[Chem. Pharm. Bull. 17(2) 242—247 (1969)]

UDC 615.451.011.4 : 547.857.4 : 09 : 547.587.11.09

Studies on Molecular Interaction of Organic Molecules in Solution. I. Effects of Solvent on Molecular Interaction of Salicylic Acid with Caffeine

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(Received August 31, 1967)[2]

A new interative method to study molecular interactions spectrophotometrically in case of two interacting species being present in comparable concentrations was developed and applied to salicylic acid–caffeine interaction to examine the effects of carbon tetrachloride, benzene, isoamyl acetate, and water on the apparent stability constant of salicylic acid–caffeine complex. The results of the present study have indicated (a) that salicylic acid interacts very strongly in the nonpolar solvents presumably by hydrogen bonding; (b) that their interaction is minimal in the moderately polar solvents, and (c) that they interact strongly in aqueous solution. These differences are discussed in terms of the difference in mechanisms of interaction in various solvents.

Because the nature of complexes in organic phase is different from that in aqueous phase, complexes themselves are not likely to penetrate through the phase boundary when caffeine and salicylic acid are allowed to partition between the organic and aqueous phase.

Although the spectrophotometric investigation is one of the most popular means of studying molecular interactions in solution, Benesi and Hildebrand's method of calculation[3] and its equivalent methods[4,5] can only be applied to the systems where the concentration of one of the interacting species is in great excess of that of the other. Thus these methods can not be used when two solutes are soluble only to a moderate extent. More serious difficulties have recently been realized when one employs a high concentration of one of solutes and a dilute solution of the other. Guttman and Higuchi[6] have noticed that some of solute molecules dimerize in moderately concentrated solution to a great extent. Moreover there is a greater chance for higher order complexes to be formed when concentration of one solute is much greater than that of the other. Emslie, Foster, Fyfe, and Horman[7] have suggested that the molar absorptivities of complexes may not be constant (that is, Beer's law does not hold) when one uses a high concentration of one of interactants.

In order to eliminate the above difficulties, a new iterative procedure was designed as described below to calculate stability constants of complexes when the concentrations of the interactants are comparable.

If two species, A and B, interact reversibly in solution to form a 1:1 complex C according to

$$A + B \rightleftarrows C \qquad (1)$$

the stability constant, K_c, in the concentration unit can be expressed by

$$K_c = \frac{C_c}{(C_a - C_c)(C_b - C_c)} \qquad (2)$$

1) Location: *Yoshida, Sakyo-ku. Kyoto.*
2) Revised from March 8, 1968.
3) H.A. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).
4) H. McConnell and N. Davidson, *J. Am. Chem. Soc.*, **72**, 3164 (1950).
5) R. Foster, D.Ll. Hammick, and A.A. Wardley, *J. Chem. Soc.*, **1953**, 3817.
6) D. Guttman and T. Higuchi, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 4 (1957).
7) P.H. Emslie, R. Foster, C.A. Fyfe, and I. Horman, *Tetrahedron*, **21**, 2843 (1965).

where C_a and C_b are the total concentrations of A and B respectively and C_c is the concentration of the complex at equilibrium. The difference, ΔA, between the measured absorbance and that expected if no interaction took place is then

$$\begin{aligned}\Delta A &= l\{a_a(C_a-C_c)+a_b(C_b-C_c)+a_aC_c-a_aC_a-a_bC_b\}\\ &= l(a_c-a_a-a_b)C_c\\ &= l\cdot\Delta a\cdot C_c \end{aligned} \quad (3)$$

where Δa represents $(a_c-a_a-a_b)$, l is the pathlength of the cell in cm, and a_a, a_b, and a_c are the molar absorptivities of A, B, and C respectively. Equation 3 can be written as

$$C_c=\frac{\Delta A}{\Delta a\cdot l} \quad (4)$$

From Eq. 2 and Eq. 4, Eq. 5 can be derived.

$$\frac{C_aC_b}{\Delta A}=\frac{1}{\Delta a\cdot l\cdot K_c}+\frac{C_a+C_b-C_c}{\Delta a\cdot l} \quad (5)$$

