A set of 30 chemicals whose octanol-water partition coefficients (log P) are known were injected on a XAD-2 column and a commercially available octadecylsilane column. Two types of mobile phase were used on the XAD-2 column: buffered and unbuffered acetonitrile in water and buffered and unbuffered octanol-saturated water passing over an octanol coated resin. With octadecylsilane column, the mobile phase consisted only of acetonitrile in water. The capacity factors (k') from different column-mobile phase systems were compared. The ability of the column system to mimic the octanol-water shake-flask method was evaluated by plotting \( \Delta \log P \) versus \( \Delta \log k' \). For each column system studied, good lines could be obtained for a homologous series of compounds, but not for the whole data set.

In general, longer retention times were observed on the octadecylsilane column relative to the XAD-2 column.
using the same mobile phase when compounds contain more polar or aliphatic character. The method and criterion to estimate the partition coefficients of organic compounds in traditional shake-flask systems by chromatographic techniques are discussed.
The Evaluation of Octanol-Water Partition Coefficients from Chromatographic Data

by

Walapa Tatong

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## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Experimental</td>
<td>18</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>23</td>
</tr>
<tr>
<td>References</td>
<td>52</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crosslinked styrene-divinylbenzene copolymer</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Log P VS log k' ACN on XAD-2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Corrected log P VS log k' pH 7 ACN on XAD-2</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>Log P VS log k' OCT on XAD-2</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>Corrected log P VS log k' pH 7 OCT on XAD-2</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>Log P VS log k' ACN on C-18</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>Corrected log P VS log k' pH 7 ACN on C-18</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>ΔLog P VS Δlog k' ACN on XAD-2</td>
<td>35</td>
</tr>
<tr>
<td>9</td>
<td>ΔCorrected log P VS Δlog k' pH 7 ACN on XAD-2</td>
<td>36</td>
</tr>
<tr>
<td>10</td>
<td>ΔLog P VS Δlog k' OCT on XAD-2</td>
<td>37</td>
</tr>
<tr>
<td>11</td>
<td>ΔCorrected log P VS Δlog k' pH 7 OCT on XAD-2</td>
<td>38</td>
</tr>
<tr>
<td>12</td>
<td>ΔLog P VS Δlog k' ACN on C-18</td>
<td>39</td>
</tr>
<tr>
<td>13</td>
<td>ΔCorrected log P VS Δlog k' pH 7 ACN on C-18</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>ΔCorrected log P VS Δlog k' pH 7 OCT on C-18 from Unger's study (reference 27)</td>
<td>41</td>
</tr>
<tr>
<td>15</td>
<td>Octanol VS ACN on XAD-2</td>
<td>47</td>
</tr>
<tr>
<td>16</td>
<td>pH 7 Octanol VS pH 7 ACN on XAD-2</td>
<td>48</td>
</tr>
<tr>
<td>17</td>
<td>ACN(XAD-2) VS ACN(C-18)</td>
<td>49</td>
</tr>
<tr>
<td>18</td>
<td>pH 7 ACN(XAD-2) VS pH 7 ACN(C-18)</td>
<td>50</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>I</td>
<td>Chemicals, pK and log P values for this study</td>
<td>19</td>
</tr>
<tr>
<td>II</td>
<td>Log k' values of various chemicals determined on the XAD-2 column</td>
<td>24</td>
</tr>
<tr>
<td>III</td>
<td>Log k' values of various chemicals determined on an octadecylsilane column</td>
<td>29</td>
</tr>
<tr>
<td>IV</td>
<td>Correlations of log P with log k' values</td>
<td>32</td>
</tr>
<tr>
<td>V</td>
<td>Correlations of log P with log k' values of each group of compound from the XAD-2 column</td>
<td>43</td>
</tr>
<tr>
<td>VI</td>
<td>Correlations of log P with log k' values of each group of compound from the reversed-phase C-18 column</td>
<td>44</td>
</tr>
<tr>
<td>VII</td>
<td>Correlations of log k' values obtained from various systems</td>
<td>46</td>
</tr>
</tbody>
</table>
THE EVALUATION OF OCTANOL-WATER
PARTITION COEFFICIENTS
FROM CHROMATOGRAPHIC DATA
INTRODUCTION

Recent studies on quantitative structure-activity relationships (QSAR) have shown the importance of the hydrophobic or lipophilic nature of drugs. The hydrophobicity of a drug is usually characterized by the partition coefficient, \( P \), which is defined as the equilibrium concentration of the nominal chemical species in the non-aqueous organic phase divided by that in the aqueous phase, \( P = \frac{[C]_o}{[C]_{aq}} \). Hence \( P \) is a pH-independent property. It has been shown that \( \log P \) is highly correlated with a variety of biological activities such as drug potency, chemical toxicity, pesticidal activity, etc. (1-3). It is evident that \( \log P \) plays a significant role in determining the extent of the interaction between chemicals and macromolecules or receptors (2). It also has been shown that the lipophilic character of drugs is an important factor in drug metabolism (4-6). The absorption, distribution, and excretion of many classes of drugs in various systems are shown to be \( \log P \)-dependent (7).

There are several methods for determining or estimating partition coefficients: shake-flask procedure which is considered the standard reference method (8,9), liquid-liquid chromatography on lipid impregnated plates (10-19), high-pressure liquid chromatography (HPLC) (20-27) and estimation using substituent constants (9).
The question of which solvent-water partitioning system best approximates biological lipophilicity has been the subject of recent debate (28). Octanol is widely accepted because of its intermediate degree of polarity, its hydroxyl group and its affinity for water. All of these seem to make it similar to biological membranes which have hydrogen-bond donating and acceptor properties (8,29-31). The octanol-water system also has its limitations, the latter appearing to be noticeable with respect to highly hydrophilic biological solutes. Recently, the aqueous two-phase polymeric system Ficoll-dextran system (or dextran-polyethylene glycol system) has been proposed by Zaslavsky et al. (32-37) for studying the relative hydrophobicities of biological solutes and particles. It has been shown (36) that log P determined in the Ficoll-dextran system can be correlated with those measured in the octanol-water system for the same solutes. It should be noted, however, that the Ficoll-dextran system cannot be used for partitioning non-polar compounds because of their low solubility in water.

The shake-flask method for determining log P can be encumbered by the difficulties in measuring solute concentration in both phases accurately, intramolecular associations, degradation of labile compounds during the shaking procedure which takes about one to two hours, and
purity requirements of the solutes (38,39). A special analytical method that can distinguish between the solutes of interest and impurities or degradation product is required.

Liquid-liquid chromatography on paper or lipid (silicone oil, liquid paraffin or 1-octanol) impregnated plates (TLC) is a useful alternative to estimate octanol-water partition coefficients. Martin (40) deduced on theoretical grounds a relationship between partition coefficient $P$ and $R_f$, expressed in the equation

$$P = a[(1/R_f) - 1]^n$$  \hspace{1cm} (1)

if $n=1$, then

$$\log P = \log a + \log [(1/R_f) - 1]$$  \hspace{1cm} (2)

where

$a$ = a constant for a compound in the system

$R_f$ = distance traveled by center of the solute zone

distance traveled by the solvent front

Bate-Smith and Westall (41) defined $R_m$ by

$$R_m = \log[(1/R_f) - 1]$$  \hspace{1cm} (3)

Substituting into equation 2
\[ \log P = \log a + R_m \]  \hspace{1cm} (4)

If "a" is constant for a selected group of compounds, a plot of \( \log P \) versus \( R_m \) will be straight line with a slope of one and an intercept equals to \( \log a \).

