Selection of a Reference Partitioning System for Drug Design Work

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Abstract □ Consideration of the structural, polar, and transport properties of water-saturated solvent and solvent-saturated water phases of three general and eight specific binary systems leads to the conclusion that the *n*-octanol–water system is a very good all-round compromise for use as a reference system for biological partitioning in drug design work.

Keyphrases □ Partitioning systems—selection of a reference for drug design work, structural, polar, and transport properties of solvent and water phases, *n*-octanol–water system and others □ Biological partitioning—reference systems, structural, polar, and transport properties of solvent and water phases □ Transport—selection of a reference partitioning system for drug design work

Apart from its intrinsic chemical ability to act at the active site, a biologically active species can exert its influence only if it can reach the active site by a transport process that involves passage through both hydrophilic and lipophilic barriers in the biological system. As a consequence, an important aspect of drug design is the modification of the hydrophobic character of compounds possessing a desired intrinsic chemical activity so as to optimize their transport to, and their binding at, the active site. This is clearly a partitioning problem, and the need for a "synthetic" reference biological system is at once apparent if such modifications and development are to be carried on expeditiously and reproducibly. As a result, various solvent–water systems have been employed with the intent that the solvent represent the lipophilic phase of biological systems and the water represent the hydrophilic phase.

The 5806 partition coefficients listed by Leo *et al.* (1) (about 9000 values are currently in the data bank) stand as some measure of the current importance of these values. The past decade has seen a very rapid development, both in magnitude and sophistication, of the correlation of molecular structure with biological activity based on a system that considers the partition coefficient of the biologically active compound in a reference partitioning system to be a major (if not *the* major) parameter in the correlation equation. Although the original concept was put forth at the beginning of this century, the recent rapid development has been facilitated by the computerization of the techniques of linear regression analysis and the inclusion of other structural parameters; the principles are now firmly established as a major tool for drug design.

A problem associated with the current situation is that of relating the correlations based on one reference system to those based on another. This problem has been dealt with statistically (1), but it is hoped that through such papers as the present one this problem can eventually be lessened by mutual agreement on one system, or type of system, that has significant advantages over the others.

At the outset, it must be realized that all biological systems, or parts of them, are far too complicated to be really closely mimicked, even with respect only to hydrophobic characteristics, by a simple two-phase system. Furthermore, it must be realized that in biological systems the lipophilic phases are not "pure hydrocarbon" in nature but are associated with significant amounts of water held by the polar and/or ionic portions of the macromolecules of which they are composed. These polar areas and their associated water molecules have a profound effect on the lipophilicity of these phases.

There is a tendency to think of partition coefficients as coming from reference systems composed of one pure solvent phase and one pure water phase, but, in fact, the partition coefficients refer to systems composed of two binary phases, a water-saturated solvent phase and a solvent-saturated water phase. One objective of this paper is to examine the effect of saturation on the structure and properties of a given phase. The eight solvents compared here vary tremendously in their hydrophobicity and in their ability to dissolve water, but on the whole they have relatively small solubility in water.

It should also be emphasized that, to be useful, a partition coefficient *(P)* must characterize the transfer of only a simple molecular form (or forms) in which it exists in these phases. To determine *P*, the experimentally determined amounts of partitioned solute per unit volume in each phase (the ratio being referred to as the *distribution* ratio) must be corrected to take account of the various molecular species with which it is in equilibrium. For many solutes, this can be achieved rigorously only by application of coupled spectroscopic–computer techniques or inferentially from the study of *P* as a function of concentration based on the *assumed* existence of molecular species. The uncertainties in calculation of true values of *P* will vary with the partitioning systems. For routine use, it is desirable to have a reference system that minimizes these uncertainties for as wide a range of solutes as possible.

This study shows the effects of saturation on the

dielectric constant, density, viscosity, and enthalpy of activation of viscous flow, and they are interpreted along with related measurements (including IR and NMR) made by others.

EXPERIMENTAL

All experimental measurements were made at temperatures within ±0.05° of the values shown, as determined against a recently factory-calibrated piezo-electric quartz thermometer[1]. The molecular composition of the olive oil[2] was determined by GC analysis of the methyl esters of the fatty acids obtained by saponification; the molecular weight was thus calculated to be 875.6 g/mole. Oleyl alcohol was reagent grade. 1-Octanol was reagent grade subjected to the following treatment: *(a)* shaking with dilute sodium hydroxide, *(b)* shaking with dilute sulfuric acid, *(c)* drying with excess anhydrous sodium sulfate, and *(d)* fractional distillation, discarding the first fraction that contains a very small concentration of UV-absorbing contaminant. All other solvents were spectroquality.

The saturated solutions were made by a succession of vigorous shakings in glass-stoppered bottles over about a day, and they were subsequently retained in their respective baths for many days before removal of samples for the required measurements. Because of slow hydrolysis, the measurements on ethyl acetate solutions were made within an hour or so of their preparation. Density (ρ) measurements were made with standard calibrated 25-ml pycnometers, dielectric constant (ϵ) measurements were made at 5 MHz with an instrument designed for capacity substitution (2), and viscosity (η) measurements were made with viscometers[3] (using sizes appropriate for the viscosities under consideration) calibrated by suitable standards. These standards[4], conforming to ASTM oil standards, had kinematic viscosities at 25.0° of 3.702, 8.904, and 34.45 centipoise ml/g; 10-sec full sweep stopwatches were used.

Values of ΔH_{vis}, enthalpies of activation of viscous flow, were obtained from the slope of the computerized least-squares fit of the plot of log (η/ρ) *versus* $1/T$, corresponding to:

$$\log(\eta/\rho) = \frac{\Delta H_{vis}}{2.303R}\frac{1}{T} + b \qquad \text{(Eq. 1)}$$

Correlation coefficients were 0.9997 or better in all cases, except for olive oil for which the correlation coefficient was 0.990. Solubility data were taken from the literature for most cases, with consensus values being used where possible. The solubilities of oleyl alcohol and olive oil in water were determined by approximation; they are very low and the values are rough. The results are tabulated in Tables I and II.