The values of C_c which are not known experimentally have to be successively approximated in order to obtain the values of K_c and a_c from Eq. 5. This can be carried out relatively simply by making use of both Eq. 4 and Eq. 5. As a first approximation, a plot of $C_aC_b/\Delta A$ against (C_a+C_b) according to Eq. 5 yields a line with a slope value approximately equal to $1/\Delta a\cdot l$. This value is substituted into Eq. 4 to obtain the first approximate values of C_c. The C_c values thus obtained are then incorporated into Eq. 5 to calculate an improved value for the slope as above. These steps are repeated until two successive cycles yield an essentially convergent value for the slope. The stability constant, K_c, is then calculated from the limiting slope and intercept values. The molar absorptivity of the complex, a_c, can also be obtained from the limiting slope value.

It can be shown that Eq. 5 can be reduced to Benesi and Hildebrand's formula[3] and its equivalent formulae[4,5] when $C_a \ll C_b$.

Experimental

Material——J.P. grade caffeine was recrystallized from water and dried at 80° for 5 hours to obtain the anhydrous compound, mp 237°. Reagent grade salicylic acid was recrystallized from methanol, mp 159°. Spectroscopic grade carbon tetrachloride and benzene, reagent grade isoamyl acetate, and redistilled water were used as the solvents. Other solvents used were also of reagent grade.

Spectrophotometric Determination of Stability Constants——A Shimadzu Spectrophotometer QV-50 with 1 cm cells was employed for measurements of absorbance at 330 mμ. The concentration of salicylic acid was kept constant at 6×10^{-4}M in carbon tetrachloride and in benzene, 8×10^{-4}M in isoamyl acetate, and 16×10^{-4}M in 0.1N H_2SO_4 aqueous solution. The concentrations of caffeine were varied between 2×10^{-3}M and 8×10^{-3}M in carbon tetrachloride, between 2.4×10^{-3}M and 16×10^{-3}M in benzene, between 3×10^{-3}M and 12×10^{-3}M in isoamyl acetate, and between 2.4×10^{-3}M and 16×10^{-3}M in 0.1N H_2SO_4 aqueous solution. The measurements were made at 25±1°.

Spectrophotometric Determination of the Stoichiometry of the Complex——The method of continuous variation[8] was employed. Various volume proportions of an equal concentration (6×10^{-4}M) of solutions of salicylic acid and caffeine were mixed. The spectrophotometric measurements were carried out at 330 mμ.

Partition Studies——The partition coefficients (P.C.) of salicylic acid and caffeine between benzene and 0.1N H_2SO_4, as defined the concentration in benzene over that in 0.1N H_2SO_4, and the apparent partition coefficient of salicylic acid in the presence of caffeine and that of caffeine in the presence of salicylic acid, were determined spectrophotometrically after equilibration in a water bath at 25°. A phase volume of 15 ml each was placed in a stoppered tube and the contents of the tube were mechanically shaken for 2 hours. Each component remaining in the aqueous phase after partitioning was determined in the following way.

8) M.M. Jones, "Elementary Coordination Chemistry," Prentice Hall, Inc., Englewood Cliffs, New Jersey, 1964, p. 282.

Caffeine in the aqueous phase was extracted into chloroform after the aqueous solution was made alkaline with 1 N NaOH. The absorbance of the chloroform solution was then determined at 272 mμ. Salicylic acid in the aqueous phase was measured at 300 mμ, the small absorbance due to caffeine in the mixed system being subtracted from the total absorbance. The difference between the amount added and that remaining in the aqueous layer is set equal to the amount in the organic phase.

Results and Discussion

The alteration in the absorption spectrum of salicylic acid in the presence of caffeine as shown in Fig. 1 has provided a basis for quantitative spectrophotometric investigation of the molecular interaction in solution. The subsequent measurements were made at 330 mμ because of maximum changes in absorbance and the absence of absorption due to caffeine in the wavelength. Utilizing this property the maximum changes in absorbance by the method of continuous variation (see Fig. 2 for the interaction in carbon tetrachloride as an example)