The ability of a TLC system to predict \( \log P \) can be determined by comparing the \( \log P \) and \( R_m \) value of a reference compound \( (\log P_r, R_{m r}) \) with the analogous values of a second compound \( (\log P_x, R_{m x}) \). Subtraction of the Eq. 4 for the reference compound from the Eq. 4 for the second compound that has the same "a" gives

\[ \Delta \log P = \Delta R_m \]  \hspace{1cm} (5)

For monosubstituted aromatic compounds, \( \Delta \log P \) is analogous to the Hansch \( \pi \) substituent value.

A plot of \( \Delta \log P \) versus \( \Delta R_m \) will show the extent of scatter about the ideal line (assuming all compounds have the same "a" value) which has a slope of one and a zero intercept. Those compounds which deviate from the ideal line have a value of \( \log a \) different from the reference compound.

Bird and Marshall (10) found that there was a near perfect linear relationship between the \( R_m \) values of
penicillins measured at pH 3 or pH 4 by a reversed-phase TLC system using n-octanol as the stationary phase and log P of penicillin free acids. Biagi et al. (11-15) found good correlations between $R_m$ values measured by a reversed-phase TLC, using silicone oil as the stationary phase and log P for a series of penicillins, cephalosporins, testosterone esters, heterocyclic substituted sulphonamides and benzodiazepines. Biagi et al. (15-19) had also found good correlations between $R_m$ values and biological activity of oligosaccharide antibiotics, benzodiazepines, steroids, phenols and naphthol and acetophenones.

There are several advantages of this method. It allows simultaneous measurement on several compounds which need not be rigorously purified, and even quite labile substances such as the penicillins which may decompose during conventional equilibration can be dealt with by this method. In general, the $R_m$ values for each compound are not used to determine the log P values, but are themselves used in QSAR studies. Most thin layer systems use a silicone oil as the stationary phase. Thus, while there is a linear relationship between $R_m$ versus log P (Eq. 4) and $\Delta R_m$ versus lipophilicity substituent constants ($\pi$), one has to be very careful at concluding that a log P value can be calculated from a $R_m$ value in a series of compounds of widely divergent structures.
According to Tomlinson (42), disadvantages of TLC are that streaking of spots is sometimes unavoidable. This is especially true in reversed-phase systems and, many times, is due to overloading of solute to obtain visualization. This effect, coupled with poor visualization, increases any subjective errors when measuring the $R_f$ values which are used to calculate $R_m$ values. Also, in reversed-phase systems, an even distribution of the non-aqueous phase upon impregnation of the support is assumed but not known for certain.

The advent of high-pressure liquid chromatography (HPLC) has provided a particularly convenient and versatile chromatographic technique of high precision. Reversed-phase HPLC has been used to estimate octanol/water partition coefficients of several classes of compounds. In HPLC, a compound's retention is expressed by the capacity factor $k'$ which is defined as:

$$k' = \frac{t_r - t_m}{t_m} = \frac{W_s}{W_m} = \frac{V_r - V_m}{V_m} = \frac{d_r - d_m}{d_m}$$  \hspace{1cm} (6)$$

where

$t_r = \text{observed retention time of the compound}$

$t_m = \text{retention time of the solvent}$

$W_s = \text{weight of compound in the stationary phase}$

$W_m = \text{weight of compound in the mobile phase}$

$V_r = \text{observed retention volume of the compound}$

$= (t_r)(\text{flow rate})$
\[ V_m = \text{retention volume of the solvent or void volume} \]
\[ = (t_m)(\text{flow rate}) \]
\[ d_r = \text{distance between the point of injection and peak of the compound} \]
\[ d_m = \text{distance between the point of injection and the peak of the solvent front} \]

The term \( k' \) and \([(1/R_f)-1]\) are analogous \((20,21,43)\) which can be proved by:

\[ R_f = \frac{d_r}{d_m} = \frac{F_t}{F_r} = \frac{t_m}{(t_m + t_s)} \quad (7) \]
\[ \frac{1}{R_f} = 1 + \frac{t_s}{t_m} \quad (8) \]
\[ \frac{1}{R_f} - 1 = k' \quad (9) \]

where
\[ d_r = \text{distance traveled by center of the solute zone} \]
\[ d_m = \text{distance traveled by the mobile phase} \]
\[ F = \text{velocity of the mobile phase} \]
\[ t_m = \text{the time a solute spends in the mobile phase} \]
\[ t_s = \text{the time a solute spends on the stationary phase} \]
\[ t_r = \text{total elution time of a solute} = t_m + t_s \]

From Eq. 1
Thus, for any chromatographic systems to be useful for estimating the partition coefficient in conventional shake-flask equilibrium systems ($P$), the factors affecting $P$ and $k'$ must be known so that the relation between $P$ and $k'$ can be evaluated. It has been shown by Chiou et al. (44)

$$P = a'(k')^n$$

(10)

$$P = \left(\frac{\gamma_w^*}{\gamma_o^*}\right)\left(\frac{\bar{V}_w^*}{\bar{V}_o^*}\right)$$

(11)

where

- $\gamma_w^*$ = activity coefficient of the solute in solvent-saturated water phase
- $\gamma_o^*$ = activity coefficient of the solute in water-saturated solvent phase
- $\bar{V}_w^*$ = molar volume of the solvent-saturated water
- $\bar{V}_o^*$ = molar volume of the water-saturated solvent

and it has been suggested by Chiou (45) that

$$k' = (\gamma_m / \gamma_s)(c)$$

(12)

where

- $\gamma_m$ = activity coefficient of the solute in the mobile phase
- $\gamma_s$ = activity coefficient of the solute in the
stationary phase
c = a constant for the specific chromatographic system incorporating the extent of loading by the stationary phase, the void space, and the nature of the stationary and mobile phases

In the dilute concentration used in chromatography and the determination of partition coefficient, the \((\bar{V}_w/\bar{V}_o)\) term is constant. Thus the \(a'\) term in equation 10 can be considered a constant incorporating the \((\bar{V}_w/\bar{V}_o)\) and \(c\) terms from equation 11 and 12, respectively. Rewriting equation 10 in logarithmic form,

\[
\log P = a + n \log k'
\]  

(13)

where

\[a = \log a'\]

A linear relationship can be expected between \(\log P\) and \(\log k'\) if all solutes have the same values of "a" and "n". In this case, a plot of \(\log P\) versus \(\log k'\) would give a straight line with a slope of \(n\) and an intercept of \(a\).

In the manner analogous to that for equation 5 and 6 for reference and second compounds that have the same value of "a", 
Thus, the prerequisite condition for predicting $P$ by using a chromatographic technique consists in finding a column system that makes "$a$" and "$n$" constant for the selected compounds. The most favorable column environment to mimic the octanol-water shake-flask system for the selected compounds is the one that gives the same value of "$a$" and gives "$n$" = 1. Under this ideal condition, $P$ is linearly related to $k'$ in equation 10.

In reversed-phase HPLC using a mixture of water and organic solvent as the mobile phase, this is equivalent to finding a stationary phase that gives $(\gamma_m/\gamma_s)/(\gamma_w^*/\gamma_o^*) = constant$ for all compounds. This study analyzes some systems with mixtures of water and organic solvent as the mobile phases and different organic stationary phases to illustrate how a selected group of compounds respond by comparing $\Delta \log P$ with $\Delta \log k'$.

Haggerty and Murrill (20) determined log $P$ values of a family of nitrosoureas using a column packed with octadecylsilane bonded to silica (Corasil C$_{18}$, Waters Associates, Inc.) eluted with 30 percent acetonitrile in pH 7.41 buffer solution. McCall (21) found that vigorously silylated octadecylsilane reversed-phase columns gave a better correlation between $k'$ and log $P$.
than untreated packing material. This can be explained by the elimination of bonding of compounds with residual active silanol sites after silylation. Carlson et al. (22) found good correlations between log $k'$ and log $P$ of some substituted phenol and aniline derivatives using bonded columns (Porasil B, Waters Associates, Inc.) with mixtures of distilled water and acetone at various concentration (mole percentage) as the eluent.

