Effect of Structural Similarity on Molecular Interaction in Aqueous Solution: Interaction of Phenazine and Tetramethylpyrimidopteridinetetrone with Alkylxanthines and Benzene Derivatives

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Abstract □ The molecular interactions in water of phenazine and tetramethylpyrimidopteridinetetrone (TMPPT) with alkylxanthines and benzene derivatives were studied by means of the phase solubility technique. Alkylxanthines showed a greater affinity toward phenazine than toward TMPPT. Benzene derivatives, on the other hand, formed more stable complexes with TMPPT than with phenazine. These results are discussed in terms of structural similarity between interacting species. Studies concerning the effects of solvent on the extent of complex formation have revealed that water plays a unique role in these interactions. The observation that complexation between two structurally dissimilar compounds is favored over that between two similar compounds suggested that some forces other than mere hydrophobic bonding should be taken into consideration. It is postulated that the results are best rationalized by hydrophobic bonding stabilized by a type of bonding similar to polarization bonding.

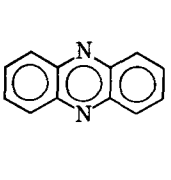
Keyphrases □ Molecular interaction, aqueous solutions—structural similarity effect □ Phenazine–alkylxanthines, benzene derivatives—interaction □ Tetramethylpyrimidopteridinetetrone–alkylxanthines, benzene derivatives—interaction □ Phase solubility—molecular interactions □ UV spectrophotometry—analysis

While molecular interaction in nonpolar solvents can best be understood on the basis of hydrogen bonding or charge transfer complexation, a mechanism of molecular interaction in aqueous solution still remains largely unsolved. Earlier workers initially believed that hydrogen bonding was responsible for complexation in aqueous solution. Considerable evidence has since been accumulated to dispute this interpretation (1). The application of the charge transfer complexation theory in nonpolar sovents (2) to aqueous solutions is not in accord with present knowledge of interactions in aqueous solution. Such an interaction has been shown to be quite weak or nonexistant in an aqueous environment (3), since a typical charge transfer band is not usually observed in water (3). It is thought that thermal electron transfer leading to the formation of radicals or reactions may be responsible for the appearance of such a band in some cases.

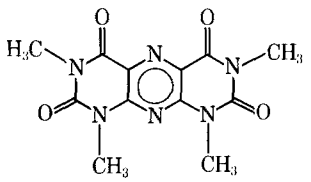
The more or less nonpolar molecules in water tend to come together, reducing the number of water-solute contacts. This phenomenon is known as hydrophobic bonding. Solvophobic bonding refers to a similar phenomenon in any solvent. The source of stability of a complex in aqueous solution has been attributed either to a favorable enthalpy term [large surface energy of water (4, 5)] or to a favorable entropy term [increased ordering of water around the hydrophobic group in a solute molecule (6, 7)]. The "squeezing-out" effect proposed by Higuchi and Lach as early as 1954 (8) is essentially the same as the theory by Sinanoğlu and Abdulnur (4, 5). Studies on the effect of solvents on the extent of complexation (9–11) generally support the hydrophobic bonding theory. Complexes stabilized in aqueous solution tend to dissociate in less polar and nonpolar solvents, except when solute molecules associate by hydrogen bonding or charge transfer interaction (12).

From a considerable amount of work concerning complex formation in aqueous solution, Higuchi (13) concluded that hydrophobic bonding alone cannot be responsible for interaction in aqueous solution. He suggested that interacting molecules may be divided into groups or classes such that compounds which belong to the same class do not interact very strongly with each other while those belonging to different classes bind strongly.

In pursuit of the elucidation of the mechanism of molecular interaction in water the present study was undertaken by selecting two model compounds, phenazine and tetramethylpyrimidopteridinetetrone (TMPPT) so that the extent of interaction can be monitored by the increase in their solubility in the presence of



phenazine



TMPPT

complexing agents. The choice of phenazine and TMPPT is based on simplicity in their spectrophotometric assay and on the polarizable nature of the former in contrast to the polar nature of the latter. The present results demonstrate that there is some discrimination in molecular interaction even in aqueous media, the extent of interaction being greater between polar and polarizable compounds than that between two polar compounds or two polarizable molecules. The results are further interpreted in terms of additional stabilization of complexes by polarization bonding (14) in the presence of hydrophobic bonding contribution due to water as solvent.