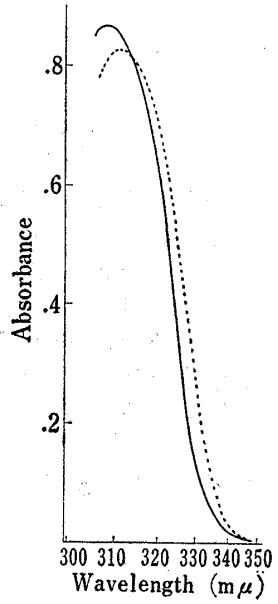

Fig. 1. Absorption Spectra of Salicylic Acid (2×10^{-4}M) with (——) and without (------) Caffeine (5×10^{-3}M) at 25° in Carbon Tetrachloride

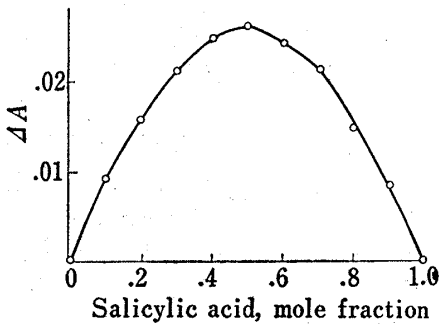

Fig. 2. Plot according to the Method of Continuous Variation for the Interaction of Salycilic Acid with Caffeine in Carbon Tetrachloride

total concentration $=6\times10^{-4}$M

were observed to correspond to the solute composition of 50% salicylic acid–50% caffeine in all solvents studied here, indicating a 1:1 complex being predominant under the experimental conditions.

Having established the stoichiometry of the major complex species present in the systems we have attempted to calculate the stability constants for the interaction by the iterative method described above. In the present successive approximation only 3 cycles of iterations were usually enough to reach convergent values for slopes. The first and the third plot of the approximations are shown in Fig. 3 for the interaction in carbon tetrachloride as an example. The fact that the final points fell well on a straight line over the concentration range satisfying the underlying assumptions on which Eq. 5 was derived together with the result of the method of continuous variation appears to confirm the stoichiometry of the complex species being predominantly 1:1 in carbon tetrachloride over the concentration range studied for determination of the stability constant. It is expected that the self-association of salicylic acid is unimportant at the concentration range employed in this study, but at higher concentrations the effect and formation of higher order complexes are necessarily to complicate the system. It is, therefore, essential to study the system under a limited

concentration range and to take account of the concentrations of the complex in order to obtain any meaningful value as the measure of interaction. Other advantages of the present interative procedure have already been pointed out in conjunction with a similar method with nuclear magnetic resonance (NMR) measurements.[9]

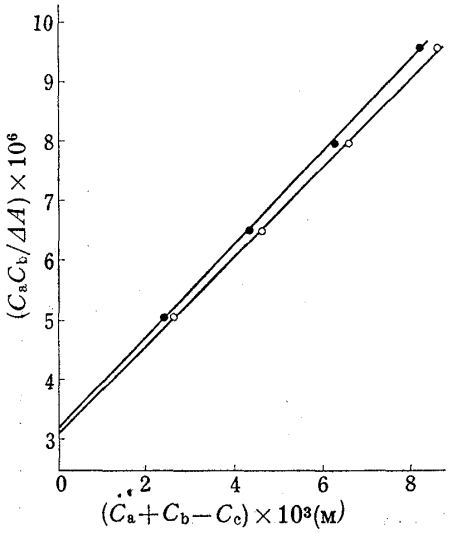

Fig. 3. Plots based on Eq. 5 for the Interaction of Salicylic Acid (6×10^{-4}M) and Caffeine ($2—8\times10^{-3}$M) in Carbon Tetrachloride at 25°

key: ○, the first approximation
●, the third approximation

Table I. Stability Constants for the Salicylic Acid–Caffeine Complexes at 25±1°

Solvent	Stability constant (M^{-1})
Carbon tetrachloride	235
Benzene	132
Isoamyl acetate	*a)*
Water	23

a) The stability constant could not be obtained because of too small change in absorbance.

