THE STABILITY OF SOME MOLECULAR COMPLEXES IN AQUEOUS MIXED SOLVENTS: CORRELATION WITH SOLVENT SURFACE TENSION

by SY-RONG SUN

(Under the supervision of Professor Kenneth A. Connors)

Earlier work in this laboratory led to the empirical linear relationship between the standard unitary free energy change for complex formation and the maximal area of plane-to-plane overlap between substrate S and ligand L in aqueous solutions. This was interpreted in terms of a simple model and the associated equation,

$$\Delta G^{o} = A\,(G^{o}_{SL} - G^{o}_{MS} - G^{o}_{ML})$$

where A is the maximal overlap area and G^{o}_{IJ} is the free energy of interaction per unit area at equilibrium between the "surfaces" I and J (M represents the medium). The empirical slope is equivalent to 64 dynes/cm. J. L. Cohen (Ph.D. dissertation, University of Wisconsin, 1969) concluded that the term for interaction between S and L, G^{o}_{SL}, is unimportant in comparison with the solvent interaction terms, G^{o}_{MS} and G^{o}_{ML}. This simplified view provided a general first-order description of molecular complex formation in aqueous solution. The deviations from the average line were ascribed to significant second-order effects, perhaps associated with more subtle variations in structure-stability relationships.

The present study started with the suggestive observation that the theoretical and empirical slopes of the area correlation both indicate possible dependence of complex stability on solvent surface tension, or some property related to it. It was found that the stability of the typical molecular complex between methyl trans-cinnamate and theophylline appeared to be determined only by the surface tension of the solvent for various mixed solvents containing water. Among the solvents used were aqueous binary mixtures with methanol, ethylene glycol, acetonitrile, and dioxane, as well as aqueous solutions of sodium chloride and lithium chloride. Deuterium oxide gave consistent results.

A linear relationship was found between standard free energy changes for the complexation equilibrium and the surface tension of the solvent. This was extrapolated to zero surface tension presumably eliminating the solvent contribution to the overall complex stability. This extrapolation procedure was applied to five complex systems: methyl trans-cinnamate-theophylline; methyl trans-cinnamate-theophylline anion; methyl trans-cinnamate-8-chlorotheophylline anion; naphthalene-theophylline; and methyl 2-naphthoate-8-nitrotheophylline anion. The extrapolated intercepts for these complexes were found to be linearly related to their vertical deviations from Cohen's average line in the maximal overlap area correlation.

If this extrapolation and separation procedure is valid, the extrapolation leads to the quantity ΔG^{o}_{SL}. Comparison of this with the overall free energy change suggests that the S - L interaction may be responsible for a significant (even a major) portion of the complex stability. Among the assumptions governing this quantitative separation of solvent and intermolecular effects on complex stability are: (1) the overall effect can be partitioned into additive contributions; and (2) the extrapolation to zero surface tension eliminates the solvent contribution. At the present time the stability-surface tension correlation appears to be a useful empirical observation, while the solvent effect separation procedure is a potentially valuable approach that must be subjected to future testing before its quantitative results can be accepted.

APPROVED Kenneth A. Connors

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I. INTRODUCTION

A. General Background

The term "molecular complex" is used to describe a variety of association products of two or more molecules. The general reaction for the molecular complex formation may be represented by the equation:

$$mS + nL \rightleftharpoons SmLn$$

where S represents the substrate and L is the ligand. (The substrate is the compound whose apparent properties are measured.) Complex formation is a reversible reaction whose rate is so fast that it can be considered to be at equilibrium.

The stability of a complex is specified in terms of its stability (association, formation) constant. The overall stability constant Kmn for the complex formation reaction is written

$$Kmn = \frac{[SmLn]}{[S]^m [L]^n}$$

where Kmn is the constant applicable to the solvent system and temperature employed (1,3,50,68,70). In the systems most desirable for fundamental studies, a 1:1 stoichiometry is sought; i.e., $m = n = 1$. The

experimentally determined 1:1 stability constant is usually labeled K'_{11} and is referred to as an apparent stability constant until evidence becomes available to suggest that it is indeed a true K_{11}. In general, these organic molecular complexes are too unstable to be easily isolated in crystalline form, but exist only in solution in equilibrium with their components, and then their existence can be inferred from the nonadditive behavior in the physical and chemical properties of solutions of the interacting species.

