolution process. The difference in the two quantities is then equal to the free energy for the transfer of the $CH_2$ group from water to the organic solvent, $-\Delta G_{CH_2}$, and is related to the slope of Fig. 7 by the equation:

$$2.303RT\pi = -\Delta G_{CH_2} \qquad \text{(Eq. 12)}$$

While the $\pi$ values in Table VII are roughly comparable to each other, the differences between them are still significant. The trend of these $\pi$ values may be related to the relative magnitude of the net interactions between the solvent–solvent and the solvent–$CH_2$ group. However, the differences are so small that they are often neglected.

The small initial slopes observed in Fig. 7 when $n$ is less than 2 or 3 may be attributed to the inductive effects of the alkyl groups. The decrease in acidity of the alkylated derivatives as compared to the parent pyridine suggests that the electron density on the nitrogen atom is higher when the ring is alkylated. This is consistent with the observation that 4-picoline has a higher dipole moment than pyridine (36). Furthermore, the polar cohesive energy of picoline, estimated from the homomorph plot of Weimer and Prausnitz (21), was 1.49 kcal/mole, which is somewhat higher than the corresponding value of 1.11 kcal/mole for pyridine. Thus, the higher ring polarity might produce a less favorable net interaction with the nonaqueous solvent and, hence, the solvent would be less likely to transfer into the oil phase. As a result, there is a partial energy cancellation in Eq. 12 and a smaller initial slope observed in the log ($k$)–chain-length plot.

On the other hand, it can be seen from Table VI that the absolute magnitudes of log ($k$) differ greatly from one solvent pair to

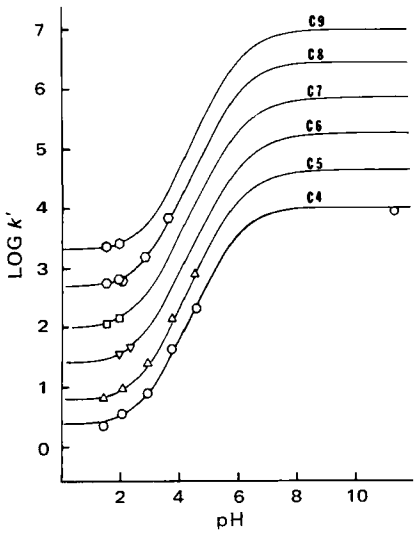


**Figure 5**—*Apparent partition coefficients of alkylpyridines between octanol and the aqueous phase as a function of pH at 25°. Curves represent theory, and points respresent experimental data.*

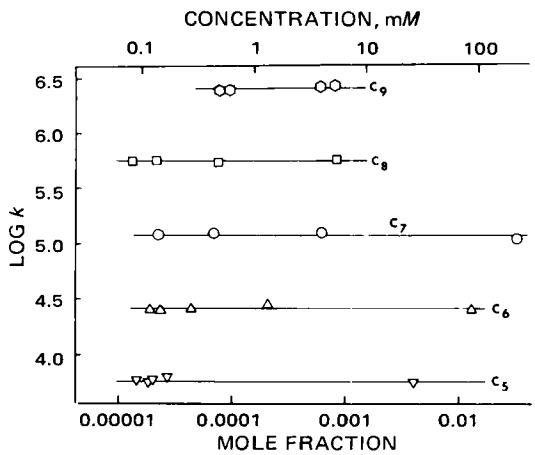


**Figure 6**—*Constancy of distribution coefficients as a function of concentration between octane and the aqueous phase.*

**Table VII—Logarithmic Distribution Coefficients of the Methylene Group at 25°**

| Organic Phase | 1 *M* NaCl | Water[a] |
|---|---|---|
| Hexadecane | 0.64 | 0.57[b] |
| Octane | 0.66 | 0.62 |
| Butyl ether | 0.63 | — |
| Octanol | 0.61[c] | 0.50 |
| Chloroform | 0.65 | 0.6–0.64 |
| Carbon tetrachloride | 0.65 | 0.62 |

[a] Reference 32. [b] 20°, Ref. 31. [c] Average of 0.62 from ion-pair and 0.60 from free base (30).

another. In general, the octane system gave the lowest values while the chloroform system gave the highest. These values may be considered as a reflection of the relative net interactions between the polar head of the alkylpyridines and the solvent molecules. Based on these data, the strengths of these net interactions may be arranged as follows: chloroform > octanol > carbon tetrachloride > butyl ether > hexadecane > octane.

The weaker interactions in hexadecane and in octane may be attributed entirely to the nonpolar nature of the solvents. In these two cases, the London dispersion force and induction force are the only attractive ones involved. With butyl ether, there are additional forces involved; besides the dispersion forces and the induction forces due to the polarization of the solvent by the polar solute, the solute molecules are also polarized by the solvent, and there are dipole–dipole interactions. Therefore, the solvent–solute interactions might be expected to be more favorable for butyl ether than for the hydrocarbons.

With chloroform and octanol, hydrogen bonding (37, 38) between the pyridine ring and the solvent molecules produces rather strong solvent–solute interactions. These solvents consequently give the highest distribution coefficients with the alkylpyridines. The net solvent–solute interactions appear to be weaker in octanol than in chloroform, even though the strength of the alcohol–pyridine hydrogen bonding (37) is greater than that between chloroform and pyridine (39). The explanation probably resides in the higher polar cohesive energy density of octanol, because more energy is required to create a cavity for the solute molecule. Therefore, the net free energy gained in the second step of the dissolution process may be less in octanol than in chloroform.