DISCUSSION

Except for special biological situations involving "active transport" or "circulation," molecular transport is heavily dependent on diffusion processes. Self-diffusion or transport of a "marked" molecule through a liquid matrix of others identical to it is very simply related to the viscosity of that liquid. The diffusivity of a dilute solute through a liquid matrix of identical but different solvent molecules is also related to the viscosity of the solvent; but, in addition, there is a component related to a concentration gradient and thus to the entropy and enthalpy of dilution. Even in the latter case, however, the solvent usually controls the motions of the isolated solute molecules in ways that relate viscosity to the intermolecular forces and to the molar ratio of free volume to intrinsic volume (3, 4). It is not immediately obvious that viscosities expressed in the traditional "kinematic viscosity" mode (η/ρ = poise × ml/g) is related to the mass transport phenomenon of diffusion. This does become evident, however, if the poise is expressed in cgs units as η = g/sec × cm; then kinematic viscosity = η/ρ = (g/sec × cm)/(g/cm^3) = cm^2/sec, the cgs units characteristic of diffusion coefficients.

The free volume of a liquid is often considered to be the sum of a large number of empty "holes" into which diffusing molecules may slip in the transport process. As one hole is filled, another is created at the location where the diffusing molecule had just been. The enthalpy of activation of viscous flow (ΔH_{vis}) is a measure of the potential barrier that a molecule must surmount as it moves from one hole in the liquid to an adjacent hole. In this sense, it is a measure of the resistance to self-diffusion in a liquid.

Table II shows that in all of the saturated binary phases, except those involving *n*-butanol and ethyl acetate, the resistance to molecular transport was changed at the most by only 3%. Thus, for the majority of these binary phases, it can be said that internal transport characteristics are not significantly different from those of the pure major component of the phase. The binary phases involving *n*-butanol and ethyl acetate, where the changes in values of ΔH_{vis} ranged from 8 to 22%, will be dealt with later.

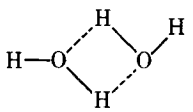
The dielectric constant (ϵ) of a liquid gives a measure of the resistance to molecular "flipping" in response to alternations of an applied electric field. For a given temperature and field frequency, the resistance to molecular rotation involves the size, polarizability, and permanent dipole (if any) of the molecules and any tendency to self-associate with other molecules to form larger aggregates. The addition of significant amounts of highly polar water to organic solvents may result in either an increase or a decrease in ϵ; as a result, molecular characteristics of liquid structure may be inferred from determination of ϵ as a function of temperature and concentration. Such inferences (based on assumed molecular models) have a greater probability of being correct if spectroscopic (usually IR and NMR) substantiation of the molecular models is possible. The following discussion, organized according to class of solvent, concerns what appears to be the appropriate aspects of the results of such measurements as they apply to the problems of determining partition coefficients.

Nonpolar and Slightly Polar Solvents—Of the solvents studied, cyclohexane is the only one that is truly nonpolar and has no capacity for hydrogen bond formation. As a result, it has no tendency for self-association into molecular aggregates, there is no liquid structure, and water is extremely insoluble in it. In a water-saturated solution, the dissolved water has no significant effect on ϵ, ρ, or η. Its solvent characteristics are typical of other saturated hydrocarbons and compounds such as carbon tetrachloride in which studies pertinent to the present discussion have been made.

Isopiestic studies (5) indicate that the very small amount of water that does dissolve in cyclohexane and carbon tetrachloride is, for all practical purposes, not associated. A recent IR study (6) of water dissolved in carbon tetrachloride indicated that about 3.6% of the dissolved water is present as a dimer, Structure I being more likely than II. The equilibrium constant for dimerization was calculated to be 2.2 M^{-1}. Since water is four times more soluble in carbon tetrachloride than in cyclohexane, it seems likely that dimerization is substantially less in cyclohexane and that, from a practical standpoint, water exists only in monomeric form in water-saturated cyclohexane.

As a partitioning liquid, this binary phase has a great affinity for nonpolar compounds and their values of P will be extremely high (the reverse is true for very polar and ionic compounds), a fact that makes the determination of P very difficult because of the very low solute solubility in one phase or the other. Partial alleviation of this difficulty is normally accomplished by selecting a suitable volume ratio of the two phases (7); but when one phase is almost totally nonpolar, this approach is quite impractical for many solutes.

This very pure nonpolar characteristic also leads to other experimental difficulties that may be impossible to overcome when measuring P values for polar solutes. To illustrate this fact, it is necessary to draw on experiments done with carbon tetrachloride, carbon disulfide, benzene, isooctane, and other nonpolar solvents. It has been found (8, 9) that when a polar solute (S) is extracted from water, there may be a many fold increase in the solubility of water in the nonpolar solvent due to the formation of hydrated solute



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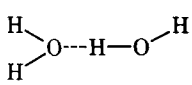

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[1] Hewlett-Packard model 2801A.
[2] Import Virgin Pompeian, Pompeian, Inc., Baltimore, MD 21224
[3] Cannon-Fenske type.
[4] Cannon Instrument Co., State College, Pa.