In this thesis, we are not including the metal coordination compounds, or inclusion compounds, in which one of the constituents of the complex is trapped in the crystal lattice of the other.

One of the primary goals in studies of complexation is to obtain information regarding the structure and the nature of interaction forces of the complex. The exact nature of the forces involved is currently a matter of considerable controversy. It was proposed in 1929 that the formation of complexes was due to covalent bonding between the two components (71); however, abundant evidence exists to show that the distance between the component molecules in crystalline complexes is only slightly less than the van der Waals distances (77-80) and is usually greater than 3A (9), and the forces of coordination are much weaker than those of covalent

bonds (1). These observations remove the possibility that any sort of normal covalent bonding could be responsible for these complexes. Covalent bonds range in energy from 50 to 200 Kcal per mole and have lengths of 1 - 2A, while intermolecular forces of the van der Waals type have energies of 0.5 - 5 Kcal per mole and usual distances of 3 - 5A (72). Therefore, it is generally accepted that the forces involved in the complexes of this type may include hydrogen bonding, van der Waals forces and charge-transfer.

Intermolecular hydrogen-bonding is negligible in our complex systems, first because the interactants are not good hydrogen donors, and second because of competition from water, which is in tremendous excess; e.g., in a dilute aqueous organic solvent mixture (for example, 30% (v/v) MeOH-aqueous mixture) S and L may be of the order of 10^{-2} M each whereas water is approximately 41.8 M (in 30% (v/v) MeOH-aqueous mixture). Support for this can be found in the work of Nakano, et al. (73), who found that for the salicylic acid-caffeine complex the stability constant was 235 M^{-1} in carbon tetrachloride and 23 M^{-1} in water, where competitive intermolecular hydrogen bonding is possible.

The theory of charge-transfer forces is due mainly to Mulliken (68,74,81-82). The charge-transfer complexes are formed by the weak interaction of electron donors

with electron acceptors and therefore are also called electron donor-acceptor complexes. In our complex systems, which are not chosen to maximize electron donor or acceptor ability, the relatively large stability constants are probably not due mainly to charge-transfer, and in many of these systems there is no detectable shift in the spectrum upon complexation. Although in Eckert's (83) complexes, as in many related examples (22,29), some charge-transfer is involved, since spectral shifts do occur in aqueous solution, there have been no satisfactory estimates of its importance toward complex stability.

Therefore in complex formation as presently viewed the net interaction may have been best described by Wallwork (84) as "polarization bonding," in which orientations favoring local dipole-dipole or dipole-induced dipole forces exist. These dipole-dipole, dipole-induced dipole and Heitler-London dispersion forces (85) are the so-called van der Waals forces.

The importance of the solvent in complex formation in aqueous solution has been largely ignored. Davis and Symons have studied the iodine-naphthalene complex (55) and Emslie and Foster (56) have studied the tetrachlorophthalic anhydride-hexamethylbenzene complex in various organic solvents. These and many other studies (57-58,64-65) deal with fully nonaqueous systems,

especially those in which hydrogen bonding is a major type of interaction (66,86); these do not concern us here. Stelmach (14) has shown the stability constant for the methyl trans-cinnamate-theophylline complex to decrease as the concentration of methanol in water increases, and the magnitude of the spectral shift decreased in the same way. Higuchi and co-workers (87-90) have undertaken a quantitative study of the influence of various organic solvents in mixed solvent systems on the extent of complexation. Moser and Cassidy have examined the extent of quinhydrone formation in various mixtures of water and organic solvents and found a rough relationship between surface tension and the extent of complex formation (91).

B. Plan of Research

This investigation was initiated with related studies in these laboratories, some results of which have been quoted above (cf. Stelmach (14), Infeld and Kline (13), and Cohen (4,30)).

