Effect of Solvent Composition on Association between Small Organic Species

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Abstract ☐ Effects of different organic solvents on the extent of complex formation in binary aqueous organic solvent mixtures were studied by means of spectrophotometry, spectropolarimetry, and solubility technique. In every instance, the stability constants of the complexes decreased as the ratio of organic solvent to water increased. The complexes were much less stable in aqueous dioxane mixtures than in similar mixtures of water and polyhydroxy compounds such as glycerin and sucrose. These studies strongly indicate the significant contribution made by hydrophobic bonding to these interactions and the major role of the water structure.

Keyphrases ☐ Organic species, small—interaction ☐ Complex formation—solvent composition effect ☐ Stability constants, complexes—solvent composition effect ☐ Solvent effect—small organic species interaction

The uniqueness of water as an environment for biological interactions has been realized in recent years (1). To account for the fact that many solute molecules, notably polymers, associate more strongly in water than in organic solvents, the concept of hydrophobic bonding has been developed (2). The exact role played by water, however, is not yet fully understood. Recent studies of the effect of various solvents on the stability constants of the tetramethylpyrimidopteridinetetrone-N,N-dimethylcinnamide complex indicated that the complex was less than one-tenth as stable in organic solvents as in water (3). Weber has reported the effect of solvents on quenching of the fluorescence of flavins in the presence of complexing agents (4). The quenching was observed only in water and not in organic solvents, including formamide which is more polar than water.

Sinanoglu and Abdulnur have undertaken theoretical studies on the effect of solvents on the denaturation of deoxyribonucleic acid (5, 6). They indicated that the main source of stability of the helical structure of the
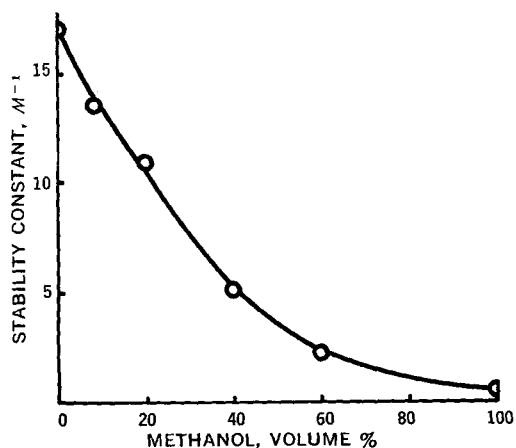
nucleic acid in water over organic solvents comes from the large surface enthalpy of water, *i.e.*, large surface tension of water. Subsequently, Moser and Cassidy have examined the extent of quinhydrone formation in various mixtures of water and organic solvents and found a reasonable correlation between surface tension and the extent of complex formation (7).

As a part of the present investigation into the nature of molecular association of small organic species in aqueous solution (3, 8–11), the authors have undertaken a quantitative study of the influence of various organic solvents in mixed solvent systems on the extent of complexation. The main purpose of the present study was to obtain additional information about the factors determining the stability of these complexes in aqueous solution.

EXPERIMENTAL

Materials—*trans*-Cinnamic acid, 3,4-dimethoxycinnamic acid, and 1,3,7,9-tetramethylpyrimido(5,4,g)pteridine-2,4,6,8(1H,3H,-7H,9H)-tetrone (abbreviated as TMPPT) were from the same commercial source[1] and were purified and used as previously described (12). Riboflavin, menadione, sodium salicylate, caffeine, sucrose, and glycerin were USP grade chemicals. Caffeine was dried at 105° overnight to obtain an anhydrous compound. Tryptophan[2] was recrystallized from ethanol–water. N,N-Dimethylcinnamide was synthesized in this laboratory (10). Methanol,[3] acetonitrile,[4] acetone,[3] and dioxane[5] were reagent grade solvents. Water was purified by distillation in an all glass still.

Determination of Stability Constants—The stability constants of 1:1 and 1:2 complexes were defined in the following way:

$$A + B = AB \qquad K_{1:1} = \frac{[AB]}{[A][B]} \qquad \text{(Eq. 1)}$$

$$AB + B = AB_2 \qquad K_{1:2} = \frac{[AB_2]}{[AB][B]} \qquad \text{(Eq. 2)}$$

where brackets refer to equilibrium concentrations. Experimental procedures and calculations of stability constants employed in this study are reported elsewhere; the procedures include spectrophotometric (13), spectropolarimetric (11), and solubility (12, 14) techniques.