Table I—Densities (ρ) and Viscosities (η) of Pure and Saturated Solvents at 15–35°

Solvent	State	ρ, g/ml			η, centipoises		
		15.00°	25.00°	35.00°	15.00°	25.00°	35.00°
Cyclohexane	Pure	0.784	0.774	0.765	1.074	0.896	0.761
	Water saturated	0.784[a]	0.774[a]	0.765[a]	1.074[b]	0.896[b]	0.761[b]
Benzene	Pure	0.884	0.874	0.863	0.700	0.602	0.524
	Water saturated	0.884	0.874	0.864	0.699	0.601	0.524
Chloroform	Pure	1.493	1.475	1.453	0.595	0.538	0.489
	Water saturated	1.498	1.479	1.459	0.596	0.539	0.490
n-Butanol	Pure	0.814	0.807	0.799	3.32	2.52	1.93
	Water saturated	0.849	0.844	0.836	3.86	2.80	2.06
n-Octanol	Pure	0.828	0.822	0.815	11.0	7.61	5.43
	Water saturated	0.834	0.829	0.824	10.6	7.26	5.17
Oleyl alcohol	Pure	0.854	0.847	0.841	45.2	28.4	18.7
	Water saturated	0.855	0.850	0.840	44.1	27.9	18.4
Ethyl acetate	Pure	0.906	0.895	0.884	0.482	0.430	0.387
	Water saturated	0.911	0.900	0.899	0.520	0.460	0.410
Olive oil	Pure	0.916	0.910	0.904	101.3	63.1	41.7
	Water saturated	0.916	0.910	0.904	100.5	62.6	41.4
Water	Pure	0.999	0.997	0.994	1.14	0.894	0.720
	Cyclohexane saturated	0.999[c]	0.997[c]	0.994[c]	1.14[c]	0.894[c]	0.720[c]
	Benzene saturated	0.998	0.997	0.994	1.14	0.896	0.721
	Chloroform saturated	0.999	0.997	0.994	1.18	0.919	0.736
	n-Butanol saturated	0.989	0.987	0.983	1.57	1.16	0.895
	n-Octanol saturated	0.998	0.997	0.994	1.13	0.887	0.714
	Oleyl alcohol saturated	0.999	0.997	0.994	1.14	0.893	0.718
	Ethyl acetate saturated	0.999	0.996	0.993	1.36	1.04	0.811
	Olive oil saturated	0.999	0.997	0.994	1.15	0.895	0.720

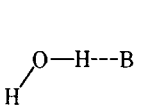
[a] Density data from Ref. 57. [b] Viscosity data from Ref. 58. [c] Density and viscosity for water-saturated cyclohexane and for cyclohexane-saturated water are assumed to be the same (to the number of significant figures given) as for the pure liquids because in each case the solubilities are less than one-fifteenth of those for the benzene–water system for which no changes in ρ and virtually no changes in η were observed.

complexes, $S(H_2O)$, $S(H_2O)_2$, *etc.*, which are in equilibrium with each other; the nonpolar character of the phase may be substantially changed by the process of partition itself. This will be especially bad with hydroxylic compounds, anilines, and other hydrogen bonding substances. This problem is in addition to the solute association (such as carboxylic acid dimerization) that often occurs and for which correction is usually made.

The extent to which the increased water content of the nonaqueous phase may also affect the solute association equilibria has not been extensively studied. However, in one study (from which it would be hazardous to generalize) it was shown (10) that in the distribution of acetamide between chloroform and water the equilibrium constant for the trimerization of acetamide in the water-saturated chloroform was not significantly different from that observed in pure chloroform. There was also no evidence for the existence of other polymers such as dimers and tetramers. This study was carried out at water phase concentrations of 0.42–9.9 *M* acetamide. However, at the solute concentrations normally used for

Table II—Dielectric Constants (ϵ) and Enthalpies of Activation of Viscous Flow (ΔH_{vis}) for Pure and Saturated Solutions at 25.0°, and the Saturation Concentrations of Water-in-Solvent and Solvent-in-Water at 25.0°

Solvent	State	Moles Water / Mole Solvent	Water Concentration, *M*	Solubility References	ϵ	ΔH_{vis}, kcal/mole
Cyclohexane	Pure				2.02	3.03
	Water saturated	0.000266	0.00245	1, 3	2.02	3.03
Benzene	Pure				2.27	2.36
	Water saturated	0.00314	0.0350	3, 45, 46	2.28	2.36
Chloroform	Pure				4.73	1.51
	Water saturated	0.00542	0.0670	1, 47	4.66	1.51
n-Butanol	Pure				17.16	4.64
	Water saturated	1.05	9.53	1, 48, 49	21.29	5.43
n-Octanol	Pure				9.78	6.07
	Water saturated	0.280	1.72	32, 34	8.58	6.23
Oleyl alcohol	Pure				3.86	7.66
	Water saturated	0.228	0.712	1	3.75	7.59
Ethyl acetate	Pure				5.98	1.73
	Water saturated	0.162	1.60	1, 50	7.41	1.87
Olive oil	Pure				3.17	5.93
	Water saturated	0.070	0.0725	1	3.17	6.07
		Moles Solvent / Mole Water	Solvent Concentration, *M*			
Water	Pure				78.54	4.03
	Cyclohexane saturated	1.18×10^{-5}	0.000651	51	76.95	4.03
	Benzene saturated	4.13×10^{-4}	0.0228	46, 51, 52, 53	76.13	4.03
	Chloroform saturated	1.16×10^{-3}	0.0637	54	75.91	4.15
	n-Butanol saturated	1.92×10^{-2}	0.977	48, 49, 55, 62	72.33	4.90
	n-Octanol saturated	7.29×10^{-5}	0.00404	49, 62, 63	76.58	4.05
	Oleyl alcohol saturated	$\sim 6.7 \times 10^{-8}$	~0.0000037		76.80	4.05
	Ethyl acetate saturated	1.64×10^{-2}	0.842	40, 50, 56	72.73	4.54
	Olive oil saturated	$\sim 2.6 \times 10^{-8}$	~0.0000014		76.18	4.07



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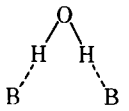

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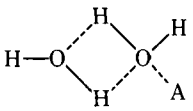

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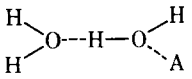

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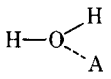


IX

drug design partition studies (10^{-2}–10^{-4} M), there would be no significant amount of trimer in the chloroform and partition would involve only the monomer.