Connors and co-workers (13,30) have derived an equation on the basis of a simplified model to correlate the complex stability and the maximal molecular overlap area. This equation is as follows:

$$\Delta G^{\circ} = A(G^{\circ}_{SL} - G^{\circ}_{MS} - G^{\circ}_{ML})$$

where ΔG^{o} is the standard unitary free energy change for complex formation, G^{o}_{IJ} is a free energy of interaction per unit area between molecular "surfaces" I and J. M represents the medium. A is the maximal overlap area (these maximal overlap areas were estimated by maximizing the overlap area between substrate and ligand molecules, oriented plane-to-plane, with CPK molecular models.[a])

They plotted $-\Delta G^{o}_{unitary}/N$ vs. maximal molecular overlap area and found that for fifty complex systems in aqueous solutions, a linear correlation was observed with a slope value equivalent to 64 dynes/cm. They further suggested that the substrate-ligand interaction is of minor importance in determining complex stability; then the average value of the G^{o}_{MS} and G^{o}_{ML} term is 32 dynes/cm. This is approximately the value of interfacial tensions between water and some typical hydrophobic organic compounds (48). Therefore, this suggests that[b]:

(1) Complex stability may be related to surface tension or interfacial tension;

(2) The study of the solvent effect on complex stability may be a useful way to approach this problem.

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[a]The Ealing Corporation, Cambridge, Mass.

[b]Professor P. Mukerjee (personal communication) has observed similar correlations.

The area correlation treatment reveals that, in the first-order approximation, all systems behave similarly, and on the average, there is no difference between neutral and ionic complexes. Deviation from the line in this area correlation represents second-order effects becoming important in complex stability considerations. It was hoped from this present study that additional information might be obtained about the relative importance of the solvent in determining complex stability.

Complex systems were chosen from those on the area correlation plot (4) to represent points on the line and with positive and negative deviations from the line. Five complex systems were chosen, and each system was studied in various aqueous organic solvent mixtures. Organic solvents in the binary aqueous organic solvent mixtures were methanol, acetonitrile, dioxane, dimethyl sulfoxide, ethylene glycol, and glycerine. The solvents deuterium oxide and aqueous sodium chloride or lithium chloride solutions were also used.

The five complex systems chosen were methyl trans-cinnamate-theophylline, methyl trans-cinnamate-theophylline anion, methyl trans-cinnamate-8-chloro-theophylline anion, naphthalene-theophylline, and methyl 2-naphthoate-8-nitrotheophylline anion. These represented both neutral and anionic complexes. The

kinetic, solubility, and spectral methods of studying the interactions were utilized. It was hoped that from this study some light might be shed on the factors determining the stability of these complexes in aqueous solution.

II. EXPERIMENTAL

A. Materials

Reagent-grade inorganic salts were used without further purification. Standard buffers were prepared according to Bates (5,6) using deionized distilled water. Other buffers were prepared according to Bates and Bower (7) or Kolthoff and Rosenblum (8). Ionic strength was adjusted by the addition of potassium chloride.

Acetonitrile (Fisher Scientific Co., Certified Reagent), isooctane (2,2,4-trimethylpentane) (Matheson Coleman and Bell, practical grade) and methyl trans-cinnamate (Matheson, Coleman and Bell or Eastman White Label) were purified as described by Mollica and Connors (12).

Methanol (Mallinckrodt), N,N-dimethylformamide (Eastman White Label), dimethyl sulfoxide (Baker Analyzed Reagent), 1,4-dioxane (Fisher Scientific Co., Certified Reagent), ethylene glycol (Fisher Scientific Co., Certified Reagent) and glycerine (Fisher Scientific Co., Certified Reagent) were all reagent grade and were used without further purification.

Deuterium oxide (Merck and Co., Inc.) with minimum isotopic purity 99.7 Atom % D, deutero sodium hydroxide

solution (Brinkmann Instruments, Inc.) (label 40% sodium deuterium oxide in D_2O, deuterium labeled portions minimum 99%), and acetonitrile-d_3 (CD_3CN, Nuclear Magnetic Resonance Specialties, Inc.) (minimum isotopic purity, 99.0% Atom % D) were used without further purification.

Methyl 2-naphthoate was prepared by treating 2-naphthoyl chloride (Eastman Organic Chemicals) with methanol in pyridine. The ester was purified by recrystallizing twice from ethanol-water, mp 78° (lit. (16), 77°).

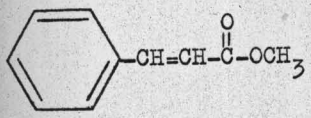
Naphthalene (J. T. Baker, resublimed) was used without further purification, mp 79° (lit. (17), 80.3°).