RESULTS

Effect of Solvent Composition on Stability Constants of Complexes—*Riboflavin–Salicylate Ion*—Apparent 1:1 stability constants of the riboflavin–salicylate-ion complex in water–methanol mixtures were determined spectrophotometrically; the results are shown in Fig. 1. Displacement of a fraction of water with methanol resulted in a steady decrease in the stability constant of the complex.

Menadione–Caffeine—Effect of solvent composition on the stability constant of the menadione–caffeine complex was also

Figure 1—*Stability constant of riboflavin–salicylate-ion complex plotted against solvent composition as measured spectrophotometrically at 25°.*
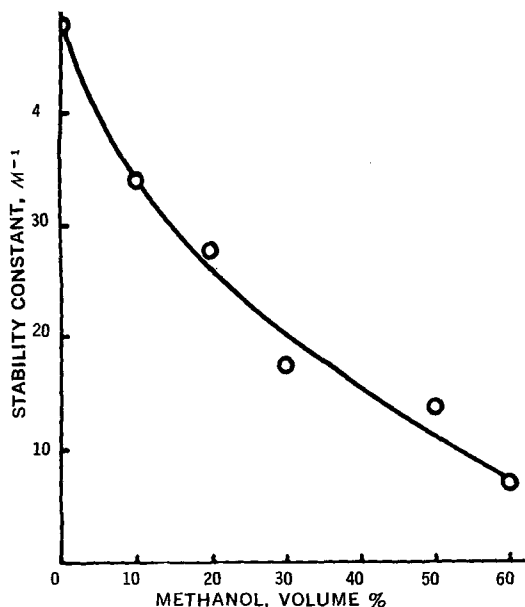
Figure 2—*Stability constant of menadione–caffeine complex plotted against solvent composition as measured spectrophotometrically at 25°.*

investigated spectrophotometrically; the results are shown in Fig. 2. A drastic decrease in stability constants with increasing methanol fraction is also apparent with this complex.

Tryptophan–Caffeine—Effects of environment on the stability constant of this amino acid–alkylxanthine complex were examined spectropolarimetrically. The results presented in Fig. 3 for this complex exhibit the same trend as observed in other systems investigated here.

TMPPT–3,4-Dimethoxycinnamate Ion—The stability constants of 1:1 and 1:2 complexes ($K_{1:1}$ and $K_{1:2}$) formed by the interaction of TMPPT with 3,4-dimethoxycinnamate ion in various compositions of methanol–water mixtures were computed from the phase-solubility diagrams. The stability constants are presented in Fig. 4 as a function of the solvent composition. It is apparent from the figure that both constants decrease with increasing fraction of methanol and the equilibria are highly dependent upon the solvent composition.
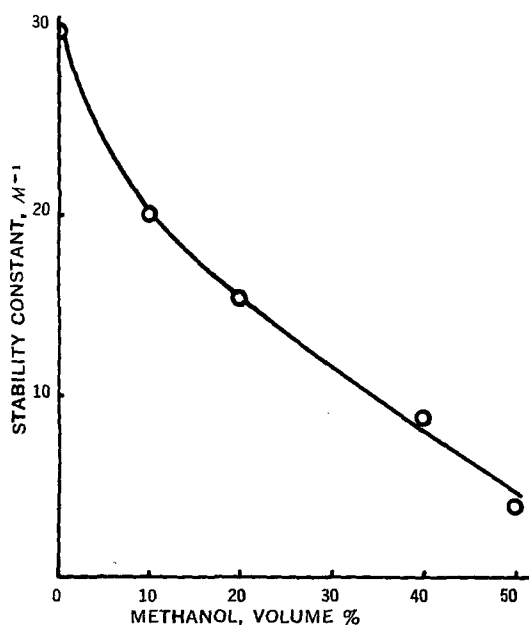

Figure 3—*Stability constant of tryptophan–caffeine complex plotted against solvent composition as measured spectropolarimetrically at 25°.*
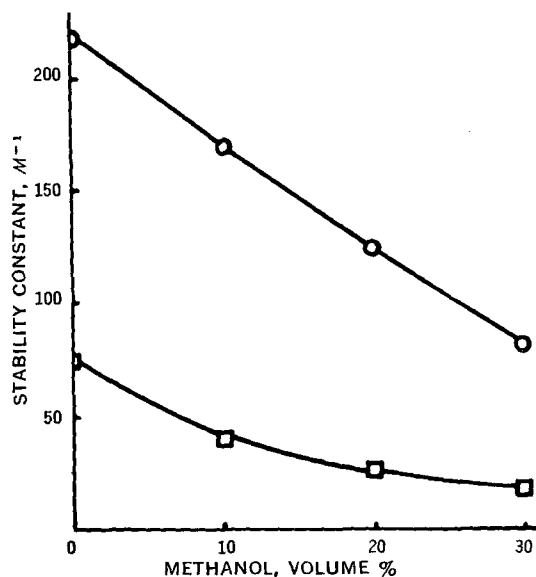