This outlook has been refined by a detailed IR study (11) of the association of water with 17 types of Lewis bases in carbon tetrachloride (taken as typical of inert nonpolar solvents). For all of the nitrogen- and oxygen-containing Lewis bases studied (some containing two nearby basic sites), a 1:1 water–base complex was formed (III) when water was in excess and a 1:2 water–base complex (a hydrated dimer) was formed (IV) when base was in excess. Presumably an effective equilibrium between the two exists when concentrations of water and base are of comparable magnitude.

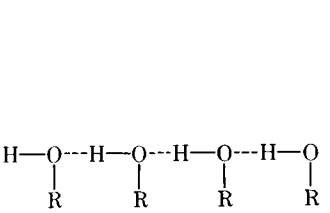
A further study of di-, tri-, and tetrabutylammonium halides also led to the conclusion that the hydrophobic cations were not hydrated at all in nonpolar solvents, but that water is hydrogen bonded to the halide ions in the same way as for the uncharged Lewis bases. Fluoride ion appears to be an exception, requiring two water molecules for hydration when water is in excess; it is suggested that this occurs through distortion of the cyclic dimer (I) and hydrogen bonding of the F^- to the two free hydrogen atoms. Normally, a water molecule is not doubly hydrogen bonded to a single base (even if there are two nearby base sites), although molecules such as 1,10-phenanthroline may represent exceptions.

NMR studies (12) have shown that when polyfunctional solutes are being partitioned, allowance must be made for intramolecular binding as well as intermolecular binding. For example, by going from 3-methyl-3-hydroxy-2-butanone to 3-methyl-4-hydroxy-2-butanone, the molecular distribution changed from 19% monomer, 30% intramolecular bound, and 51% dimer to 12% monomer, 8% intramolecular, and 80% dimer.

Although the arguments are by no means clearly settled (13, 14), it appears (15–17) that nonsterically hindered alcohols tend to form a fairly cleancut monomer–tetramer equilibrium in solution, with the tetramer being partly in a linear form (V) and partly in a cyclic form (VI). At 30°, for *n*-octanol in *n*-decane, the monomer–tetramer association constants are 163 M^{-3} for linear and 324 M^{-3} for cyclic to give an overall value of 490 M^{-3}. With increasing temperature, the extent of polymerization decreases and the proportion of cyclic to linear forms also decreases; at 75°, the equilibrium constants are 5.46 M^{-3} for linear and 4.89 M^{-3} for cyclic to give an overall value of 10.35 M^{-3}.

A comparison of overall association constants [490 for *n*-octanol in *n*-decane at 30°, 430 for *n*-butanol in *n*-decane at 30°, and 312 for *tert*-butanol in cyclohexane at 27° (15, 18)] shows that there is not a great difference in degree of association for nonsterically hindered alcohols in the same solvent; the significantly lower value for *tert*-butanol in cyclohexane is probably due more to some degree of steric hindrance than to the difference in solvent. Sterically hindered alcohols, such as di-*tert*-butylcarbinol (19) and 2,5-substituted phenols (20), cannot form tetramers and in this case dimers are formed in equilibrium with monomers. Partially sterically hindered alcohols will participate in a more complicated set of polymeric equilibria. For *n*-octanol in octane at 25° (14), it has been estimated that the maximum proportion of dimer is just over 5% at a concentration of 0.13 M and that this amount decreases to less than 2% at 1 M and to less than 1% at 5 M; otherwise, the only associated species is thought to be the tetramer.

To date, the structural studies of associated polar molecules in nonpolar solvents have not considered the effect of the water that



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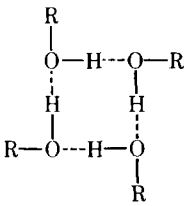


VI

is drawn into the nonpolar solvent by a polar solute when it is partitioned between water and the solvent.

The water concentration and all equilibria between solute hydrates, solute polymers, hydrated solute polymers, and intramolecularly bound molecules are dependent on solute concentration so that the determination of true partition coefficients corrected for these effects becomes a very formidable task. For many solutes, these effects could be minimized or eliminated by never permitting the solute concentration to exceed 10^{-3} M in the nonpolar solvent phase, but this may, in turn, introduce analytical difficulties, especially in the aqueous phase. In any case, when the biological activity for a series of compounds is compared with log P values determined in, say, an alkane–water system, the correlations are often not nearly as good as those obtained using log P values determined in the more polar *n*-octanol–water system (7, 21). The simplest binary liquids (water-saturated alkanes, cycloalkanes, carbon tetrachloride, *etc.*) thus have properties that lead to great difficulties when determining true partition coefficients and getting the best correlations with biological activity.

In many ways the properties of benzene are comparable to cyclohexane. It is a nonassociated, nonpolar liquid that has a great affinity for lipophilic hydrocarbon-like substances. However, it does differ in one important respect; its π-electron system can act as an electron pair donor for hydrogen bond formation. As a consequence, water is 15 times more soluble in benzene than in cyclohexane. The net result is that the binary phase, water-saturated benzene, is definitely more polar than cyclohexane, yet its dielectric constant is barely larger than that of pure benzene and its density and viscosity are the same as pure benzene. A variety of measurements, cryoscopic (22), isopiestic (3, 23–25), and NMR (26), leads to the conclusion that up to saturation the water dissolved in benzene is not self-associated in any polymeric form. It is believed (11, 27) to exist primarily in a hydrogen bonded solvated form such as IV, where B represents benzene, the base donor of an electron pair.