EXPERIMENTAL

Materials—Phenazine (Aldrich) was recrystallized from methanol–water, m.p. 171°. 1,3,7,9-Tetramethylpyrimido(5,4-*g*)pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetrone (abbreviated as TMPPT; Alfred Bader Chemicals, Aldrich) was used without further purification since its NMR spectrum indicated no impurity; m.p. >340°. With the exception of *N*-substituted amides (15), complexing agents were obtained from commercial sources and purified whenever necessary. Water was purified by distillation. Organic solvents employed were of spectroscopic grade.

Methods—Phenazine (or TMPPT) in excess of the solubility was added to vials containing aqueous solutions (5 ml.) of varying concentrations of the complexing agent. Complexing agent concentrations were obtained by dilution of a stock solution. The vials were

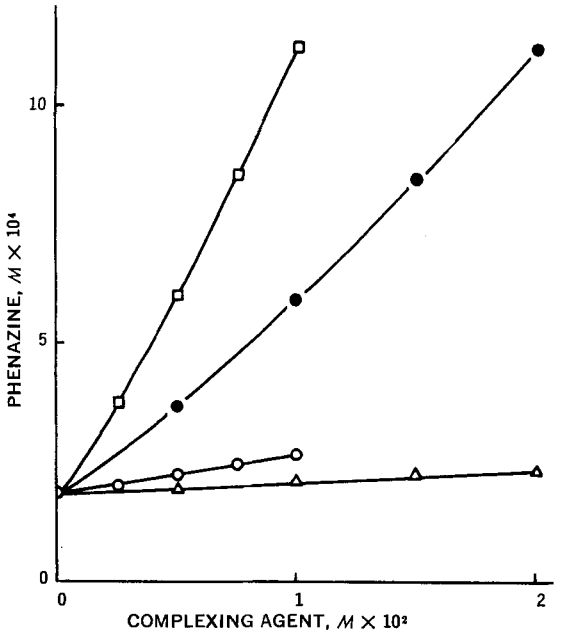


Figure 1—*Solubility diagrams of phenazine in the presence of 8-methoxycaffeine* (□), *theophylline* (●), N,N-*dimethylcinnamamide* (○), *and sodium salicylate* (△) *in water at 25°.*

closed and shaken in a constant-temperature water bath at 25° for about 40 hr. The equilibrated contents of the vials were quickly filtered through sintered-glass funnels (medium porosity) under reduced pressure. Samples (2 ml.) were then diluted with water and the total concentration of phenazine (or TMPPT) solubilized was measured spectrophotometrically at 366 mμ (or at 360 mμ for TMPPT) employing a spectrophotometer (Shimadzu QV-50). The observed solubility of phenazine (or TMPPT) was plotted against the concentration of complexing agent added. Stability constants were calculated from the solubility diagrams according to the following manner.

1:1 Complex—Where a straight line was obtained in the plot, the formation of a 1:1 complex was assumed and the stability constant was calculated from Eq. 1 (16);

$$K_{1:1} = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})} \qquad \text{(Eq. 1)}$$

1:1 and 1:2 Complexes—Where an upward curve was obtained in the plot, the formation of both 1:1 and 1:2 complexes given by

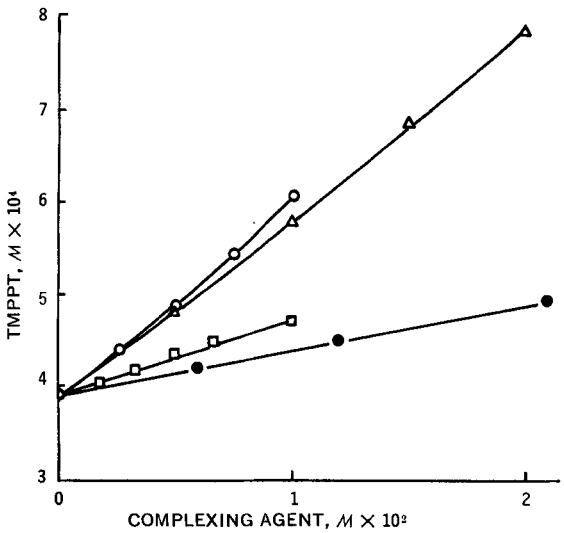


Figure 2—*Solubility diagrams of TMPPT in the presence of* N,N-*dimethylcinnamamide* (○), *sodium salicylate* (△), *8-methoxycaffeine* (□), *and theophylline* (●) *in water at 25°.*