Stability constants obtained in four solvents for the interaction of salicylic acid with caffeine at 25° are given in Table I. Little interaction was also observed in other moderately polar solvents such as methanol, acetonitrile, and chloroform. The observed value of stability constant in 0.1N H_2SO_4 is smaller than those obtained by other investigators utilizing the solubility technique.[10,11] Disagreements in the values of stability constants determined by different techniques are often reported in such studies particularly in aqueous media.[11]

Although molecules of salicylic acid exist in intramolecularly hydrogen bonded forms in nonpolar solvents, their intermolecular bindings may also be ascribable primarily to hydrogen bonding at the carboxylic hydrogen atom with one of carbonyl oxygens of caffeine in such solvent systems. Since excellent hydrogen acceptor ability of amides has been demonstrated,[13,14] caffeine may well behave similarly. Hydrogen bonding, because of the essentially electrostatic nature, is expected to decrease in its importance as the solvent becomes polar. Thus in polar solvents this type of interactions is not expected to play a significant part. Nevertheless experimental results have shown that they interact comparatively strongly in aqueous media. This fact rules out a significant contribution of hydrogen bonding as the intermolecular force between the solute species in aqueous media. Although the exact nature of interaction in water is not wholly understood, its plane to plane stacking[15] and some kind of hydrophobic nature[16] has been noted. If the interaction is of solvophobic

9) M. Nakano, N.I. Nakano, and T. Higuchi, *J. Phys. Chem.*, **71**, 3954 (1967).
10) T. Higuchi and D.A. Zuch, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 138 (1953).
11) G. Levy and R.H. Reuning, *J. Pharm. Sci.*, **53**, 1471 (1964).
12) D.A. Wadke and D.E. Guttman, *J. Pharm. Sci.*, **54**, 1293 (1965).
13) T. Higuchi and S. Chulkaratana: cited in T. Higuchi and K.A. Connors, *Advan. Anal. Chem. Instr.*, **4**, 117 (1965).
14) S. Mizushima, M. Tuboi, T. Shimanouchi, and Y. Tsuda, *Spectrochim. Acta*, **7**, 100 (1955).
15) O. Jardetzky, *Biopolymers Symposia*, **1**, 501 (1964).
16) M. Nakano, Ph. D. Thesis, University of Wisconsin, 1967.

nature this contribution will decrease in its importance as the solvent becomes less polar.[17,18] Our experimental results appear to support this view indicating little interaction in isoamyl acetate, a solvent of intermediate polarity. The relationship between the extent of interaction as measured by the stability constant and the polarity of the solvent may be depicted only schematically as shown in Diagram 1 for cases when two solutes can interact by different mechanisms in the extremes of solvent polarity.

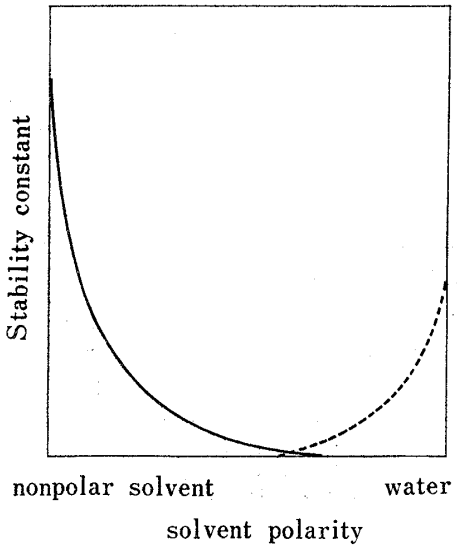

Diagram 1. Schematic Representation of the Relationship between Stability Constant and Solvent Polarity (See a Text for Detail)

key: —— hydrogen bonding
----- solvophobic bonding

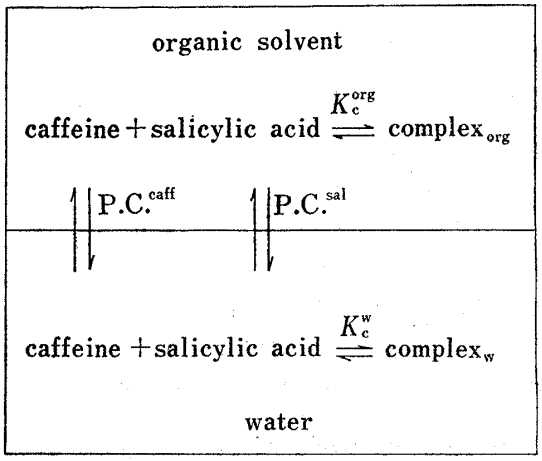

Diagram 2. Partition between and Interaction within the Solvents for the Caffeine–Salicylic Acid System

In this hypothetical representation, microscopic polarity of solvents, characterized by the magnitude of interaction between a solvent and solutes as well as the gross dielectric constant of the solvent are meant to be the measure of solvent polarity. Thus a large difference was observed between the effects of carbon tetrachloride and benzene which show but a little difference in magnitude of dielectric constants, on the degree of interaction between the two solutes, because of the difference in the extents of the solute–solvent interactions.