All structural and partitioning problems associated with cyclohexane also hold for benzene: the great increase in water solubility caused by partitioned polar solutes, the multiequilibria between hydrated species and multimeric species, and the effects of steric hindrance. In addition, there are the complications introduced by solutes such as alcohols competing with water for hydrogen bonding with the benzene. A thermodynamic study (28) of this competition indicated that, for the simple *n*-alkanols up through *n*-pentanol, there is a dramatic drop in the enthalpy of solution of water in alcohol–benzene solutions if the alcohol concentration exceeds a mole fraction of 0.025; larger *n*-alkanols do not show this effect. Clearly, the nature of the binary phase, water-saturated benzene, varies with the nature and concentration of partitioned alcohols and is bound to be reflected in values of partition coefficients. It has been suggested (29) that the larger alcohol molecules adopt a coiled configuration in benzene that results in a partial screening of the —OH interaction with benzene. Since more recent studies (16, 18) indicate the importance of cyclic tetramers in alcohol solutions, it may be that the proposed "shielding" comes from the long alkyl chains of these cyclic tetramers rather than from the coiling of monomers.

There can frequently be an analytical problem with the benzene–water system over and above that caused by the extreme solubility of lipophilic solutes in benzene and the insolubility in water (and the reverse for hydrophilic solutes). The most common analytical tool for determination of P is UV spectrophotometry; the very strong UV absorption by benzene renders this method entirely inapplicable, even in the benzene-saturated water phase, at wavelengths shorter than 270 nm.

As with cyclohexane, the conclusion must be reached that the benzene–water system leaves much to be desired as a reference partitioning system.

Chloroform is a slightly polar, nonassociated liquid which has a great affinity for lipophilic materials. Its lone proton is an effective electron pair acceptor in hydrogen bond formation; as a result, the

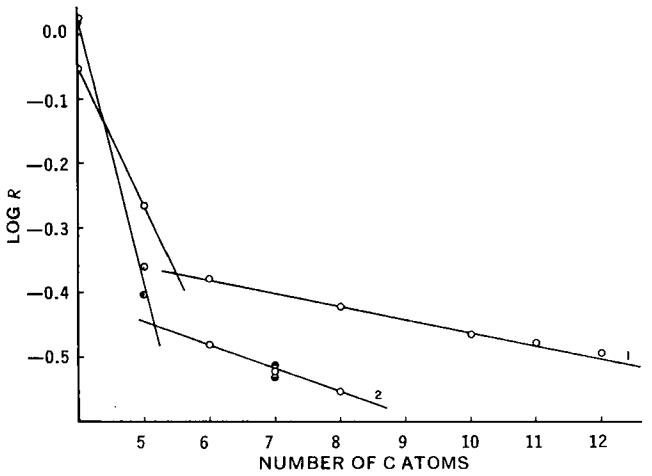


Figure 1—*Log* R versus *number of carbon atoms in* n-*alkanol chain. In curve 1,* R = *(H₂O area)/(CH₂ area), the ratio of areas under NMR peaks corresponding to dissolved water (H₂O area) and the two protons in the alcohol to which the OH is attached (CH₂ area) for alcohols saturated with water at 25°. In curve 2,* R = *moles of water/moles of alcohol in* n-*alkanols saturated with water at 25°. Key:* ○, *from Ref. 32;* ◐, *from Ref. 59;* ●, *from Refs. 48 and 49;* ◒, *from Ref. 61; and* ◓, *from Ref. 60.*

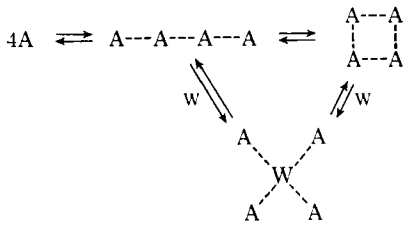
solubility of water is almost two times greater than it is in benzene. This greater solubility leads to a small but significant *decrease* in the dielectric constant of chloroform, yet it is still too small to have a significant effect on the density or viscosity of chloroform. Nevertheless, the amount of dissolved water is sufficiently large to introduce a structural complexity not observed in benzene or cyclohexane.

NMR studies (25, 29) revealed that a small amount of water dimer is hydrogen bonded to chloroform, probably as a mixture of I and II, as in VII and VIII. The majority is monomerically hydrogen bonded as in IX where A represents chloroform, the electron pair acceptor. The equilibrium dimerization constant for water in chloroform is 0.437 M^{-1} at 25° (corresponding to about 5.2% dimer at saturation), a value somewhat smaller than for other partially chlorinated hydrocarbons in which the range is usually about 8–15% dimerization (9). Since ϵ is smaller for water-saturated chloroform than for pure chloroform, the water–chloroform complexes must have a smaller μ than pure chloroform and the complexes must be sufficiently stable to behave as a polarizable species; for dimer dissociation, ΔH° = 1.8 kcal/mole.

The analytical application of UV spectrophotometry is not affected by chloroform, but all other partition problems associated with benzene apparently are also associated with chloroform, although perhaps in less extreme form. There also is the added complication of water association.

Alcohols—The problems associated with partitioning of alcohols *as solutes* between nonpolar or slightly polar solvents and water have already been discussed. Because of the highly polar nature of the hydroxy group and its great capacity for hydrogen bonding, the structure of the pure liquids and their solutions have been the object of extensive investigation. The pure alcohols are associated liquids with multiple equilibria existing between monomers and linear and cyclic multimers. For nonsterically hindered alcohols, the present IR spectral evidence heavily favors the predominance of linear and cyclic tetramers (V and VI) in equilibrium with monomers, with very little evidence for other polymeric forms (14, 16, 17).

Earlier temperature-dependent dielectric dispersion data were interpreted (30, 31) in terms of a shift in equilibria to smaller linear polymers and fewer with increasing temperature, with something of the order of four to six monomeric units per polymer at a maximum at room temperature (*n*-hexanol to *n*-decanol). However, it appears than an equally good explanation could be made in terms of decreasing the relative proportions of cyclic to linear tetramers and decreasing the proportion of tetramers to monomers with increasing temperature. Heavily substituted alcohols (especially on the α-carbon) have severe steric hindrance, which tends to reduce the concentration of monomers and to encourage the formation of cyclic polymers (32). In extreme cases, such as di-*tert*-butylcarbinol, the dimer is the largest polymer formed (17, 19).