Hulshoff and Perrin (23) determined the lipophilicities of 1,4-benzodiazepine derivatives using oleyl alcohol supported on a porous silica (Porasil C, Waters Associates, Inc.) as an HPLC procedure and compared their results with oleyl alcohol reversed-phase TLC system. Using TLC, they found that their $R_m$ values correlated well with log $P$ values determined directly in the oleyl-water system and with the literature values for the 1-octanol-water system. In contrast, using HPLC, the correlations of log $k'$ with log $P$ oleyl-water and log $P$ octanol-water were not as good as those found for the $R_m$ values. Mirrlees et al. (24), using 1-octanol supported on silanized diatomaceous earth with octanol-saturated water as the mobile phase, obtained good linear relationships between log $P$ in a range of -0.3 to +3.7 and log $t_c$ (corrected elution time) for a number of compounds (i.e., caffeine, aniline, acetanilide, acridine).

Henry et al. (25) compared various HPLC techniques
using three different columns: a bonded reversed-phase pellicular silica (Corasil $C_{18}$, Waters Associates, Inc.), a nonbonded pellicular silica (Corasil II, Waters Associates, Inc.) coated with 1-octanol or squalene, and a nonbonded porous silica (Porasil A, Waters Associates, Inc.) coated with 1-octanol or squalene. They found several good correlations between log $V_c$ (corrected retention volume) and log $P$ as well as correlations between log $V_c$ and log biological activity for sulfonamides and barbiturates. They also warned investigators using these techniques to be careful in interpreting these correlations. Baker (26) developed a method of predicting the HPLC retention indices ($I$) using a standard set of reference compounds similar to Kovat indices used in gas chromatography. He estimated the retention indices ($I$) of a series of propranolol, anthranilic acid and barbiturate analogues on C-18 reverse phase column ($\mu$-Bondapak $C_{18}$, Waters Associates, Inc.) and found that there were correlations between $I$ and log $P$ values or lipophilicity substituent constants ($\pi$). The excellent relationship between $I$ and log $P$ could be expected only if the two chromatographic phases were precisely identical with the two phases in the classical "shake-flask" experiment. He concluded that the prediction of the HPLC retention properties based on the use of the retention index scale and lipophilicity
substituent constants proved to be very reliable in most cases.

Unger (27) obtained an excellent agreement between 1-octanol shake-flask partition coefficients and a reversed-phase HPLC procedure over a log P range of 3.5 units using 1-octanol coated, persilated octadecyl bonded silica (Corasil C₁₈, Waters Associates, Inc.) as the stationary phase and 1-octanol-saturated pH 7.0 (0.01 M) phosphate buffer as the mobile phase. He also found that only relatively unhindered basic pyridines deviated considerably from the regression line and were retained on the column much longer than expected. This result was attributed to adsorption phenomena due to free silanol sites and was corrected by adding N,N-dimethyloctylamine (DMOA) to the mobile phase (46).

In contrast with experimentally derived partition coefficients, the partitioning character of a molecule can be estimated from appropriate hydrophobic substituent or fragment constants. It is quite reliable for many common organic compounds that require only simple addition of fragment values for the appropriate atoms and bonds. However, the estimation of log P from these fragment values can be very involved with complex molecules because many correction terms (such as branching, chain length, steric factor, etc.) have not been well established (47). Therefore, experimental techniques to determine partition coefficients are still needed.
In theory HPLC and TLC should be equally useful in estimating partitioning behavior. In practice HPLC has proved more useful and versatile. The detecting systems available to HPLC permit good sensitivities without having to overload the system. The permanently bonded reversed-phase columns available from several manufacturers are much easier to use than impregnating a TLC plate with silicone oil, mineral oil or similar nonpolar material. Bonded phases have the advantages of good reproducibility and high efficiency. They often require only aqueous-alcohol or aqueous-acetonitrile eluting mixture. Their main disadvantages are that they have a silica support which can be used only over a narrow pH range of about 2 to 8, and have residual active silanol sites which would interact with the compounds being chromatographed. Therefore, some type of silylation (21) is required to block these free silanol sites.

In contrast Amberlite XAD-2 is a rigid, nonionic, macroreticular copolymer of styrene and divinylbenzene (Figure 1) which is stable at all pH's in aqueous solution and in most organic solvents (48-50). It has large surface area (about 300 m²/gm) and a rigid, near permanently porous structure (90 Å average pore diameter). It is all organic and has been shown to withstand the pressure common to low flow rates (51). It is capable of retaining both neutral and ionic chemical species from
Figure 1. CROSSLINKED STYRENE-DIVINYLBENZENE COPOLYMER
solution and has been shown to be suitable as a stationary phase for liquid chromatography of a variety of chemical classes (52-54). XAD-2 has been used to chromatographically determine the dissociation constants of acids and bases (55). Since it is totally organic and nonionic, it should eliminate the active adsorption sites characteristic of the silica-based internal phase.

The purpose of this study is to evaluate the feasibility of the XAD-2 column with different compositions of the mobile phase as a mean of estimating log P. When the data obtained using XAD-2 as the stationary phase do not conform to the single "a" and "n" values (Eq. 13), i.e., $\Delta \log k'$ values are not linear to $\Delta \log P$ for many compounds, the pattern of the observed scatter provide a basis to analyze the affinity of compounds for the XAD-2 relative to that for octanol, with water as the eluting phase.
EXPERIMENTAL

APPARATUS

The high-pressure liquid chromatograph (Waters Associates Model ALC/GPC 201) consisted of a pump (Waters Associates Model M 6000A) and a sample injection system (Waters Associates Model U6K). A variable wavelength UV-visible spectrophotometer (Varian Model 635 LC) equipped with low dead volume flow cell was used as detector.