Figure 4—*Stability constant of 1:1 complex (O) and 1:2 complex (□) of TMPPT with 3,4-dimethoxycinnamate ion plotted against solvent composition as measured by solubility technique at 25°.*

TMPPT–Cinnamate Ion—Figure 5 presents the stability constants of the TMPPT–cinnamate-ion complex ($K_{1:1}$) in both water–methanol mixtures and water–dioxane mixtures. These stability constants were determined by the solubility technique and are shown in Fig. 5 as a function of solvent composition. These results again indicate that environment plays a major role in the stability of the complex. Dioxane was more effective in dissociating a complex than methanol. This point was further examined as presented in the next section.

Comparison of Effects of Various Solvents at 10% Level on Stability Constants—Since the stability constants of the complexes tend to be quite low in most organic solvents, the effects of such solvents on stability constants can be more easily evaluated at lower solvent concentrations in water. For this reason, the effects of various organic solvents on the stability constants of complexes were investigated in 10% v/v organic solvent in water. The TMPPT–N,N-dimethylcinnamide and menadione–caffeine complexes were chosen as model complexes for this purpose and studied by the solubility method and spectrophotometry, respectively.

The results are shown in Table I together with the values of surface tension of the media. Compounds such as sucrose and glycerin exhibited a much less destabilizing effect on the complexes than the other solvents investigated. Acetonitrile, acetone, and dioxane displayed a larger dissociating effect than the hydroxy compounds, dioxane exhibiting the greatest effect among the solvents investigated.

DISCUSSION

As amply demonstrated in this study, organic solvents have an unfavorable effect on the stacking interactions of organic planar molecules in water. Thus, irrespective of the solutes investigated here and of the methods employed to study these interactions, a steady decrease in the stability of a complex was observed with increasing fractions of organic solvent in aqueous media. Further, the comparison of various organic solvents at the 10% organic solvent–water mixture level indicates that the destabilizing effect of these organic solvents depends upon the nature of solvents and also upon the nature of interacting molecules.

Although interactions in pure organic solvents are greatly reduced in comparison with those in water, there nevertheless exists some associative tendency in pure organic solvents. For instance, the TMPPT–N,N-dimethylcinnamide complex has a stability constant of 60.0 M^{-1} at 25° in water, whereas in pure organic solvents such as methanol, acetone, and dioxane, the values range between 2.5 and 4.6 M^{-1} at 25° (3). Yet it is evident that an aqueous environment is necessary for significant interaction to take place between these species. The considerable stability of these complexes
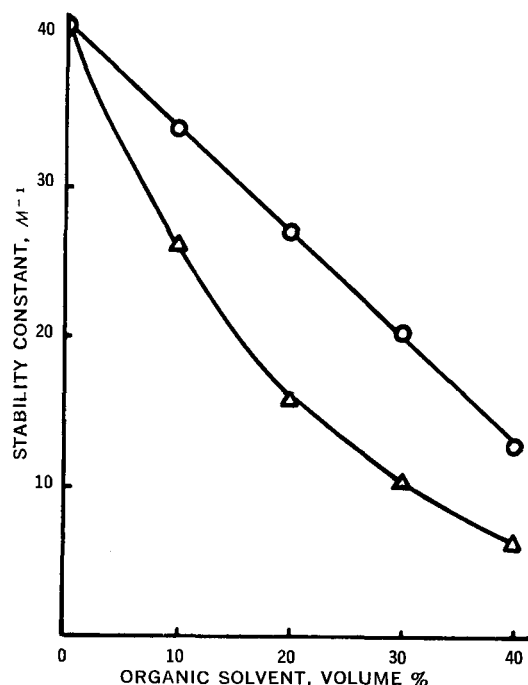
Figure 5—*One-to-one stability constant of TMPPT–cinnamate-ion complex plotted against solvent composition as measured by solubility technique at 25°. Key: O, methanol–water mixture; and △, dioxane–water mixture.*

Table I—Stability Constants of Complexes in 10% v/v Organic Solvent in Water at 25°