Eqs. 2 and 3 was assumed and the stability constants defined by Eqs. 4 and 5 were computed.

$$A + B \rightleftharpoons AB \qquad \text{(Eq. 2)}$$

$$AB + B \rightleftharpoons AB_2 \qquad \text{(Eq. 3)}$$

$$K_{1:1} = \frac{C_{AB}}{C_A^\circ(C_B - C_{AB} - 2C_{AB_2})} \qquad \text{(Eq. 4)}$$

$$K_{1:2} = \frac{C_{AB_2}}{C_{AB}(C_B - C_{AB} - 2C_{AB_2})} \qquad \text{(Eq. 5)}$$

where C_A°, C_B, C_{AB}, and C_{AB_2} are the solubility of A in the absence of B, the total concentration of B, the equilibrium concentration of AB, and that of AB_2, respectively. From the material balance, the apparent solubility of A, C_A, is given by

$$C_A = C_A^\circ + C_{AB} + C_{AB_2} \qquad \text{(Eq. 6)}$$

From Eqs. 4–6 the following equation was obtained (17):

$$\frac{C_A - C_A^\circ}{C_A^\circ(C_B - C_{AB} - 2C_{AB_2})} = K_{1:1} + K_{1:1}K_{1:2}(C_B - C_{AB} - 2C_{AB_2}) \qquad \text{(Eq. 7)}$$

Since C_{AB} and C_{AB_2} were not known experimentally, they had to be calculated by an iteration (17). The initial values for $K_{1:1}$ and $K_{1:1}K_{1:2}$ were obtained from Eq. 7 by ignoring the C_{AB} and C_{AB_2} terms. The first approximate values for C_{AB} and C_{AB_2} were obtained from Eqs. 4 and 5, *i.e.*,

$$C_{AB} = K_{1:1}C_A^\circ C_B \qquad \text{(Eq. 8)}$$

$$C_{AB_2} = K_{1:1}K_{1:2}C_A^\circ C_B^2 \qquad \text{(Eq. 9)}$$

The C_{AB} and C_{AB_2} values were then put into Eq. 7 to obtain better values for $K_{1:1}$ and $K_{1:1}K_{1:2}$. These steps were repeated until a convergent straight line was obtained.

RESULTS

The solubility diagrams of phenazine in the presence of theophylline, 8-methoxycaffeine, *N,N*-dimethylcinnamamide, and sodium salicylate are shown in Fig. 1. The solubility diagrams for the TMPPT systems are shown in Fig. 2.

Similar solubility measurements were carried out for both phenazine and TMPPT in the presence of 1,3-dimethyluracil, β-hydroxyethylphthalimide, cinnamamide, sodium benzoate, sodium cinnamate, benzoic acid, phenol, benzamide, anisamide, and *N*-methylbenzamide. The solubility diagram of phenazine in the presence of caffeine is shown in Fig. 3, while that of TMPPT is presented in Fig. 4.

A downward trend in the solubility diagram of the TMPPT–caffeine system is apparent in Fig. 4. Guttman (18) earlier found that the solubility diagram for the interaction of riboflavin with caffeine was similarly convex. He has explained this tendency on the basis of the dimerization of caffeine (19). When the dimerization of caffeine was taken into consideration for the present system, a straight line was obtained giving $K_{1:1}$ of 19 M^{-1}. In this calculation, equilibria $2C \overset{K_d}{\rightleftharpoons} C_2$ and $T + C \overset{K_{1:1}}{\rightleftharpoons} TC$ were taken into consideration, while the contribution from $2T \rightleftharpoons T_2$ was neglected because of low concentrations of T present, where C = caffeine and T = TMPPT. A value of K_d = 12.7 M^{-1} (19) was used in the calculation. The upward curve in Fig. 3, on the other hand, clearly demonstrates the presence of higher order complexes as well as a 1:1 complex. Stability constants computed from such solubility data are presented in Table I for the complexes formed in each system. A typical example of the iterative procedure used, when applicable, for calculating $K_{1:1}$ and $K_{1:2}$ values is illustrated in Fig. 5 for the interaction of phenazine with 8-methoxycaffeine.