REAGENTS

The choice of chemicals (Table I) was the same as those used in Unger's study (27). This set contains a variety of substituted benzenes and, taken as a whole, is not a homologous series. All chemicals were used as received. Solvents were of analytical reagent quality, and the 1-octanol was further purified according to a reported procedure (56). Chemicals to be chromatographed were dissolved in 20 percent acetonitrile. Sodium nitrate (0.01 M) in 20 percent acetonitrile was used as a suitable nonretained compound to define dead volume. Sample concentrations were adjusted so that the relative peak areas remained approximately constant. The wavelength of the detection was adjusted to the appropriate maximum wavelength for each compound.
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Log ( p^a )</th>
<th>Corrected Log ( p^b )</th>
<th>( pK_a^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acetanilide ( ^f )</td>
<td>1.16</td>
<td>1.16</td>
<td>11.35</td>
</tr>
<tr>
<td>2. Acetone</td>
<td>-0.24</td>
<td>-0.24</td>
<td></td>
</tr>
<tr>
<td>3. Acetophenone ( ^f )</td>
<td>1.58</td>
<td>1.66</td>
<td>19.20</td>
</tr>
<tr>
<td>4. 2-Acetylpyridine ( ^e )</td>
<td>0.84</td>
<td>0.85</td>
<td>2.64</td>
</tr>
<tr>
<td>5. 4-Acetylpyridine ( ^e )</td>
<td>0.51</td>
<td>0.48</td>
<td>3.51</td>
</tr>
<tr>
<td>6. Acridine</td>
<td>3.40</td>
<td>3.39</td>
<td>5.58</td>
</tr>
<tr>
<td>7. 2-Amino-4-picoline ( ^e )</td>
<td>1.15</td>
<td>0.56</td>
<td>7.48</td>
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<tr>
<td>8. Aniline ( ^f )</td>
<td>0.90</td>
<td>0.93</td>
<td>4.63</td>
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<td>9. o-Anisidine ( ^f )</td>
<td>0.95</td>
<td>1.23</td>
<td>4.52</td>
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<td>2.08</td>
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<td>1.45</td>
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<td>2.15</td>
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<td>3.18</td>
<td>-6.0</td>
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<td>15. 2-Butanone</td>
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<td>0.29</td>
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<td>18. 2-Chloropyridine ( ^e )</td>
<td>1.34</td>
<td>1.34</td>
<td>7.85</td>
</tr>
<tr>
<td>19. 4-Cyanophenol ( ^d )</td>
<td>1.63</td>
<td>1.58</td>
<td>5.15</td>
</tr>
<tr>
<td>20. N,N-Dimethylaniline ( ^f )</td>
<td>2.51</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>21. m-Dinitrobenzene ( ^f )</td>
<td>1.49</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>22. 2-Ethylpyridine ( ^e )</td>
<td>1.69</td>
<td>1.69</td>
<td>5.89</td>
</tr>
<tr>
<td>23. 4-Fluorophenol ( ^d )</td>
<td>1.79</td>
<td>1.79</td>
<td>9.81</td>
</tr>
<tr>
<td>24. 2,6-Lutidine ( ^e )</td>
<td>1.87</td>
<td>1.68</td>
<td>6.64</td>
</tr>
<tr>
<td>25. 4-Nitrophenol ( ^d )</td>
<td>1.91</td>
<td>1.68</td>
<td>7.07</td>
</tr>
<tr>
<td>26. Phenol ( ^d )</td>
<td>1.46</td>
<td>1.48</td>
<td>9.89</td>
</tr>
<tr>
<td>27. Phenylacetonitrile ( ^f )</td>
<td>1.56</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>28. 4-Picoline ( ^e )</td>
<td>1.33</td>
<td>1.18</td>
<td>6.32</td>
</tr>
<tr>
<td>29. Pyridine ( ^e )</td>
<td>0.64</td>
<td>0.63</td>
<td>5.23</td>
</tr>
<tr>
<td>30. Quinoline</td>
<td>2.03</td>
<td>2.04</td>
<td>4.80</td>
</tr>
</tbody>
</table>

\( ^a \) reference 57  
\( ^b \) corrected for ionization; reference 27  
\( ^c \) references 58 and 59  
\( ^d \) phenolic compounds  
\( ^e \) substituted pyridine compounds  
\( ^f \) substituted nonphenolic benzene compounds
XAD-2 copolymer (Bio-Beads SM-2, Bio-Rad Laboratories) was obtained as 20 to 60 mesh (500 micron average) beads. The resin was repeatedly washed in a Soxhlet extractor with acetonitrile until the washings yielded a negligible UV absorption and then dried in a vacuum oven at 25 mm Hg at 120°C for 24 hr. Then the resin was ground and wet sieved (using acetone as the solvent) through two U.S. Standard screens. The sieve with mesh size 230 (63 micron) was on top of the one with size 325 (45 micron). The particles staying on the second sieve (mesh size 325 or 45 micron) would have a particle size of 45-63 micron. Many fines were produced during size reduction and were not completely removed by sieving since they tended to aggregate. Hence settling techniques had to be used to remove these fines prior to packing the column. This was done by placing the sieved particles (45-63 micron) in ethyl acetate, stirring, and allowing to settle overnight. Particles that did not settle were discarded. This procedure was repeated several times. The particles were then dried under vacuum at 120°C for 24 hr.

PROCEDURE

A 9 cm long chromatographic grade stainless steel column with a polished 0.23 cm i.d. was packed by a dry-packing technique with 45-63 micron particles. The
column was attached to the pump and washed with octanol-saturated water. The column was loaded with purified 1-octanol by injecting directly into the column under pressure until droplets appeared and then washed with octanol saturated mobile phase until clear eluate appeared. Four mobile phases were used: 20 percent acetonitrile in water, 20 percent acetonitrile pH 7.0 in phosphate buffer (0.01 M), 1-octanol-saturated water, and 1-octanol-saturated pH 7.0 phosphate buffer (0.01 M). The column did not contain any octanol when the acetonitrile containing mobile phases were used. The flow rate was 1 mL/min. Sodium nitrate and aniline retention distances were determined daily at constant flow rate. Good reproducibility was found between days. The column was repacked after the pressure drop across the column was greater than 2,500 psi. Excellent agreement was found between columns.

Log $k'$ values of the samples were also determined on a 10 micron octadecylsilane column ($\mu$ Bondapak C-18, Waters Associates, Inc.), 30 cm long and 3.9 mm i.d., using 20 percent acetonitrile in water and 20 percent acetonitrile pH 7.0 in phosphate buffer (0.01 M) as the mobile phases with flow rate 2 mL/min.

For both columns, all solutions were first filtered (Fritted Disc, 10-15 micron, Pyrex) to reduce contamination or column clogging. Water-acetonitrile and
buffer-acetonitrile mixtures were expressed as percent by volume. The pH values of solvents containing the buffer were determined by a pH meter before and after adding acetonitrile. The pH of 20 percent acetonitrile pH 7.0 in phosphate buffer (0.01 M) was adjusted after adding acetonitrile. The saturation with octanol of the aqueous buffer had no effect on pH. The pH meter was standardized each day at pH 4 and pH 7. All experiments were performed at ambient temperature (25±1°C). Each sample was injected three times. The capacity factor (k') was determined from equation 6 using distances (d_r, d_m). The k' values were averaged and the standard deviation were determined (Tables II and III). Statistical analyses were performed on the Oregon State University Cyber 170 using the Oregon State University Statistical Interactive Programming System (SIPS). All plots were performed on Tektronix Model 4010-1 and Tektronix Interactive Digital Plotter Model 4663 using Easy Graphing (Tektronix).
RESULTS AND DISCUSSION

A summary of the experimental results obtained from the XAD-2 column is seen in Table II and in Figures 2-5. Each log k' value represents an average of three determinations. The experimental results determined on a commercially available octadecylsilane column are shown in Table III and Figures 6-7. There was no attempt to evaluate the effect of octanol coating on an octadecylsilane column because this has been thoroughly done by Unger (27). The linear relationships between log P and log k' for both columns can be seen in Table IV.

Log k' values for very hydrophobic compounds such as acridine, benzophenone and chlorobenzene (log P: 3.40, 3.18, and 2.84, respectively) could not be determined on the XAD-2 columns, using the octanol-saturated water and octanol-saturated pH 7.0 buffer systems as the mobile phases. These lipophilic compounds were retained so long that it was not possible to obtain a peak definitive enough to measure a retention distances (d_r). Increasing the flow rate caused the resin packing to collapse.