Scheme I

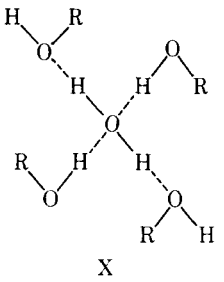
When water is added to alkanols, the simpler members of the family undergo much more profound intermolecular interactions, especially if water-saturated solutions are considered as is the case in partition measurements. In a sense, the water-saturated alkanols fall into two major categories, C_4–C_5 and C_6–C_{12}, as illustrated by water solubility in Fig. 1. C_3-Alkanols and below are miscible with water in all proportions, and C_{13}-alkanols and above are solids at room temperature. Curve 1 in Fig. 1 was determined from NMR measurements on water-saturated *n*-alkanols by taking the ratio of the area under the water peak to the area under the CH_2 peak of the carbon to which the OH is attached. Once the molar ratio of water to alcohol (W/A) exceeds ~0.40, the structure and properties of the binary phase are so greatly altered as to permit the association of a great deal more water, probably in a three-dimensional hydrogen bonded network. When the water-to-alcohol ratio in saturated solution is below ~0.40, the structure and properties of the saturated solution undergo a rather predictably slow change with increasing chain length of the alcohol.

Dielectric constant and viscosity measurements (33–35) offer some insight into the significance of this ratio. At room temperature, when water is added to the *n*-alkanols (C_1–C_5), 3-ethylpentan-3-ol, ethylene glycol, and glycerol (36), there is a steady increase in ϵ and η with increasing water concentration; but for the *n*-alkanols (C_6–C_{10}) and cyclohexanol, there is an initial decrease in ϵ and η with increasing water concentration. In the cases of 1-heptanol (at ~45°) and cyclohexanol (at ~25°), the values of ϵ go through a minimum at W/A = 0.25; in the case of 1-octanol, an apparent minimum is just reached at room temperature at W/A = 0.28 when saturation is reached. NMR data for 1-heptanol indicate (33) a relatively stable molecular aggregate at W/A = 0.25, and this finding is supported by less detailed studies with *n*-octanol (34). Added to the foregoing is the IR information about nonsterically hindered alkanols that indicates an equilibrium between monomers and linear and cyclic tetramers, an equilibrium that shifts in favor of monomers and linear tetramers with increasing temperature. The more sterically hindered an alcohol is near the α-carbon, the less able it is to form the tetramers, and cyclic dimers tend to be the preferred aggregate. The very simplest alcohols tend to form long linear multimers (37).

An oversimplified set of equilibria, which can be modified in detail for individual alcohols and which offers a general explanation for the properties of the higher alcohols and their water solutions, is shown in Scheme I, where A represents an alcohol monomer, W represents water, and the linear and cyclic tetramers are Structures V and VI. These equilibria are not meant to imply any particular kinetic mechanism of interconversion among the different forms. The water-centered complex, A_4W, is believed to have tetrahedral orientation of hydrogen bonded alcohols about the oxygen atom of water, as in X.

Qualitatively, A and cyclic A_4 have very low values of ϵ, linear A_4 has a high value of ϵ, and A_4W has a low value of ϵ that is greater than cyclic A_4. In the absence of W, only the horizontal equilibria exist; with increasing temperature, these equilibria shift to the left, causing a decrease in the amounts of both linear and cyclic A_4 and an increase in A. As a result, ϵ decreases because of the relative loss of linear A_4. If the alcohol is seriously hindered sterically, the horizontal equilibria lie far to the right and the linear and cyclic multimers are probably not tetramers but more likely dimers or trimers. In this case, an increase in temperature also shifts the equilibria to the left but ϵ increases because of the relative increase in the amount of linear multimer at the expense of cyclic multimer.

When water is added to these alcohols at room temperature,



X

there is a very strong tendency to form A_4W and all equilibria shift *in that direction; the net result is a decrease in* ϵ *for the solution* due to the loss of high ϵ linear A_4. If it is possible to add more water than corresponds to stoichiometric A_4W (*e.g.*, cyclohexanol and *n*-heptanol and smaller), ϵ will rise again due to the formation of larger aggregates of cross-linked, water-bridged A_4W units. In the case of the sterically hindered alcohols where there is a relatively small amount of linear multimer in the first place, the increase in ϵ is due to the formation of A_4W at the expense of low ϵA. The effect of adding water at each temperature must be assessed in terms of the relative amounts of A, linear A_4, and cyclic A_4 (or their counterparts in sterically hindered alcohols) that exist in the horizontal equilibria.

Although the increase in NMR line broadening for both OH and water on addition of water does indicate increased proton exchange, this exchange rate is relatively small even at the maximum corresponding to A_4W. The presence of the merest trace of many impurities will so accelerate the exchange as to cause the merging of the two peaks. The implication of this observation for partitioning is that a large fraction of solutes partitioned will probably initiate very rapid exchange, perhaps due to minor changes in pH. It is unlikely that this exchange will influence the values of *P*.

Although water–alcohol solutions are obviously complex, it would appear that a water-saturated *n*-octanol binary phase may have some advantages for partitioning. With a value of W/A = 0.28, almost all of the alcohol and water molecules are involved in the same type of A_4W complex, with but a small excess of water probably involved in cross-linking some of these complexes. Because of the large quantity of dissolved water, it is unlikely that the water concentration or the structural characteristics of this binary phase will be significantly changed as it is in the case of the nonpolar solvents. This means that low but convenient analytical concentrations of partitioned solute may be used, regardless of the polar functional groups it may possess. The balance of polarity and nonpolarity is such as to minimize the need for concern about solute self-association while simultaneously retaining the predominantly lipophilic character required for a reference biolipid system.