Carbon tetrachloride is another interesting case. Its dispersive and inductive interactions with the solute would be expected to be similar to those in octane or hexadecane. Yet this solvent apparently is involved in stronger interactions with alkylpyridines than even butyl ether. This finding probably is in accord with the observations that pyridine has a higher dipole moment in carbon tetrachloride than in benzene (36) and that its heat of solution at infinite dilution in carbon tetrachloride is lower than in hexane (40).

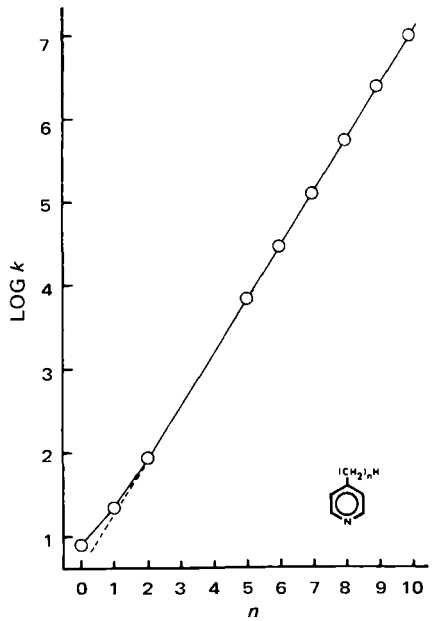


**Figure 7**—*Intrinsic distribution coefficients of alkylpyridines between hexadecane and the aqueous phase as a function of the chain length.*

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* Present address: Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

[x] To whom inquiries should be directed.

# Fully Automated Analysis of Phenylbutazone in Plasma and Urine

GEORGE LUKAS [x], CATHERINE B. BORMAN, and SYLVIA B. ZAK

**Abstract** □ A rapid, automated method for the determination of phenylbutazone in plasma and urine was developed. The method offers distinct advantages over earlier procedures and is particularly suitable for large-scale bioavailability studies.

**Keyphrases** □ Phenylbutazone—analysis, automated method, human plasma and urine □ Automated methods—analysis of phenylbutazone in human plasma and urine □ Anti-inflammatory agents—phenylbutazone, analysis, automated method, human plasma and urine

Phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidinedione) has been used extensively during the past 2 decades in the treatment of rheumatoid and other inflammatory conditions. Several methods have been developed for the analysis of the drug in body fluids in conjunction with investigations on its biological disposition.

The plasma levels of phenylbutazone were measured spectrophotometrically following its extraction (1), and several modifications were introduced (2, 3) to reduce the interference caused by oxyphenbutazone, the principal metabolite. The procedure also was modified for small samples (4). Another method based on hydrolysis of phenylbutazone to hydrazobenzene, with subsequent rearrangement to benzidine, was developed (5) and modified (6, 7). Also, phenylbutazone was oxidized to azobenzene, and the UV absorbance of the latter compound was measured (8, 9). Several workers (10–15) used GC for the assay of the drug in biological fluids or solid dosage forms. A method using liquid chromatography was reported recently (16).

Although most of these methods have merit in terms of specificity and/or sensitivity, they are rather time consuming. Ahuja *et al.* (17) developed an automated method capable of assaying solid dosage forms at the rate of 13 samples/hr. Unfortunately, their method lacks the sensitivity required for the analysis of phenylbutazone in biological fluids.

Analysis of plasma levels in conjunction with large-scale bioavailability assessments of phenylbutazone dosage forms after a therapeutic dose in humans requires speed, sensitivity, and specificity. The automated method presented here satisfies these criteria.

**Table I—Standard Curve of Phenylbutazone from Aqueous Solutions**[a]

| Concentration, μg/ml | Peak Height[a] |
|---|---|
| 0 | 0.0 ± 0.0 |
| 0.5 | 0.3 ± 0.1 |
| 1 | 0.6 ± 0.1 |
| 2 | 1.4 ± 0.2 |
| 3 | 2.5 ± 0.1 |
| 4 | 3.3 ± 0.2 |
| 5 | 4.1 ± 0.1 |
| 7 | 5.6 ± 0.3 |
| 10 | 8.0 ± 0.2 |
| 20 | 15.5 ± 0.4 |
| 30 | 25.4 ± 0.6 |
| 40 | 32.2 ± 0.5 |
| 50 | 38.5 ± 1.5 |
| 60 | 48.0 ± 2.7 |

[a] Each value represents the average ± *SD* of five determinations (arbitrary units).

## EXPERIMENTAL

**Reagents and Standards**—All chemicals except heptane (chromatoquality) were reagent grade or of equivalent purity. The following were used: aminoacetic acid–hydrochloric acid buffer (pH 1.2), prepared by dissolving 25 g of aminoacetic acid and 50 ml of 6 *N* HCl in a final volume of 1000 ml of water; heptane–isopentyl alcohol (98.5:1.5); and 2.5 *N* NaOH.

The following standard solutions, preferably prepared daily, were used: (*a*) phenylbutazone, 1 or 10 mg/ml in 1.0 *N* NaOH; and (*b*) phenylbutazone, 2–80 μg/ml in plasma[1].

**Procedure**[2]—The flow diagram for the automated manifold is depicted in Fig. 1. At the beginning of the day's analyses, the re-

[1] Plasma was purchased from the American Red Cross and was mixed with varying amounts of phenylbutazone.

[2] The following equipment was used: Technicon sampler IV, Technicon proportioning pump III, Technicon continuous filter with hydrophobic filter paper (Product No. 518-3041), Beckman model DB-G grating spectrophotometer equipped with a 6-mm rectangular flowcell, and Technicon single-pen linearized recorder with linear-scale chart paper.