It is obvious that the results from the XAD-2 systems are inferior to those obtained from the reversed-phase octadecylsilane column. The inconsistencies in the results can be explained in terms of the differences in the retention properties of the stationary phases.
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Log $k_1$&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Log $k_2$&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Log $k_3$&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Log $k_4$&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Anisamidine&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.468 ± 0.011</td>
<td>0.545 ± 0.001</td>
<td>0.386 ± 0.008</td>
<td>0.512 ± 0.006</td>
</tr>
<tr>
<td>2. Acetone</td>
<td>-0.359 ± 0.001</td>
<td>-0.273 ± 0.038</td>
<td>-0.532 ± 0.002</td>
<td>-0.426 ± 0.025</td>
</tr>
<tr>
<td>3. Acetophenone&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.368 ± 0.005</td>
<td>1.737 ± 0.005</td>
<td>1.433 ± 0.009</td>
<td>1.704 ± 0.009</td>
</tr>
<tr>
<td>4. 4-Acrylpyridine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.620 ± 0.005</td>
<td>0.840 ± 0.001</td>
<td>0.553 ± 0.009</td>
<td>0.899 ± 0.010</td>
</tr>
<tr>
<td>5. 4-Acrylpyridine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.176 ± 0.003</td>
<td>0.296 ± 0.001</td>
<td>0.212 ± 0.002</td>
<td>0.201 ± 0.003</td>
</tr>
<tr>
<td>6. Acrizine</td>
<td>2.292 ± 0.002</td>
<td>-</td>
<td>2.321 ± 0.005</td>
<td>-</td>
</tr>
<tr>
<td>7. 2-Amino-4-picoline&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.239 ± 0.009</td>
<td>0.528 ± 0.003</td>
<td>0.205 ± 0.001</td>
<td>0.306 ± 0.012</td>
</tr>
<tr>
<td>8. Aniline</td>
<td>2.747 ± 0.004</td>
<td>0.830 ± 0.002</td>
<td>0.747 ± 0.004</td>
<td>0.806 ± 0.006</td>
</tr>
<tr>
<td>9. 2-Anisidine&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.106 ± 0.003</td>
<td>1.313 ± 0.003</td>
<td>1.033 ± 0.010</td>
<td>1.224 ± 0.009</td>
</tr>
<tr>
<td>10. Anisole&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.665 ± 0.001</td>
<td>1.012 ± 0.001</td>
<td>0.692 ± 0.004</td>
<td>0.894 ± 0.007</td>
</tr>
<tr>
<td>11. Benzaldehyde&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.310 ± 0.005</td>
<td>1.530 ± 0.005</td>
<td>1.239 ± 0.009</td>
<td>1.553 ± 0.020</td>
</tr>
<tr>
<td>12. Benzene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.789 ± 0.003</td>
<td>1.910 ± 0.005</td>
<td>1.579 ± 0.009</td>
<td>1.906 ± 0.009</td>
</tr>
<tr>
<td>13. Benzonitrile&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.454 ± 0.004</td>
<td>1.520 ± 0.001</td>
<td>1.499 ± 0.010</td>
<td>1.490 ± 0.009</td>
</tr>
<tr>
<td>14. Benzonaphene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.238 ± 0.001</td>
<td>-</td>
<td>2.193 ± 0.008</td>
<td>-</td>
</tr>
<tr>
<td>15. 2-Butanone</td>
<td>0.131 ± 0.002</td>
<td>0.139 ± 0.002</td>
<td>0.052 ± 0.002</td>
<td>0.097 ± 0.013</td>
</tr>
<tr>
<td>16. Catechol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.111 ± 0.003</td>
<td>0.233 ± 0.006</td>
<td>0.033 ± 0.010</td>
<td>0.170 ± 0.008</td>
</tr>
<tr>
<td>17. Chlorobenzene&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.145 ± 0.001</td>
<td>-</td>
<td>2.192 ± 0.008</td>
<td>-</td>
</tr>
<tr>
<td>18. 1-Chloropyridine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.532 ± 0.004</td>
<td>1.282 ± 0.001</td>
<td>0.856 ± 0.010</td>
<td>1.286 ± 0.010</td>
</tr>
<tr>
<td>19. 1-Cyanophenol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.439 ± 0.003</td>
<td>1.036 ± 0.004</td>
<td>0.658 ± 0.006</td>
<td>0.753 ± 0.007</td>
</tr>
<tr>
<td>20. N-N-Dimethylaniline&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.803 ± 0.004</td>
<td>0.979 ± 0.003</td>
<td>0.677 ± 0.009</td>
<td>0.840 ± 0.005</td>
</tr>
<tr>
<td>21. m-Dinitrobenzene&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.748 ± 0.002</td>
<td>1.877 ± 0.001</td>
<td>1.719 ± 0.010</td>
<td>1.825 ± 0.016</td>
</tr>
<tr>
<td>22. 1-Ethylpyridine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.764 ± 0.003</td>
<td>1.222 ± 0.002</td>
<td>0.833 ± 0.012</td>
<td>1.279 ± 0.010</td>
</tr>
<tr>
<td>23. m-Fluorophenol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.796 ± 0.002</td>
<td>1.552 ± 0.005</td>
<td>0.863 ± 0.008</td>
<td>1.116 ± 0.010</td>
</tr>
<tr>
<td>24. p-Lutidine&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.559 ± 0.002</td>
<td>1.171 ± 0.005</td>
<td>0.615 ± 0.009</td>
<td>1.081 ± 0.010</td>
</tr>
<tr>
<td>25. 4-Hydroxybenzene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.885 ± 0.002</td>
<td>1.447 ± 0.004</td>
<td>0.704 ± 0.007</td>
<td>0.796 ± 0.010</td>
</tr>
<tr>
<td>26. Phenol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.673 ± 0.003</td>
<td>0.931 ± 0.008</td>
<td>0.696 ± 0.009</td>
<td>0.871 ± 0.009</td>
</tr>
<tr>
<td>27. Phenylecetonitrile&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.496 ± 0.004</td>
<td>1.460 ± 0.002</td>
<td>1.564 ± 0.007</td>
<td>1.470 ± 0.006</td>
</tr>
<tr>
<td>28. 4-Picoline&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.432 ± 0.002</td>
<td>0.781 ± 0.001</td>
<td>0.345 ± 0.008</td>
<td>0.833 ± 0.008</td>
</tr>
<tr>
<td>29. Phenol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.075 ± 0.003</td>
<td>0.388 ± 0.004</td>
<td>0.217 ± 0.004</td>
<td>0.560 ± 0.009</td>
</tr>
<tr>
<td>30. Quinoline</td>
<td>1.216 ± 0.004</td>
<td>1.925 ± 0.004</td>
<td>1.284 ± 0.002</td>
<td>1.916 ± 0.011</td>
</tr>
</tbody>
</table>

a mobile phase: 20 percent acetonitrile in water; Figure 2
b mobile phase: octanol-saturated water; Figure 3
c mobile phase: 20 percent acetonitrile pH 7.0 in phosphate buffer (0.01 M); Figure 4
d mobile phase: octanol-saturated phosphate buffer pH 7.0 (0.01 M); Figure 5
e substituted phenolic compounds
f substituted pyridine compounds
g substituted nonphenolic benzene compounds
FIGURE 2 LOG P VS LOG $K'_{ACN}$ ON XAD-2

- EQUATION 15
- PHENOLS
- PYRIDINES
- NONPHENOLIC BENZENES
- MISCELLANEOUS
FIGURE 3 CORRECTED LOG P VS LOG K' PH 7 ACN ON XAD-2

- EQUATION 16
- * PHENOLS
- ▲ PYRIDINES
- ○ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
FIGURE 4 LOG P VS LOG K'OCTANOL ON XAD-2