The complicated association equilibria that exist for alcohol and hydroxylic solutes partitioned into nonpolar or slightly polar solvents are virtually eliminated in water-saturated *n*-octanol, with each such partitioned molecule undoubtedly replacing one octanol in an A_4W complex to maintain the equivalent of a monomeric dispersion of the partitioned solute. The A_4W complex also offers, in very high concentration (~6.2 *M*), the opportunity for a highly lipophilic solute simply to "dissolve" monomerically in the matrix of highly lipophilic carbon chains of neighboring A_4W complexes. The preceding statements are supported by the long experience in this laboratory with the use of *n*-octanol, which reveals that only with certain very hydrophobic ionic species will partition measurements need to be made over a range of solute concentrations to get a true value of *P* by extrapolation to infinite dilution. This one problem may actually have as its origin the nature of the aqueous phase *(vide infra)*.

n-Heptanol and *n*-nonanol might be considered as comparable alternatives to *n*-octanol for partitioning. Water-saturated *n*-heptanol has the advantage of being somewhat less viscous but the disadvantage of having a little too much water (W/A = ~0.30, with dielectric and viscosity properties corresponding to those past the optimum A_4W). Water-saturated *n*-nonanol has the advantage of having an even more ideal water concentration (W/A = ~0.26) but the disadvantage of being more viscous.

With a molar water-to-alcohol ratio of ~1.0, it is not surprising that the structure of water-saturated *n*-butanol is extremely complex. It has been interpreted (38, 39) in terms of a whole variety of hydrogen bonded species, none of them predominant. The really serious disadvantage to the use of water-saturated butanol in a reference partitioning system is the fact that it offers so little sensitivity for discriminating between the relative hydrophobicities of solutes; the water-saturated alcohol is too similar in solvent properties to the alcohol-saturated water phase in which W/A = ~52.

Oleyl alcohol, *cis*-9-octadecen-1-ol, has been used for partition work by some investigators in the belief that it closely resembles biological phases. With an exposed double bond at the *cis*-9,10-position, it has additional hydrogen bonding potential compared to the saturated alcohols, and this, no doubt, accounts for the high water-to-alcohol ratio of 0.23 in contrast to the value of 0.17 expected from Fig. 1. At the moment, there is no experimental basis for speculating about how much of the dissolved water is associated with the double bond and how much with the hydroxy group. Also, with the bulky carbon chains, it is difficult to assess the importance of the A_4W or other complexes. However it is associated, the dissolved water has negligible effects on the dielectric and transport properties. From an experimental standpoint, water-saturated oleyl alcohol is difficult to work with because of its high viscosity (about four times that of water-saturated 1-octanol) and its tendency to form stable emulsions.

Esters—As a class, esters are polar Lewis bases whose oxygen atoms have a high potential for hydrogen bonding. As a result, they tend to dissolve more water than do the slightly polar hydrogen bonding solvents like chloroform or benzene. IR studies (11) in nonpolar solvents indicate that when water is in excess, a 1:1 ester–water complex is formed (Structure III, with B representing the ester); but when the ester is in excess as it always is in water-saturated ester, a 2:1 ester–water complex is formed (Structure IV). The increase in ϵ with an increase in water concentration also indicates an increase in concentration of bulky polar aggregates. Being more polar than water-saturated benzene or chloroform, the binary ester phase will tend to discourage the formation of self-association complexes of the partitioned solute, but these same solutes may well carry additional water into the solvent phase. It seems likely that hydroxylic solutes will participate in different varieties of association complexes with water, ester, and alcohol, depending on the degree of steric hindrance, hydrophobicity, and concentration; therefore, the determination of true partition coefficients would be very difficult.

Water-saturated ethyl acetate has been the most widely used ester for biological partitioning and is typical of the simple esters. In addition to the uncertain and variable structural problems just mentioned, it seems to be undesirably lacking in hydrophobic character for use as a reference system for a biological lipid phase. (When using values of *P* for the octanol–water system, log *P* for ethyl acetate = 0.71 compared to 3.15 for *n*-octanol.) From an analytical standpoint, it suffers from the fact that it slowly hydrolyzes, a process that may likely be catalyzed by some partitioned solutes. For example, physical measurements such as ϵ must be made within an hour to be significant (40). Therefore, immediate attention must be given to the analysis of the partitioned phases and it is not possible to keep a stock solution of water-saturated ethyl acetate for convenient use over a period of time as is customarily done in partitioning work for solvents that dissolve appreciable quantities of water. The hydrolysis products may achieve a concentration as great or greater than the solute being partitioned, the pH will be lowered by the acetic acid that is formed, and many basic solutes will form a salt which will drastically alter the apparent partition coefficient.

Being derived directly from a natural source, olive oil is in principle the best qualified of the systems considered in this paper for use as a reference biolipid phase. It is a triglyceryl ester. Other than its natural source, however, water-saturated olive oil has nothing but discouraging attributes. It does not dissolve much water. The amount that it does dissolve at 25°, 0.07 mole of water/mole of olive oil, must be considered as a third of that, on the average, per ester group. And when it is realized that a significant fraction of the long chain fatty acids esterified with the glycerol are unsaturated, then the fraction of ester groups and double bonds that are hydrogen bonded to water molecules (probably as Structure III) must be between 0.01 and 0.02. The low dielectric constant and water solubility indicate that structural problems, problems of self-association of partitioned solute, and problems of

varying degrees of hydration of polar partitioned solutes by additional water brought in by the partitioning process associated with nonpolar or slightly polar solvents will be involved here.

From a practical standpoint, there are additional difficulties. Olive oil is very difficult to work with because of its very high viscosity (about nine times that of *n*-octanol) and its discouraging tendency to form stable emulsions. Other problems also should be considered from the standpoint of its use as a reference system. Its composition varies appreciably with the source of the olive oil, as do certain of the natural contaminants which are extremely difficult to remove. It has poor storage stability, and the resulting rancidity involves changes in color, acidity, and odor, the formation of oxidation products and peroxides, and the process of slow polymerization (41–43). Other vegetable oils that have been considered for use in a reference partition system for biological lipids suffer in varying degrees from the same difficulties.