The effects of solvent on molecular interaction were examined for the interaction of TMPPT with *N,N*-dimethylcinnamamide. The results shown in Table II clearly indicate the important part played by water as a solvent. Studies with less polar solvents than those employed, such as carbon tetrachloride and cyclohexane, could not be made because of insolubility of TMPPT in such solvents.

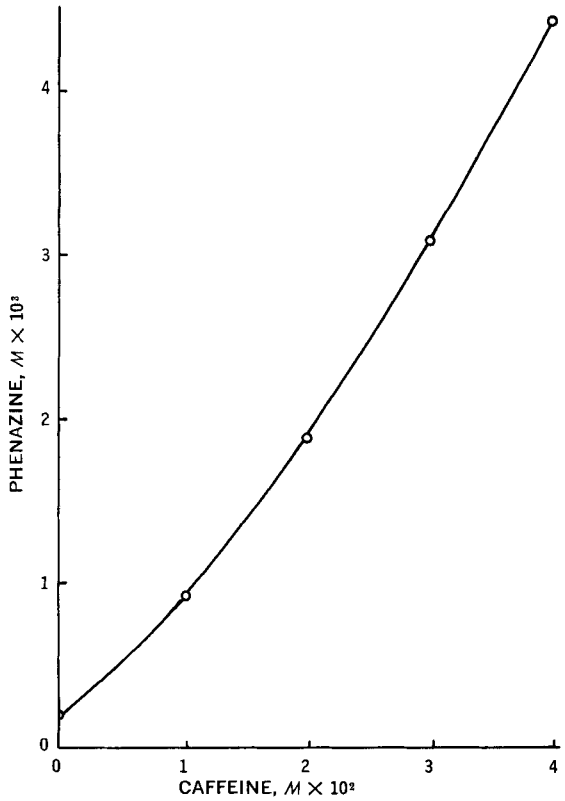
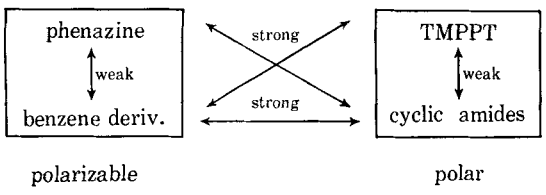


Figure 3—*Solubility diagram of phenazine in the presence of caffeine in water at 25°.*

DISCUSSION

Results shown in Figs. 1–4 and Table I clearly indicate that cyclic amides (alkylxanthines, 1,3-dimethyluracil, and β-hydroxyethylphthalimide) interacted much more strongly with phenazine than with TMPPT, while benzene derivatives (with the exception of benzamides) interacted more strongly with TMPPT than with phenazine. Although benzamides appeared to complex with both phenazine and TMPPT to the same extent, the complexing behavior of other agents toward these model compounds unequivocally supports the view that compounds belonging to the same class (benzene derivatives and phenazine on the one hand and cyclic amide and TMPPT on the other) do not interact very strongly, while those belonging to different classes (cyclic amides and phenazine on the one hand and benzene derivatives and TMPPT on the other) interact strongly in aqueous solutions (13). Phenazine and benzene derivatives may be classified as polarizable since the charge distribution in phenazine and in the benzene ring of the benzene derivatives are expected to be more or less uniform, although the benzene derivatives have net dipole moment. Cyclic amides including TMPPT, on the other hand, are classified as polar because the charge distribution in 1,3-dimethyluracil, the six membered ring of the alkylxanthines, and the maleimide group of β-hydroxyethylphthalimide, are considered to be very much irregular (20). The observed complexing behavior between these compounds may be summarized as in Scheme I.