EQUATION 17

- PHENOLS
- PYRIDINES
- NONPHENOLIC BENZENES
- MISCELLANEOUS
FIGURE 5 CORRECTED LOG P VS LOG K' PH 7 OCTANOL ON XAD-2

**EQUATION 18**

- **PHENOLS**
- **PYRIDINES**
- **NONPHENOLIC BENZENES**
- **MISCELLANEOUS**
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Log $k'$&lt;sub&gt;ACN&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Log $k'$&lt;sub&gt;ACNBUF&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acetanilide&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.558 ± 0.005</td>
<td>0.496 ± 0.007</td>
</tr>
<tr>
<td>2. Acetone</td>
<td>-0.039 ± 0.012</td>
<td>-0.140 ± 0.013</td>
</tr>
<tr>
<td>3. Acetophenone&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.945 ± 0.007</td>
<td>0.912 ± 0.008</td>
</tr>
<tr>
<td>4. 2-Acetylpyridine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.465 ± 0.009</td>
<td>0.422 ± 0.009</td>
</tr>
<tr>
<td>5. 4-Acetylpyridine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.398 ± 0.010</td>
<td>0.342 ± 0.008</td>
</tr>
<tr>
<td>6. Acridine</td>
<td>1.857 ± 0.008</td>
<td>1.882 ± 0.007</td>
</tr>
<tr>
<td>7. 2-Amino-4-picoline&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.378 ± 0.003</td>
<td>0.579 ± 0.006</td>
</tr>
<tr>
<td>8. Aniline&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.552 ± 0.009</td>
<td>0.460 ± 0.009</td>
</tr>
<tr>
<td>9. o-Anisidine&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.744 ± 0.009</td>
<td>0.657 ± 0.008</td>
</tr>
<tr>
<td>10. Anisole&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.220 ± 0.008</td>
<td>1.176 ± 0.007</td>
</tr>
<tr>
<td>11. Benzaldehyde&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.827 ± 0.009</td>
<td>0.792 ± 0.001</td>
</tr>
<tr>
<td>12. Benzene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.138 ± 0.008</td>
<td>1.095 ± 0.008</td>
</tr>
<tr>
<td>13. Benzonitrile&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.954 ± 0.008</td>
<td>0.929 ± 0.007</td>
</tr>
<tr>
<td>14. Benzophenone&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.969 ± 0.004</td>
<td>1.979 ± 0.002</td>
</tr>
<tr>
<td>15. 2-Butanone</td>
<td>0.205 ± 0.006</td>
<td>0.127 ± 0.007</td>
</tr>
<tr>
<td>16. Catechol&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.287 ± 0.009</td>
<td>0.263 ± 0.010</td>
</tr>
<tr>
<td>17. Chlorobenzene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.555 ± 0.007</td>
<td>1.619 ± 0.006</td>
</tr>
<tr>
<td>18. 2-Chloropyridine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.613 ± 0.007</td>
<td>0.553 ± 0.005</td>
</tr>
<tr>
<td>19. 4-Cyanophenol&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.660 ± 0.009</td>
<td>0.492 ± 0.009</td>
</tr>
<tr>
<td>20. N,N-Dimethylaniline&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.445 ± 0.008</td>
<td>1.370 ± 0.003</td>
</tr>
<tr>
<td>21. m-Dinitrobenzene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.140 ± 0.007</td>
<td>1.103 ± 0.008</td>
</tr>
<tr>
<td>22. 2-Ethylpyridined</td>
<td>1.109 ± 0.008</td>
<td>0.922 ± 0.008</td>
</tr>
<tr>
<td>23. 4-Fluorophenol&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.743 ± 0.009</td>
<td>0.695 ± 0.007</td>
</tr>
<tr>
<td>24. 2,6-Lutidine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.226 ± 0.003</td>
<td>0.858 ± 0.005</td>
</tr>
<tr>
<td>25. 4-Nitrophenol&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.805 ± 0.008</td>
<td>0.269 ± 0.008</td>
</tr>
<tr>
<td>26. Phenol&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.579 ± 0.009</td>
<td>0.532 ± 0.008</td>
</tr>
<tr>
<td>27. Phenylacetonitrile&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.042 ± 0.009</td>
<td>1.109 ± 0.007</td>
</tr>
<tr>
<td>28. 4-Picolined</td>
<td>1.013 ± 0.007</td>
<td>0.726 ± 0.008</td>
</tr>
<tr>
<td>29. Pyridine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.546 ± 0.003</td>
<td>0.389 ± 0.009</td>
</tr>
<tr>
<td>30. Quinoline</td>
<td>1.108 ± 0.008</td>
<td>1.085 ± 0.007</td>
</tr>
</tbody>
</table>

<sup>a</sup> mobile phase: 20 percent acetonitrile in water; Figure 6

<sup>b</sup> mobile phase: 20 percent acetonitrile pH 7.0 in phosphate buffer (0.01 M); Figure 7

<sup>c</sup> substituted phenolic compounds

<sup>d</sup> substituted pyridine compounds

<sup>e</sup> substituted nonphenolic benzene compounds
FIGURE 6 LOG P VS LOG K' ACN ON C-18

- EQUATION 19
- PHENOLS
- PYRIDINES
- NONPHENOLIC BENZENES
- MISCELLANEOUS
FIGURE 7 CORRECTED LOG P VS LOG K' PH 7 ACN ON C-18

- EQUATION 20
- * PHENOLS
- ♦ PYRIDINES
- ◦ NONPHENOLIC BENZENES
- △ MISCELLANEOUS

CORRECTED LOG P

LOG K' PH 7 ACN
Table IV. Correlations of Log P with Log k' Values

<table>
<thead>
<tr>
<th>Eq.</th>
<th>HPLC Column</th>
<th>Mobile Phase</th>
<th>Log P = a + b(Log k')</th>
<th>n</th>
<th>r</th>
<th>$s_b$</th>
<th>$F_{1,n-2}$</th>
<th>$F_{0.05,1,n-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>XAD-2</td>
<td>20% acetonitrile in water</td>
<td>0.66(0.15) + 0.94(0.13)</td>
<td>30</td>
<td>0.8113</td>
<td>0.47</td>
<td>53.92</td>
<td>4.20</td>
</tr>
<tr>
<td>16</td>
<td>XAD-2</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>0.66(0.12) + 0.91(0.11)</td>
<td>29</td>
<td>0.8432</td>
<td>0.41</td>
<td>66.40</td>
<td>4.21</td>
</tr>
<tr>
<td>17</td>
<td>XAD-2</td>
<td>octanol-saturated water</td>
<td>0.50(0.17) + 0.81(0.13)</td>
<td>27</td>
<td>0.7747</td>
<td>0.38</td>
<td>37.53</td>
<td>4.24</td>
</tr>
<tr>
<td>18</td>
<td>XAD-2</td>
<td>octanol-saturated phosphate buffer pH 7.0</td>
<td>0.55(0.15) + 0.79(0.13)</td>
<td>27</td>
<td>0.7774</td>
<td>0.39</td>
<td>38.19</td>
<td>4.24</td>
</tr>
<tr>
<td>19</td>
<td>µC-18</td>
<td>20% acetonitrile in water</td>
<td>0.14(0.14) + 1.53(0.14)</td>
<td>30</td>
<td>0.8994</td>
<td>0.35</td>
<td>118.54</td>
<td>4.20</td>
</tr>
<tr>
<td>20</td>
<td>µC-18</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>0.31(0.11) + 1.51(0.12)</td>
<td>30</td>
<td>0.9197</td>
<td>0.32</td>
<td>153.69</td>
<td>4.20</td>
</tr>
<tr>
<td>21</td>
<td>C-18e</td>
<td>octanol-saturated phosphate buffer pH 7.0</td>
<td>0.80(0.03) + 1.03(0.03)</td>
<td>30</td>
<td>0.9890</td>
<td>0.12</td>
<td>1254.95</td>
<td>4.20</td>
</tr>
</tbody>
</table>

$^a$ ( ) = standard error of the regression coefficient

$^b$ $s = standard error of the regression

$^c$ calculated F value

$^d$ critical F value

$^e$ reference 27, using compounds listed in Table I
Subtle differences exist between XAD-2 and octadecylsilane stationary phases. In the partition process with XAD-2, the aromatic portion of the solute may be preferentially sorbed into the polystyrene network of the resin while the hydrophilic section of the molecule remains oriented in the aqueous phase. From the structural point of view, XAD-2 should have a property close to toluene or benzene. Alteration in the aromatic/hydrophilic balance within the solute or within the solvent mixture in comparison to the resin will affect the partitioning of the solute.

On bonded octadecylsilane supports, silylation with hexamethyldisilazane (HMDS) or trimethylsilyl chloride (TMSCl) has been carried out (21) to block unbonded silanol sites. Nevertheless, a few of the silanol sites on chemically bonded phases may still be sterically protected from silylating reagents. Therefore, the reversed-phase octadecylsilane stationary phase is not entirely nonpolar but still has some degree of polarity. This polarity coupled with the aliphatic chain may make the bonded octadecylsilane similar to water-saturated octanol in octanol/water system. A column of XAD-2 resin coated with octanol does not have polar sites and therefore, is minimally hydrated.