Solvent-Saturated Water—Tables I and II show the principal physical properties of the solvent-saturated water phases considered in this paper. With the exception of *n*-butanol and ethyl acetate, the solvents have very little impact. Their low water solubility causes only a small decrease in ϵ and a negligible effect on density and viscosity (diffusion and transport phenomena). Although the ratio of moles of water to moles of solvent is over 50:1 for *n*-butanol and ethyl acetate, this still corresponds to a concentration in excess of 1.1 *M*, and the physical and structural characteristics of the hydrogen bonded water molecule network are markedly affected. The unusually large increase in both η and ΔH_{vis} probably results from the water structure-forming influence of the nonpolar hydrocarbon portion of the molecules present in such a high concentration (44).

As mentioned earlier, the difference in solvent characteristics of the two phases in the *n*-butanol–water system is too small to make this system suitably sensitive as a reference system for discriminating between compounds over a wide range in hydrophobicity. Ethyl acetate has the problem of hydrolysis. Both have problems of structural interpretation in the solvent phase. The water solubility of oleyl alcohol and olive oil is so low that the effect on partitioning in the water phase can be ignored, but the usefulness of these two is limited for reasons given earlier.

The other four solvents shown in the tables have solubilities in the range of from 6×10^{-4} to 6×10^{-2} *M*. Although these concentrations have an almost negligible effect on the structure and properties as such, they may have a significant effect on partitioning. For example, hydrophobic interaction between solute and solvent in the water phase probably will result in a higher solute solubility in solvent-saturated water than in pure water. As a consequence of this enhanced water solubility, the observed values of log *P* may be lower than expected, especially for those nonpolar solutes whose water solubility is comparable to or less than the water solubility of the solvent.

Measurements carried out in this laboratory show that the increased solubility in octanol-saturated water is approximately 4.7% for naphthalene, 5.2% for phenanthrene, and 67% for anthracene. Since it has also been shown that the octanol–water partition coefficients for phenanthrene and anthracene are the same (~4.45), as expected, it is clear that solute–solvent interaction in the water phase does not necessarily lead to poor values of *P*. In the phenanthrene and anthracene cases, the great differences in effect of octanol on solubility may only reflect unusual differences existing in their *solid* states that affect the solid–dissolved solid equilibrium; even in pure water, the solubility of phenanthrene is about 20 times greater than anthracene.

Partition coefficient determinations do not (or should not) involve phases saturated with the partitioned solute or in equilibrium with its solid form. The practice of calculating values of *P* by taking the ratio of solute solubility in the separate pure phases for solutes that are fairly soluble in both (1) is a poor one from the standpoint of drug design because it ignores *(a)* the importance of activity coefficients which must be considered at high solute concentration, and *(b)* the effect that each solvent has on the solvent power of the other.

Another example of the possible importance to partitioning of the low concentration of solvent dissolved in water is the following. With increasing chain length in a homologous series of partitioned compounds, the point may be reached where the water phase solubility is so low that it is comparable to or lower than the concentration of the dissolved solvent. With still further increases in chain length, the solute solubility in the water phase may not drop off as fast as might have been expected because its solubility may be primarily determined by hydrophobic interaction with the dissolved solvent molecules. The result will be seen as a "break" in the log *P*–chain length relationship.

Such breaks have been observed with certain series of compounds, but their interpretation has not been satisfactorily resolved. Should such a break be interpreted as *(a)* hydrophobic interaction of solute with dissolved solvent as just described, *(b)* "folding" of the solute molecules with increasing hydrophobicity so as to present a smaller hydrophobic interface with water, *(c)* hydrophobic self-association equilibria between solute molecules, or *(d)* some combination of these three? Additional work is needed to clarify this point. It would seem that one way to minimize this problem in determining a true value of *P* would be to perform all partition experiments at solute concentrations smaller than the concentration of the dissolved solvent. All of the above statements presuppose the use of solute concentrations below the critical micelle concentration for those compounds that tend to form micelles.

CONCLUSIONS

Of the types of solvents considered, the best choices for a reference biolipid phase are water-saturated *n*-heptanol, *n*-octanol, and *n*-nonanol. Considering the number of compounds for which *P* values have already been determined in *n*-octanol (~3000 in the data bank of this laboratory compared to a very small number for the other two), water-saturated *n*-octanol is much the better choice. Its advantages seem to stem from having most of the *n*-octanol and water tied up in a tetrahedral hydrogen bonded complex that retains a high degree of hydrophobicity because of the four 8-carbon nonpolar chains surrounding the polar center. It discourages self-association of partitioned polar solutes by having a reasonable degree of polarity and by providing an opportunity for a solute molecule to exchange with an alcohol molecule in the tetrahedral complex. Because it contains a relatively high concentration of water, its structure, polarity, and partitioning properties will not be altered by additional water introduced to the phase by polar solutes as is the case for nonpolar and slightly polar solvents. No analytical or storage problems are associated with water-saturated *n*-octanol.

To minimize problems due to possible hydrophobic interaction of partitioned solutes with dissolved solvent in the solvent-saturated water phase, solute concentrations should be kept less than solvent concentrations in the determination of *P* values. In octanol-saturated water, solute concentrations of 10^{-3} *M* or less are normally recommended.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 12, 1974, from the *Seaver Chemistry Laboratory, Pomona College, Claremont, CA 91711*

Accepted for publication October 30, 1974.

The work upon which this publication is based was performed pursuant to Contracts NIH-69-2112 and NIH-NCI-C-73-3703 with the National Cancer Institute, Department of Health, Education, and Welfare.

The authors are indebted to Professor C. Freeman Allen for his determination of the molecular composition of the olive oil sample and to Dr. Albert Leo for valuable discussions.

[x] To whom inquiries should be directed.