Scheme I

Though the interaction of TMPPT with phenazine cannot be determined experimentally because of the low solubility of these compounds, the interaction of cyclic amides with benzene derivatives has been reported to be appreciable (16). It should be pointed out here that distinction between polar and polarizable molecules may become obscure when a compound carries both polar and polarizable groups.

Table I—Stability Constants of Phenazine and TMPPT Complexes in Water at 25°

Complexing Agent	—Phenazine— $K_{1:1}$ M^{-1}	$K_{1:2}$ M^{-1}	—TMPPT— $K_{1:1}$ M^{-1}	$K_{1:2}$ M^{-1}
Theophylline	166	24	13	—
Caffeine	314	20	19	—
8-Methoxycaffeine	345	42	21	—
1,3-Dimethyluracil	23	—	2.3	—
β-Hydroxyethylphthalimide	42	27	10	—
Cinnamamide	28	—	69	—
N,N-Dimethylcinnamamide	37	—	44	33
Sodium salicylate	12	—	43	8
Sodium cinnamate	17	—	46	—
Sodium benzoate	4.7	—	8.7	—
Benzoic acid	9.8	—	23	—
Phenol	5.5	—	18	—
Benzamide	11	—	13	—
Anisamide	34	—	31	—
N-Methylbenzamide	15	—	13	—

The present studies concerning the effects of solvent on complex formation (Table II) have quantitatively demonstrated the specific role played by water as a solvent. Since both TMPPT and *N,N*-dimethylcinnamamide have no hydrogen capable of forming hydrogen bonds, the possibility of direct hydrogen bonding between the interactants can be ruled out. The results do not appear to support charge transfer as the major force responsible for the interaction since there seems little tendency for stabilization of the complex in less polar solvents (21).

A valid mechanism for this associative behavior of organic molecules in aqueous media should, therefore, take into account both the selective nature of interaction and the important role of water as the environment which facilitates such interactions. The following hypothesis is proposed in order to give a rationale to the present knowledge of interactions. Water seems to have a role of bringing solute molecules together (hydrophobic bonding). When two solute molecules are brought together, a bonding similar to polarization bonding may become operative and stabilizes the complex. The detailed reasoning on which the hypothesis is based follows.

If one of the direct interactive mechanisms such as hydrogen bonding, charge transfer complexation, or interactions due to orientation forces and induction forces were the primary forces which bind two solutes together, even larger stability constants in less polar solvents than in water would be expected. The authors' results (9) as well as others' (10, 11), however, showed that the extent of interaction is greatest in water, decreasing with increasing

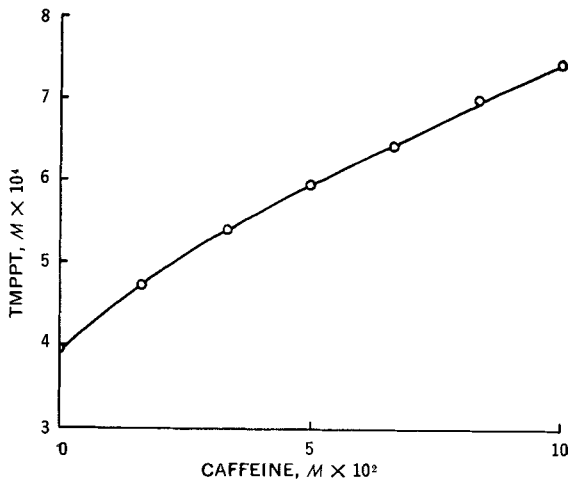


Figure 4—*Solubility diagram of TMPPT in the presence of caffeine in water at 25°.*