A better way of evaluating whether a chromatographic system mimics the octanol-water shake-flask system is to
utilize equation 14. If the chromatographic system accurately models the shake-flask system, a scatter of points on a $\Delta \log P$ versus $\Delta \log k'$ plot should fall on the ideal line which goes through the origin and has a slope of one. Any compound can function as the reference. In this work the convention of using benzene as the reference was followed. Thus $\Delta \log P$ is equivalent to $\pi$. Because of the non-additivity of $\pi$, log P is used so that the disubstituted compounds (compound 9,16,19,21,23,25), the aliphatic ketones (compound 2,15) and the heterocyclic compounds (compound 4-7,18,22,24,28-30) may be included without misinterpretation. Chiou et al. (60) have used this approach to compare the effects of substituents on partition coefficients of substituted benzenes in heptane-water and octanol-water systems. They found that heptane-water is more sensitive than octanol-water to the polarity of the substituents presumably due to the fact that octanol is partially polar which can accommodate a wide range of compounds of varying polarities whereas heptane is extremely nonpolar responding favorably to nonpolar compounds and unfavorably to polar compounds.

Plots of $\Delta \log P$ versus $\Delta \log k'$ for the XAD-2 column will be found in Figures 8-11 and for the commercial octadecylsilane column in Figures 12-13. Essentially a compound that deviates from the linear correlation line between log P and log k' differs from the reference compound, benzene, in "a" and "n".
FIGURE 8 △LOG P VS △LOG K' ACN ON XAD-2

- IDEAL LINE
- * PHENOLS
- ○ PYRIDINES
- ○ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
FIGURE 9 ΔCORRECTED LOG P VS ΔLOG K' PH 7 ACN ON XAD-2

- IDEAL LINE
- * PHENOLS
- ♦ PYRIDINES
- ○ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
FIGURE 10 ΔLOG P VS ΔLOG K' OCTANOL ON XAD-2

- IDEAL LINE
- * PHENOLS
- ○ PYRIDINES
- ○ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
FIGURE 11 ΔCORRECTED LOG P VS ΔLOG K' PH 7 OCTANOL ON XAD-2

- IDEAL LINE
- * PHENOLS
- ♦ PYRIDINES
- ○ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
FIGURE 12 ΔLOG P VS ΔLOG K′ ACN ON C-18

---

- IDEAL LINE
- * PHENOLS
- ♦ PYRIDINES
- ○ NONPHENOLIC BENZENES
- △ MISCELLANEOUS

Δ LOG P

Δ LOG K′ ACN
FIGURE 13 ΔCORRECTED LOG P VS ΔLOG K' PH 7 ACN ON C-18

- IDEAL LINE
- * PHENOLS
- ◇ PYRIDINES
- ◇ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
FIGURE 14 ∆CORRECTED LOG P VS ∆LOG K' PH 7 OCTANOL ON C-18 FROM UNGER'S STUDY (REFERENCE 27)

--- IDEAL LINE
* * PHENOLS
▽ ▽ PYRIDINES
○ ○ NONPHENOLIC BENZENES
△ △ MISCELLANEOUS
These results are consistent with previous reports where most investigators have found the correlations between log P and log k' on reversed-phase HPLC are obtained mostly only for distinct classes of compounds. The slopes and/or intercepts can differ between classes of compounds. The reason that the results reported by Mirrlees et al. (24) and Unger et al. (27) give a slope of one and a common intercept of "a" for different classes of compounds is due to the use of octanol-saturated C-18 and octanol-saturated water as the mobile phase, satisfying the requirement of equation 14. In this study, regression equations were obtained for three subgroups of the test set: phenols, substituted pyridines and substituted nonphenolic benzenes. The results are summarized in Tables V and VI. In general, compounds in a homologous series such as the phenols show a good to excellent relationship between log P and log k'. A similar statement can be made for the pyridines. The substituted nonphenolic benzenes only show a good correlation on the commercial octadecyilsilane column. The scatter plots and regression data show that the octadecyilsilane column better mimic the octanol-water shake-flask system. However, using the group data, the XAD-2 system generally gave better correlations for the polar substituted phenols and pyridines. This can be explained by the XAD-2 stationary phase lacks polar sites
Table V. Correlations of Log P with Log k' Values of Each Group of Compound from XAD-2 Column

<table>
<thead>
<tr>
<th>Eq.</th>
<th>Compound</th>
<th>Mobile Phase</th>
<th>Log P = a + b(Log k')</th>
<th>n</th>
<th>r</th>
<th>s</th>
<th>F_{1,n-2}</th>
<th>F_{0.05,1,n-2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Substituted Phenols</td>
<td>20% acetonitrile in water</td>
<td>0.69(0.11) + 1.15(0.16)</td>
<td>5</td>
<td>0.9805</td>
<td>0.09</td>
<td>74.84</td>
<td>10.13</td>
</tr>
<tr>
<td>23</td>
<td>Substituted Phenols</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>0.82(0.08) + 1.11(0.13)</td>
<td>5</td>
<td>0.9011</td>
<td>0.08</td>
<td>77.17</td>
<td>10.13</td>
</tr>
<tr>
<td>24</td>
<td>Substituted Phenols</td>
<td>octanol-saturated water</td>
<td>0.64(0.06) + 0.89(0.06)</td>
<td>5</td>
<td>0.9936</td>
<td>0.05</td>
<td>232.57</td>
<td>10.13</td>
</tr>
<tr>
<td>25</td>
<td>Substituted Phenols</td>
<td>octanol-saturated phosphate buffer pH 7.0</td>
<td>0.75(0.16) + 0.99(0.19)</td>
<td>5</td>
<td>0.9465</td>
<td>0.14</td>
<td>25.81</td>
<td>10.13</td>
</tr>
<tr>
<td>26</td>
<td>Substituted Pyridines</td>
<td>20% acetonitrile in water</td>
<td>0.68(0.28) + 1.03(0.51)</td>
<td>8</td>
<td>0.6370</td>
<td>0.40</td>
<td>4.10</td>
<td>5.99</td>
</tr>
<tr>
<td>27</td>
<td>Substituted Pyridines</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>0.54(0.16) + 1.22(0.10)</td>
<td>8</td>
<td>0.8551</td>
<td>0.28</td>
<td>16.32</td>
<td>5.99</td>
</tr>
<tr>
<td>28</td>
<td>Substituted Pyridines</td>
<td>octanol-saturated water</td>
<td>0.33(0.24) + 1.04(0.27)</td>
<td>8</td>
<td>0.8387</td>
<td>0.28</td>
<td>14.23</td>
<td>5.99</td>
</tr>
<tr>
<td>29</td>
<td>Substituted Pyridines</td>
<td>octanol-saturated phosphate buffer pH 7.0</td>
<td>0.20(0.16) + 1.08(0.19)</td>
<td>8</td>
<td>0.9207</td>
<td>0.21</td>
<td>33.40</td>
<td>5.99</td>
</tr>
<tr>
<td>30</td>
<td>Substituted Non-phenolic Benzenes</td>
<td>20% acetonitrile in water</td>
<td>0.82(0.36) + 0.73(0.26)</td>
<td>14</td>
<td>0.6293</td>
<td>0.54</td>
<td>7.87</td>
<td>4.75</td>
</tr>
<tr>
<td>31</td>
<td>Substituted Non-phenolic Benzenes</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>1.06(0.34) + 0.51(0.26)</td>
<td>13</td>
<td>0.5078</td>
<td>0.47</td>
<td>3.82</td>
<td>4.84</td>
</tr>
<tr>
<td>32</td>
<td>Substituted Non-phenolic Benzenes</td>
<td>octanol-saturated water</td>
<td>1.22(0.43) + 0.26(0.31)</td>
<td>12</td>
<td>0.2523</td>
<td>0.45</td>
<td>0.68</td>
<td>4.96</td>
</tr>
<tr>
<td>33</td>
<td>Substituted Non-phenolic Benzenes</td>
<td>octanol-saturated phosphate buffer pH 7.0</td>
<td>1.28(0.37) + 0.24(0.28)</td>
<td>12</td>
<td>0.2672</td>
<td>0.42</td>
<td>0.77</td>
<td>4.96</td>
</tr>
</tbody>
</table>