Organic Solvent	TMPPT–DMCA[a] Complex	Menadione–Caffeine Complex	Surface Tension[b]
None	60.0	48	72.0
Sucrose[c]	56.7	—	72.4
Glycerin	51.0	38	72.8
Methanol	48.7	34	58.2
Acetonitrile	39.9	25	53.8
Acetone	41.5	22	50.9
Dioxane	36.1	—	45.7[d]

[a] N,N-Dimethylcinnamide. [b] "Handbook of Chemistry and Physics," 49th ed., R. C. Weast, Ed., The Chemical Rubber Co., Cleveland, Ohio, pp. 28–30. [c] 10% w/v. [d] J. Timmermans, "The Physical-Chemical Constants of Binary Systems in Concentrated Solutions," vol. IV, Interscience, New York, N. Y., 1960, p. 14.

in water may then be regarded as medium-facilitated interactions. Although the exact mechanism by which water uniquely facilitates this type of association is not known, it may be speculated that the structural feature of water plays a major role.

With the exception of the existence of a hydrogen-bonded network, the structure of water still remains controversial. A popular view in recent years is to consider water as a mixture of bulky four-coordinated cluster and dense monomeric water (15, 16). Regarding the influence of solutes upon water structure, Frank outlined the most widely held view (15, 16). Hydrogen-bonding molecules are, in general, expected to alter water structure comparatively little. Those that cannot participate in the four-coordinated clusters, however, tend to break water structure. Sucrose and glycerin are considered to be capable of hydrogen bonding with water because of their polyhydroxyl groups, whereas others such as acetonitrile, acetone, and dioxane can be looked upon as the breakers of the regular structure of water. This may explain the reason why sucrose and glycerin are less effective in dissociating complexes than the nonhydroxyl solvents.

It is interesting to compare the effect of organic solvents on the extent of complexation with that on the micellar properties of surfactants in aqueous solution. It has been observed that critical micelle concentration increases in a monotonic manner with increasing fraction of organic solvent in aqueous media (17, 18). The effect was found to be more pronounced in dioxane–water mixtures than in ethanol–water mixtures. Thus, the relative effects of dioxane and the alcohol on the micellar properties of surfactants are similar to those observed in the present study of complex formation. Since hydrophobic interaction is supposed to be responsible for micelle formation, a similar effect of organic solvents on both micelle and complex formations may be reasonable, and hydrophobic interactions may be considered to be of importance for the association of solute molecules in water.

There are two theories as to the cause of hydrophobic interaction. Hydrophobic bonding is attributed by some workers to the structural restriction of water around nonpolar groups of a solute molecule; i.e., nonpolar groups are surrounded by an "iceberg" zone of water in which the orientation disorder is smaller than bulk water (19, 20). According to this theory, the total number of structurally restricted water molecules will be smaller when two molecules are brought together than when they are separated. This leads to an increase in entropy upon complexation. This positive entropy term

is considered to be the major contribution to the free energy of hydrophobic interaction.

The second school of thought ascribes hydrophobic interaction to the energy gained when two cavities accommodating two single molecules coalesce to one when a complex is formed (5, 6). In this theory the enthalpy term (i.e., negative ΔH) is largely responsible for the favorable interaction in water. This theory thus places a particular emphasis upon the large surface tension of water as being responsible for the unique behavior of water in stabilizing complexes of this nature.

Experimentally, however, negative ΔS for the interactions of simple organic species are more common than positive ΔS as the first theory suggests (11). Fitness of the second theory (enthalpy contribution), on the other hand, gave some suggestive trend. Although the extent of interaction cannot be quantitatively correlated with the surface tension of the media (Table I), surface tension probably is nevertheless related to a common physical property which also influences the intensity of hydrophobic bonding.

Hydrophobic bonding is generally recognized as important for the stability of protein conformations in aqueous solution (2, 20) and is referred to as the intramolecular force operating between the nonpolar side chains of these macromolecules. It is important to emphasize that the systems studied in the present work are interactions between small organic species, and it may be far from secure at this stage to correlate the observed binding in this case with the behavior of large molecules in solution. While the proteins are denatured to varying extent in aqueous organic solvents and in pure organic solvents, the same cannot, of course, take place for the aromatic and heterocyclic species studied here.

As was pointed out in the studies of complex formation between small organic molecules in aqueous solution (8, 9, 12), variation in the ability to bind cannot be certainly explained by hydrophobic forces alone. For example, it has been shown that hydroxy groups on benzoates and cinnamates had a strong enhancing effect on the observed binding in water (8, 9, 12). It is difficult to attribute this effect to a higher hydrophobic character of the interacting species. It has also been demonstrated that the contribution from flexible alkyl side chains was very small (12).