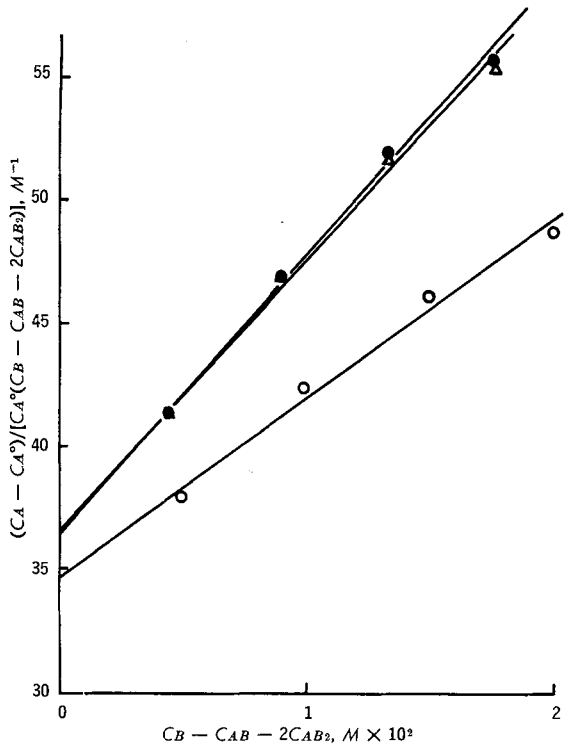


Figure 5—*Analysis of the solubility data for the interaction of phenazine* (A) *with 8-methoxycaffeine* (B) *in water at 25°. Key:* O, *first iteration;* Δ, *second iteration; and* ●, *third and further iterations.*

percentage of polar organic solvents in water–organic solvent mixtures, and it is very small in pure organic solvents. The extent of interactions in various solvents was correlated with the surface free energy of the solvent (10, 22). Sinanoğlu and Abdulnur attributed the main interactive force to the large enthalpy of water (4, 5). Observed results (Table I), however, cannot be rationalized merely by solvophobic bonding. If the bonding were merely due to the squeezing-out property of a solvent, it would not be expected to discriminate among polar and nonpolar molecules to such an extent as was observed since the solvophobic bonding is not affected very much by the nature (polar or nonpolar) of the molecule (5).

The present data cannot be explained by dispersion force alone. The dispersion interaction energy, E, is given by (23)

$$E = -\frac{3}{2r^6} \cdot \frac{I_1 I_2}{I_1 + I_2} \alpha_1 \alpha_2 \qquad \text{(Eq. 10)}$$

where α_i = polarizability of Molecule i, I_i = ionization potential of Molecule i, and r = intermolecular separation. Since the ionization potential varies but little for organic molecules (23), the dispersion energy is mainly affected by polarizability. Then a complexing agent with a large polarizability would bind with both phenazine and TMPPT strongly. This is not the case. Thus forces such as the dispersion force do not seem to be the sole source of stability of the complex in aqueous solution. It is important to note here that the present finding of favorable binding between structurally dissimilar molecules over that between structurally similar molecules is contrary to what is expected on the basis of dispersion interaction (24). The theory provides for a self-recognition of a molecule by an identical molecule. If the dispersion force is responsible for the stability of the complex, the difference in energy, E, between the interaction of Molecule A with Molecule B, A-B, and self-interactions, A-A and B-B, as depicted by Arrangements 1 and 2:

A-A	*A-B*
B-B	*A-B*
Arrangement 1	Arrangement 2

(E in Arrangement 1) − (E in Arrangement 2)

is proportional to

$$-(\alpha_A^2 + \alpha_B^2) - (-2\alpha_A\alpha_B)$$
$$= -(\alpha_A - \alpha_B)^2 \leq 0$$

Then it would be concluded that Arrangement 1 is more energetically stable than Arrangement 2 (24). Again this has been found not to be the case.

Thus a bonding with some degree of selectivity between dissimilar molecules must be sought. Polarization bonding (14) describes weak interactions between polar groups of one component and a polarizable second component in crystalline state. This kind of bonding in a crystalline xanthine complex has recently been postulated by Shefter (25). He also pointed out that in order to propose molecular models for xanthine complexes of pharmaceutical interest, one should take into account polarization interactions. The exact nature of polarization bonding is not known, although it has been proposed that polycyclic hydrocarbon–tetramethyluric acid complexes in the crystalline state (26–28) are due to this bonding. The interactive force seems to be very weak and effective only when the polar and polarizable molecules are in close proximity as in the crystalline lattice. The bonding was not observable in nonpolar solvents such as cyclohexane and benzene (29). It may be speculated, however, that molecular interactions similar to polarization bonding in crystalline complexes may become operative in water when two molecules are brought together by hydrophobic bonding. Polarization bonding, however, has to be distinguished from the classical induction interaction described by (23)