If it is the solvent which decides the nature of binding forces in solution as discussed above, in systems consisting of two immisible liquid phases little partition of complex species across the boundary is expected. It is likely that only free forms penetrate the phase boundary and equilibrium to form complex species is set up in each phase. Levy and Reuning[11] have attempted to explain earlier the observed absorption behavior of salicylic acid from the rat stomach in the presence of caffeine by assuming the complex species themselves move across the boundary at a slower rate than that of free salicylic acid. The same model system as the one used for the partition study by them can be alternatively interpreted according to the scheme shown in Diagram 2.

If the partition coefficient of salicylic acid is defined to be concentration in organic phase over that in water, the apparent partition coefficient, $P.C._{app}^{sal}$, in the presence of caffeine is equal to

$$P.C._{app}^{sal} = P.C.^{sal} \frac{1 + K_c^{org}(caff)_{org}}{1 + K_c^{w}(caff)_{w}} \qquad (6)$$

17) O. Sinanoglu and S. Abdulnur, *Photochem. Photobiol.*, **3**, 333 (1964).
18) O. Sinanoglu and S. Abdulnur, *Federation Proc.*, **24**, s-12 (1965).

where K_c^{org} and K_c^w are the stability constants in organic solvent and in water respectively, and $(caff)_{org}$ and $(caff)_w$ are the concentrations of free caffeine in organic and in water respectively. Similarly the apparent coefficient of caffeine in the presence of salicylic acid is expressed by

$$P.C._{app}^{caff} = P.C.^{caff} \frac{1+K_c^{org}(sal)_{org}}{1+K_c^w(sal)_w} \qquad (7)$$

Thus for the given system the reduced partition behavior of salicylic acid is considered mainly due to the large value for K_c^w in comparison with that for K_c^{org} and a high concentration of free caffeine in aqueous phase (caffeine is more soluble in water than in isoamyl acetate). On the other hand their experimental observation on the increased partition behavior of caffeine may be rationalized by the extremely higher solubility of salicylic acid in the organic phase than that in water although the value of K_c^{org} is significantly small in comparison with that of K_c^w. This interpretation may thus explain Levy and Reuning's observation of a large decrease in the apparent partition coefficient of salicylic acid in the presence of caffeine and the corresponding increase in the apparent partition coefficient of caffeine being not so significant.

The apparent partition coefficient of salicylic acid between benzene and 0.1 N H_2SO_4 in the presence of caffeine and that of caffeine in the presence of salicylic acid were determined as shown in Table II. In the present choice of organic solvent, the partition coefficients of

Table II. Partition Coefficients (Benzene/0.1 N H_2SO_4) at $25 \pm 0.1°$

Initial composition of aqueous phase		($\times 10^3$ M)	P.C.
Salicylic acid	salicylic acid	5	2.4
	salicylic acid	5	2.6
	and caffeine	5	
Caffeine	caffeine	5	0.69
	caffeine	5	0.86
	and salicylic acid	5	

both compounds increased in the presence of the other. The larger apparent partition coefficient of salicylic acid in the presence of caffeine may be due to the much larger K_c in benzene than that in water, this effect overbalancing the lower solubility of caffeine in benzene than that in water, *i.e.* $K_c^{org}(caff)_{org} > K_c^w(caff)_w$ in Eq. 6.

Although *in vitro* models to study the partition behavior of drugs are essential for elucidation of the mechanisms governing drug absorption the actual situation seems to be far more complex.[19] Disagreement between the results of partition studies and those of absorption studies may arise from the fact that equilibrium states are not usually reached *in vivo* as well as the reason discussed above among others.

19) R.H. Reuning and G. Levy, *J. Pharm. Sci.*, **56**, 843 (1967).