Theophylline (Merck and Co., NF) was recrystallized from water, mp 270-271°. The compound was then dried at 150° for 4 hours, ground to a fine powder, and the drying continued for 24 hours to insure the anhydrous form. 8-Bromotheophylline (Aldrich Chemical Co.) was used without further purification. 8-Chlorotheophylline (Aldrich Chemical Co.) was purified as described by Mollica and Connors (12). 8-Nitrotheophylline dihydrate (K and K Laboratories) was used without further purification, mp 282-283° d (lit. (18), 282-283° d).

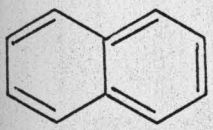
Table I shows structures of the substrates and ligands. Table II gives pertinent ultraviolet spectral data for some of the compounds described in this section.

TABLE I

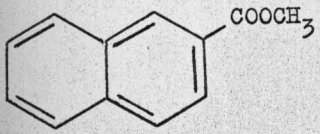
Substrates



Methyl trans-cinnamate



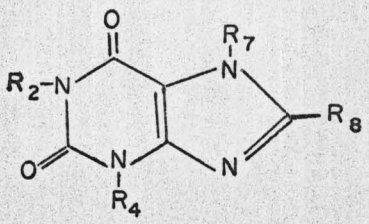
Naphthalene



Methyl 2-naphthoate

TABLE I - Cont.

Ligands



R_2	R_4	R_7	R_8	Compound
CH_3	CH_3	H	H	Theophylline
CH_3	CH_3	H	Cl	8-Chlorotheophylline
CH_3	CH_3	H	Br	8-Bromotheophylline
CH_3	CH_3	H	NO_2	8-Nitrotheophylline

TABLE II

Ultraviolet Spectrophotometric Data

Compound	λ_{max} (nm)	$10^3 \epsilon_{max}$	Solvent
Methyl *trans*-cinnamate[a]	279	21.9	pH 6.5 buffer
	272	21.5	isooctane
Methyl 2-naphthoate	279	6.82	isooctane
Naphthalene[b]	275	5.66	isooctane
Theophylline	272	10.0	pH 6.75 phosphate buffer

[a] lit. (19), ϵ_{279} 2.21×10^4.

[b] lit. (20), ϵ_{275} 5.63×10^3.

B. Apparatus

Constant temperatures were achieved with a water bath controlled to a claimed precision of ±0.01° by Sargent Thermonitor Electronic Relays. Some solubility determinations were made in a bath controlled to ±0.05° by a mercury column thermo-regulator (Bronwill Scientific Co.). Thermometers were calibrated against thermometers carrying either A.S.T.M. or National Bureau of Standards calibration certificates.

pH measurements were made with a Sargent Model DR pH meter equipped with either a universal range glass-calomel combination electrode S-30072-15 (Sargent) or a universal range miniature combination glass-calomel electrode S-30070-10 (Sargent), or on a Radiometer model 25 pH meter with scale expander and a wide range glass electrode G202B. The meter electrode systems were standardized with NBS standard buffers.

Solubility determinations were made with an apparatus described by Mollica and Connors (12). Some studies were also performed in a large water bath equipped with a rotating shaft to which 15 ml screw-capped vials could be attached and tumbled end-over-end. The temperature of this bath was held to 25±0.05° by a relay and a mercury column thermoregulator (Brownell Scientific Co.).

Most spectrophotometric measurements were obtained on a Cary Recording Spectrophotometer, model 14; some measurements were made on a Cary Recording Spectrophotometer, model 15 or Cary Spectrophotometer, model 16. Each instrument was fitted with a thermostatted cell compartment and circulating water bath that maintained temperature to $\pm 0.1°$.

Melting points were determined on a Thomas-Hoover Capillary Melting Point apparatus. A 2.00 ml microburet (Roger Gilmont Instruments) was used for accurate delivery of small volumes. Infrared spectra were obtained on a Beckman IR-5A model spectrometer. A 0.20 ml blow-out volumetric pipette (Kimax-51, No. 37034) was used in spectral studies for precise delivery.

Specific gravity measurements were carried out with the Sargent pycnometer (S-9225) (50.0 ml) which is double walled and vacuum jacketed, with ground-in perforated stopper and a ground glass cap to minimize evaporation. Capacities are adjusted to ± 0.3 ml at 20°C.

Surface tension and interfacial tension measurements were made with a Cenco-Du Nouy ring tensometer (Cenco 70535) having a platinum-iridium ring 5.992 cm in circumference. The ratio of ring diameter to wire diameter, R/r, was 53.6. Samples were kept in a constant-temperature water bath until just before measurement.