The observation that the binding between organic species dissolved in water apparently takes place most effectively between members of two large, distinct classes of structures (12) has strongly suggested that some selectivity must be operating among the interactants. This again tends to indicate that hydrophobic forces alone cannot be largely responsible, in most instances, for the observed binding, but rather a mechanism of some nonclassical "donor-acceptor" type may be operating. Also the fact that a slight change in molecular structure, notably by introducing substituents having mesomeric effects, results in substantial changes in the observed stability constants (8, 9, 12) appears to support this hypothesis.

Since water may be considered to participate in these interactions, a possible mechanism of the donor–acceptor type apparently should be classified as being not of the classical type but peculiar to water. The authors would expect that the effect of different solvents on the overall stability of a classical charge–transfer complex should reflect competitive solvation effects. Thus, in polar solvents that can act as both donor and acceptor, the medium would be expected to solvate both free solute species and the com-

plex itself. The overall net effect would probably render complex formation less favorable in the presence of such solvents than in an inert solvent.

Such available data concerning the influence of solvents on the stability of charge–transfer complexes suggest that these classical complexes in general are formed in appreciable amount only in nonpolar solvents. For example, the stability constants for the trinitrobenzene–N,N-dimethylaniline complex decrease with the solvents in the following order (21): cyclohexane $>$ n-hexane, n-heptane $>$ carbon tetrachloride $>$ chloroform $>$ s-tetrachloroethane $>$ 1,4-dioxane. The stability constants in cyclohexane and n-hexane were 9.5 and 8.2 M^{-1}, respectively. In chloroform, s-tetrachloroethane, and dioxane, they were of much less magnitude, being 1.3, 0.2, and 0.15 M^{-1}, respectively.

The iodine–naphthalene complex decreases in stability with the solvents in the following order (22): n-heptane $>$ cyclohexane $>$ carbon tetrachloride $>$ n-hexane $>$ chloroform. Similarly, it has been shown that the stability constants for the complex between tetrachlorophthalic anhydride and hexamethylbenzene decrease when the solvents are varied in the following order (23): n-hexane $>$ carbon tetrachloride $>$ dibutyl ether $>$ benzotrifluoride $>$ fluorobenzene $\simeq$ benzene $\simeq$ cyclohexanone. The magnitude of the stability constants were reported to be 260 M^{-1} in n-hexane and 145 M^{-1} in carbon tetrachloride. For comparison, the constants in dibutyl ether, benzotrifluoride, fluorobenzene, and cyclohexanone were 77, 52, 29, and 23 M^{-1}, respectively.

In all the cited examples it is seen that in going from nonpolar solvents to typical polar solvents such as chloroform, s-tetrachloroethane, dibutyl ether, fluorobenzene, benzotrifluoride, cyclohexanone, and dioxane, there is a substantial decrease in the stability of the charge–transfer complexes. If donors and acceptors interact to form complexes that are appreciably ionic in character, these generalizations may no longer apply; in such cases, complex formation may be facilitated in media that promote ionization (24, 25). However, this has by no means been found to be a general rule (25), and the type of complexes which are more stable in polar solvents presumably involve ion radicals and are not simple donor–acceptor complexes. With the exception of some complexes of tetramethylphenylenediamine with various tetrahaloquinones, tetracyanoethylene, and related acceptors, it appears that the known classical charge–transfer complexes are in general formed in appreciable amounts only in nonpolar solvents (25).

Although this discussion suggests that contribution from classical charge–transfer complexes may not be a major factor in aqueous solution because of the competitive participation of water, the authors cannot exclude the possibility that a donor–acceptor mechanism, peculiar to water, may contribute to the stability of the complexes studied. Such a speculation may be justified, since hydrophobic forces alone appear to fail to account for all the observed effects.

CONCLUSIONS

From the preceding discussion, it is apparent that the favorable interaction between small organic species in water may be due to a summation of several factors. The net enhancement in binding by increasing the content of water in the environmental solvent cannot be rationalized on the basis of any single binding mechanism alone. Although hydrophobic bonding and a nonclassical donor–acceptor mechanism may have been the major forces involved, with the contributions from the former possibly of lesser magnitude, further investigations along these lines may shed light on additional factors which have not been evident in this limited study. Since

the balance of forces operating between the complex components in pure aqueous solution is not known, it is difficult to separate and interpret the gross effect seen in mixtures of organic solvents with water.

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