\(^a\) ( ) = standard error of the regression coefficient
\(^b\) s = standard error of the regression
\(^c\) calculated F value
\(^d\) critical F value
Table VI. Correlations of Log P with Log k' Values of Each Group of Compound from Reversed-Phase C-18 Column

<table>
<thead>
<tr>
<th>Eq.</th>
<th>Compound</th>
<th>Mobile Phase</th>
<th>Log P = a + b(Log k')</th>
<th>n</th>
<th>r</th>
<th>a</th>
<th>b</th>
<th>F₁,n₋₂</th>
<th>F₀.₀₅ ₁,n₋₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Substituted Phenols</td>
<td>20% acetonitrile in water</td>
<td>0.27(0.01) + 2.05(0.02)</td>
<td>5</td>
<td>0.9998</td>
<td>0.01</td>
<td>9682.68</td>
<td>10.13</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Substituted Phenols</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>0.95(0.44) + 1.17(0.92)</td>
<td>5</td>
<td>0.5948</td>
<td>0.34</td>
<td>1.64</td>
<td>10.13</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Substituted Pyridines</td>
<td>20% acetonitrile in water</td>
<td>0.37(0.32) + 0.95(0.34)</td>
<td>8</td>
<td>0.7464</td>
<td>0.35</td>
<td>7.55</td>
<td>5.99</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Substituted Pyridines</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>-0.13(0.28) + 1.97(0.45)</td>
<td>8</td>
<td>0.8726</td>
<td>0.26</td>
<td>19.15</td>
<td>5.99</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Substituted Non-phenolic Benzenes</td>
<td>20% acetonitrile in water</td>
<td>0.15(0.18) + 1.54(0.16)</td>
<td>14</td>
<td>0.9400</td>
<td>0.24</td>
<td>91.07</td>
<td>4.75</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Substituted Non-phenolic Benzenes</td>
<td>20% acetonitrile pH 7.0 in phosphate buffer</td>
<td>0.37(0.14) + 1.41(0.13)</td>
<td>14</td>
<td>0.9515</td>
<td>0.21</td>
<td>114.73</td>
<td>4.75</td>
<td></td>
</tr>
</tbody>
</table>

a ( ) = standard error of the regression coefficient  
b = standard error of the regression  
c = calculated F value  
d = critical F value
such as the silanol groups as on a C-18 column. To date, the octanol coated column described by Unger (27) is superior to any other commercial column. Adding a suitable counter ion (N,N-dimethyloctylamine) to compete with small basic molecules, i.e., substituted pyridines, in binding with free silanol sites, these relatively basic unhindered pyridines will behave normally and move onto the regression line (46).

When the partition coefficient of a solute is near or greater than that of octanol \( \log P = 3.15 \), octanol coated column cannot be used. Then compounds with known \( \log P \) values and properties similar to the one to be determined can be used as standards to obtain a regression model using \( \log k' \) as the independent variable.

The comparative behavior on the XAD-2 column of octanol-saturated mobile phase and 20 percent acetonitrile can be seen in Figures 15 and 16. In both cases the slope is one and parallels the theoretical line. The intercept represents constant difference resulting from the change in mobile phase. Adjusting concentration of acetonitrile should move the regression line onto the theoretical line.

A similar comparison using the same mobile phase but changing the stationary phase can be seen in Figures 17 and 18. There is more of a scatter and the slope of the overall regression line (not shown) is only 0.5 (Eq. 42,43). This slope could well be dependent on the
<table>
<thead>
<tr>
<th>Eq.</th>
<th>Column</th>
<th>$y = a + bx$</th>
<th>$n$</th>
<th>$r$</th>
<th>$s_b$</th>
<th>$F_{1,n-2}$</th>
<th>$F_{0.05,1,n-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>XAD-2</td>
<td>$\log k'^{'}<em>{\text{oct}} = 0.26(0.07) + 1.01(0.07)\log k'^{'}</em>{\text{acn}}$</td>
<td>27</td>
<td>0.9417</td>
<td>0.20</td>
<td>195.80</td>
<td>4.24</td>
</tr>
<tr>
<td>41</td>
<td>XAD-2</td>
<td>$\log k'^{'}<em>{\text{oct}, \text{pH 7}} = 0.23(0.05) + 1.00(0.06)\log k'^{'}</em>{\text{acn}, \text{pH 7}}$</td>
<td>27</td>
<td>0.9569</td>
<td>0.18</td>
<td>271.66</td>
<td>4.24</td>
</tr>
<tr>
<td>42</td>
<td>XAD-2 and µC-18</td>
<td>$\log k'^{'}<em>{\text{acn}, \text{C-18}} = 0.43(0.10) + 0.51(0.08)\log k'^{'}</em>{\text{acn}, \text{XAD-2}}$</td>
<td>30</td>
<td>0.7473</td>
<td>0.32</td>
<td>35.44</td>
<td>4.20</td>
</tr>
<tr>
<td>43</td>
<td>XAD-2 and µC-18</td>
<td>$\log k'^{'}<em>{\text{acn}, \text{pH 7}, \text{C-18}} = 0.28(0.07) + 0.55(0.06)\log k'^{'}</em>{\text{acn}, \text{pH 7}, \text{XAD-2}}$</td>
<td>29</td>
<td>0.8542</td>
<td>0.24</td>
<td>72.94</td>
<td>4.21</td>
</tr>
</tbody>
</table>

\(^a\) = standard error of the regression coefficient  
\(^b\) = standard error of the regression  
\(^c\) = calculated F value  
\(^d\) = critical F value
FIGURE 15 OCTANOL VS ACN ON XAD-2

- THEORETICAL LINE
- EQUATION 40
- • PHENOLS
- □ PYRIDINES
- ● NONPHENOLIC BENZENES
- △ MISCELLANEOUS

LOG K' OCTANOL vs LOG K' ACN
FIGURE 16 PH 7 OCTANOL VS PH 7 ACN ON XAD-2

- THEORETICAL LINE
- EQUATION 41
* * PYRIDINES
◊ ◊ PYRIDINES
○ ○ NONPHENOLIC BENZENES
△ △ MISCELLANEOUS

LOG K' PH 7 OCTANOL

LOG K' PH 7 ACN
FIGURE 7 ACN(XAD-2) VS ACN(C-18)

- THEORETICAL LINE
- ★ PHENOLS
- ♦ PYRIDINES
- ○ ⋄ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
FIGURE 18 PH 7 ACN(XAD-2) VS PH 7 ACN(C-18)

- THEORETICAL LINE
- * PHENOLS
- ◇ PYRIDINES
- ○ NONPHENOLIC BENZENES
- △ MISCELLANEOUS
selection of compounds used in this study. In general, compounds with log P less than 1.0-1.2 are above the theoretical line and are not as likely to partition into the relatively non-polar XAD-2 stationary phase. Addition of aliphatic substituents (compound 20.22,24) or non-aromatic (compound 2,15) tend to partition towards the aliphatic octadecylsilane stationary phase relative to the aromatic XAD-2. Also, use of a buffered mobile phase reduces the scatter about the theoretical line.
REFERENCES


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57. Pomona College Medicinal Chemistry Project, Pomona College, Claremont, CA, July 1978.