C. Spectral Measurements

The evaluation of complexation equilibrium constants by spectroscopic measurements has been described by Mollica and Connors (12). Their procedures were utilized here but some minor improvements will be discussed. The substrate stock solution was prepared by dissolving a weighed amount of ester (substrate) in the organic solvent of interest so that 0.2 ml of substrate stock solution added to 25.0 ml would give the desired concentration of substrate. Buffer solutions were prepared by dissolving the appropriate amount of buffer reagents with different percentages (v/v) of aqueous organic solvent mixture. A typical preparation of a 10% (v/v) CH_3CN-aqueous phosphate buffer solution with pH = 6.6, μ = 0.3 was conducted as follows: To a 250 ml volumetric flask, 3.225 gm KCl, 1.256 gm KH_2PO_4 and 1.065 gm Na_2HPO_4 were added. A suitable amount of water was added to the salts. After the salts were dissolved, an exact amount (25.0 ml) of acetonitrile, which had been pre-equilibrated at 25.0° was added to the 250 ml volumetric flask, then water to almost 250 ml. It was mixed well and again placed into the 25.0° water bath to equilibrate. After the equilibrium temperature was reached, water, which had been equilibrated at 25.0° was added to bring the volume exactly to the mark.

Unless otherwise stated, ligand stock solutions were prepared by adding a weighed quantity to a volumetric flask, then the ligand was dissolved in buffer solution which was prepared as described previously. Again the buffer solution had been pre-equilibrated at 25.0° before using it to dissolve the ligand.

If the ligand was ionized at the pH of the study an equivalent amount of standard sodium hydroxide or hydrochloric acid was added. Potassium chloride was used to bring the buffer solutions to the desired ionic strength.

Solutions with different concentrations of ligand, but identical pH, could then be prepared by serial dilution of the ligand stock solution with the buffer stock solutions. Then 0.20 ml of substrate stock solution was added to each ligand solution. 0.20 ml of organic solvent was added to the same amount of buffer solution (i.e., without the ligand) as the blank solution. Then the absorbances of the ligand solutions were measured against the blank solution, usually at 320 nm. The small absorbances due to the presence of ligands were substracted out. The measurements of the absorbances of the ligand solution can be done by measuring the absorbance of the ligand solution against the blank solution. All the spectral studies were carried out at 25.0° unless otherwise stated.

For the spectral studies in D_2O solvent, NaOD solution in D_2O was used to bring the solutions to desired pD values, CD_3CN was used to dissolve the substrate, and potassium chloride was used to control the ionic strength. To measure pD in D_2O solutions, the electrodes were standardized against the usual standard buffers in ordinary water, then, at 25°, pD is obtained as (36)

$$pD = \text{"measured pH"} + 0.41$$

(for the pD range 2 - 9). In these studies it was only necessary to ensure that all solutions had the same pD.

D. Kinetic Measurements

All kinetic studies were done at 25.0° and ionic strength 0.3 unless otherwise indicated. Hydroxide ion activity or deuteroxide ion activity was established by potentiometric measurement as described. pH's or pD's were maintained, generally, between 12 and 13 using hydroxide-chloride or deuteroxide-chloride buffer solutions described earlier.

Procedures used in spectrophotometric measurements of kinetic studies depend upon the half-life of the reaction, as well as the pathlength of the spectrophotometer cell used. Details of such studies involving

reactions with various half-lives, using a 1 cm cell, have been described (12) and were followed during this study with minor improvements.

A typical kinetic study for the system between methyl trans-cinnamate and 8-chlorotheophylline anion in D_2O solution was conducted as follows: a stock solution of the ligand (8-chlorotheophylline anion) was prepared by adding a weighed quantity to a volumetric flask in D_2O. Sufficient potassium chloride to provide the desired ionic strength was added and the solution was brought to volume with an appropriate buffer solution. The buffer solution was also brought to the desired ionic strength by the addition of potassium chloride. Both solutions were then brought to temperature (25.0°C) and adjusted to the same pD by the addition of small volumes of deutero sodium hydroxide solution in D_2O (40% sodium deuterium oxide in D_2O). Solutions with different concentrations of ligand, but identical pD, could then be prepared by serial dilution of the ligand stock solution with the buffer stock solution. Then 0.1 ml of acetonitrile-d_3 solution of substrate was added by pipet to a 10 ml volumetric flask containing buffer and ligand that had previously been equilibrated at 25.0°. The contents were mixed and were then added directly to the sample cell, which was temperature equilibrated in the spectrophotometer cell compartment.