$$E = -\frac{\mu_1^2\alpha_2 + \mu_2^2\alpha_1}{\epsilon^2 r^6} \qquad \text{(Eq. 11)}$$

where μ_i = dipole moment of Molecule i and ϵ = effective value for the dielectric constant of the medium. The formula describes the energy of interaction when the size of a molecule is smaller than the intermolecular distance. Thus the formula is not applicable to the case when the size of the molecule is larger than the intermolecular distance as is the case of complexes of planar molecules in aqueous solution. The most favorable relative orientation of two flat molecules seems to be that of stacking one of the molecules on top of the other (30, 31). A new theoretical development which describes an induction interaction applicable to such a vertically stacked complex of large flat molecules seems to be essential in order to understand theoretically the observed results.

Current works in the authors' laboratories have revealed that cyclic amides employed in the present studies are salted-out by tetramethylammonium chloride while benzene derivatives are salted-in by the same salt (32). Again differences in nature of these molecules in aqueous solution seem to play a part.

Table II—Stability Constants for the TMPPT–*N*,*N*-Dimethylcinnamamide Complex in Several Solvents at 25°

Solvent	Stability Constant, $K_{1:1}$, M^{-1}
Water	44 ($K_{1:2}$ = 33 M^{-1})
Methanol	3.6
Acetone	2.5
Chloroform	2.1
Dioxane	4.6
Benzene	4.7

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Binding Specificity between Small Organic Solutes in Aqueous Solution: Classification of Some Solutes into Two Groups According to Binding Tendencies

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Abstract □ Experimental data have been obtained which appear to show that the binding between organic species dissolved in water apparently takes place most effectively *between* members of two large, distinct classes of structures, classified arbitrarily as A and B types. Typical examples of Class A are the uncharged alkylxanthines and tetramethylpyrimidopteridinetetrone. Among the compounds in Class B are various benzene derivatives, salicylates, and *trans*-cinnamic acid anions. Many drugs may be included in the present classification system; some examples for which data are available are caffeine, theophylline, and prednisolone in Class A, and phenacetin, promethazine, and menadione in Class B. The complexing tendencies of series of systems involving pairs of interacting organic molecules in aqueous solution were investigated by the phase-solubility technique. Stability constants for some caffeine interactions were evaluated by means of partitioning studies.

Keyphrases □ Organic solute binding specificity—aqueous solution □ Solutes, small organic—binding tendency classification □ Stability constants—solute binding □ Solubility—solute interaction effect □ Spectrophotometric analysis—organic solutes

Water strongly stabilizes a large number of molecular complexes, apart from its participation in hydrophobic bonding (1). Water provides a medium which seems to be unique for the molecular binding tendencies of organic molecules (2–10), many of them of great biological and pharmacological importance. In the last few years a number of papers have reported the properties of pyrimidines, purines, and the important nucleoside and nucleotide polymers in the aqueous environment. It has conclusively been shown, for example, that the bases associate to varying degree in aqueous solution, evidently through plane-to-plane stacking (11–16). It is believed that molecular interactions between adjacent bases in nucleic acid strands to a major extent are responsible for the structural stability of nucleic acids in solution (17–19).

The exact nature of the force or balance of forces operating between the complex components in aqueous solution still is the subject of controversial discussions in the literature. The observed binding between organic molecules in water is, however, firmly believed to be strictly physical in nature. As pointed out earlier (1, 5, 6), the observed intensity of binding cannot be rationalized on the basis of simple charge-transfer-type interactions (the binding constants are extremely low in alcohol, dioxane, and purely nonpolar solvents), dispersion forces (little or no interactive tendency is evident among systems of low polarizability), hydrophobic associations (very small contributions from flexible alkyl side chains), or hydrogen bonding alone. The matter is, of course, complicated by the possible interplay of different interacting forces. The problem is obviously related to the structure of liquid water, which in itself is a very intricate one, and many safe conclusions have not been made so far (20–25).

In this article the authors present their most recent observations carried out on series of systems involving pairs of interacting molecules in aqueous solution. These results have strongly reinforced a growing belief