Recording of absorbance as a function of time at 320 nm was begun within 15 seconds of the initiation of the reaction.

Rate constants were determined from plots of log $(A_t - A_\infty)$ *vs.* time or by the method of Guggenheim (67). Reactions were followed for a minimum of two half-lives unless the Guggenheim method of plotting was employed, in which case data were obtained over at least six half-lives. All "infinity" values were taken after ten half-lives and the spectra at this time were shown to match those of the expected products.

E. Solubility Measurements

An amount of substrate known to be in excess of its solubility at the temperature of the study was added to each of several 4-dram screw-capped vials. To each vial was added 10.0 ml of buffer solution containing varying amounts of ligand. The ligand concentrations were obtained by serial dilutions of stock ligand solution with buffer. All concentrations used were below the solubility of the ligand. The vials' covers were lined with a piece of teflon®. Parafilm® was used in addition to the screw-cap to seal the vials. The vials were then tumbled in a constant-temperature water bath for at least 48 hours. (This time was sufficient for equilibration as determined by heating one of the

blanks (zero ligand concentration) to supersaturate it with respect to substrate and comparing its final concentration with the other blanks.)

Aliquots of the supernatant were withdrawn by a pipette, the tip of which had been wrapped in glass wool. The substrate was then extracted with isooctane and the isooctane phase was measured spectrophotometrically, or, when necessary, diluted and then measured.

All the solubility studies were carried out at 25.0°C.

F. Density Measurements

Density measurements were carried out with the Sargent pycnometer. A typical measurement was conducted as follows:

To measure the density of 40% (v/v) CH_3CN-aqueous mixture, 100 ml of the mixture sample was prepared, since the volume of the pycnometer is 50 ml. To a 100 ml volumetric flask, 40.0 ml of CH_3CN, which had been pre-equilibrated at 25.0°, was added by volumetric pipette. Then water was added almost to the mark. It was mixed well and then put back into the 25.0° water bath. After the equilibrium temperature was reached, water was added to the mark.

The weight of water plus the pycnometer, at 25.0°, was determined. Let this weight equal W_1. Similarly,

let W_2 be the weight of the 40% (v/v) CH_3CN aqueous mixture, and the pycnometer, at 25.0°. Then the density (d_4^{25}) of the 40% (v/v) CH_3CN aqueous mixture was calculated as follows:

$$d_4^{25} = \left(\frac{\text{weight of 40\% (v/v) } CH_3CN \text{ aqueous mixture, at 25.0°, inside the pycnometer}}{\text{weight of water, at 25.0°, inside the pycnometer}}\right) \times \left(\frac{\text{weight of water, at 25.0°, inside the pycnometer}}{\text{weight of water, at 4.0°, inside the pycnometer}}\right)$$

$$= \frac{W_2 - W}{W_1 - W} \times (\text{density of water at 25.0°})$$

$$= \frac{46.6872}{50.0167} \times 0.99708 \text{ (lit. (23))}$$

$$= 0.9307,$$

where W is the weight of the empty pycnometer at 25.0°. To convert the 40% (v/v) CH_3CN-aqueous mixture to weight by weight percentage the following equation was used:

$$\%(w/w)CH_3CN = \frac{(\text{ml of } CH_3CN \text{ in 1 L. mixture})(d_4^{25} \text{ for pure } CH_3CN)}{1000 \times (d_4^{25} \text{ for this mixture})} \times 100\%$$

$$= \frac{400 \times 0.7777^a}{1000 \times 0.9307} \times 100\%$$

$$= 33.42\%.$$

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[a] lit. (24), d_4^{25} for pure CH_3CN = 0.778.

That is, 40% (v/v) CH_3CN aqueous solution corresponds to 33.42% (w/w) CH_3CN aqueous solution.

G. Surface Tension and Interfacial Tension Measurements

Surface tension and interfacial tension measurements were made with a Cenco Du Nouy ring tensiometer and were carried out at 25.0°C.

The true surface tension or interfacial tension, γ, was calculated by the following equation (59):

$$\gamma = P \times F$$

where P is the apparent surface tension, or reading on the tensiometer, and F is a correction factor (59). Zuidema and Waters (59) have published a formula for the correction factor. They have plotted the correction factor, F, against P/(D - d), where P is the apparent surface tension. When making a surface tension measurement D is the density of the liquid and d is the density of saturated air. d is so small in comparison with the density of most liquids that it may be ignored. When making an interfacial tension measurement D is the density of the lower liquid, d the density of the upper liquid.

When the measurement is to be made on the interface between water or H_2O-organic solvent mixture and a liquid lighter in density than water the ring pull is

upward and the procedure is as follows. With water or water-organic solvent mixture only in the clean dish, the platform is raised until the ring is immersed from 5 to 7 mm in the water or the water-organic solvent mixture. A quantity of the liquid is then carefully poured on the surface of the water or the water-organic solvent mixture. This should be to a depth of 5 to 10 mm depending on the liquid, but deep enough to prevent the ring from entering the upper surface before the film breaks. The position of the dish is adjusted until the ring is in the interface and the lever arm in the neutral position. Then the torsion of the wire is increased and the dish lowered, keeping the index of the lever arm at zero. The reading when the film at the interface breaks is the apparent interfacial tension, P.

In all of the above measurements the scale reading on the instrument is the apparent surface or interfacial tension. According to Harkins and Jordan (25) and Freud and Freud (26) this value may differ from the true value by as much as 30% in extreme cases. For most measurements the difference is probably less than 5%. To obtain the true surface or interfacial tension, γ, it is necessary to correct the apparent surface or interfacial tension, P, by a factor, F, so that $\gamma = P \times F$, as described previously.

A typical calculation of the interfacial tension, γ, at 25.0° for the system between benzene and water was conducted as follows:

P = apparent interfacial tension reading on the tensiometer, 32 dynes/cm

D = density of the lower phase (the aqueous phase), 0.9971 gm/ml (61)

d = density of the upper phase (the organic phase), 0.8748 gm/ml (61).

Then,

$$\frac{P}{D - d} = \frac{32.0}{0.9971 - 0.8748} = 262$$

and F = 1.070 (59);

therefore,

$$\gamma = P \times F = 32.0 \times 1.070 = 34.24 \text{ dynes/cm}^{a}$$

H. Treatment of Data

A comparison of the mathematical methods, assumptions, and approximations for the evaluation of stability constants by spectral, solubility and kinetic methods has been made by Connors and Mollica (50). Cohen and Connors (35) have recommended an experiment as a straightforward introduction to the solubility

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[a] lit. (61), = 34.3 dynes/cm.; lit. (62,63), = 34.18 dynes/cm.

method for studying complex formation between sodium salicylate and theophylline. Kramer and Connors (69) have described the determination of a stability constant for the interaction of sodium cinnamate with theophylline utilizing spectral techniques. Cohen and Connors (76) have introduced an experiment for the study of complex formation between methyl trans-cinnamate and theophylline anion by the kinetic method.

Higuchi and Connors (2) have reviewed in detail the solubility method for studying complexes. Equations for the analysis of spectral data have been proposed by Benesi and Hildebrand (21) and Ketelaar (31), while Colter (32) employs a relationship utilizing rate constants for both free and complexed ester and an apparent equilibrium constant to analyze kinetic data.

An extensive review of all methods of stability constant analysis has been compiled by Rossotti and Rossotti (42). In all cases the data were treated in the manner described by Connors and Mollica (50), and only a summary of the final equations, plotting forms, and methods of computing stability constants from the plots will be presented here. All concentrations are molar; all methods assume a 1:1 stoichiometry.

(a) Kinetic method. Two reactions were considered in interpreting kinetic results:

$$S + R \xrightarrow{k_s} \text{Products}$$

$$SL + R \xrightarrow{k_{11}} \text{Products}$$

where S is the uncomplexed substrate, R is the attacking agent and SL is the complex. The pertinent equation involving the apparent second-order rate constant (k_s) and ligand concentration is

$$\frac{k_s}{k_s - k_s'} = \frac{1}{q_{11} K_{11} (L)} + \frac{1}{q_{11}}$$

where,

k_s = rate constant for reaction of substrate in the absence of ligand

k_s' = rate constant for reaction of substrate in the presence of ligand

q_{11} = fractional decrease in reactivity of complexed substrate = $(1 - k_{11}/k_s)$

(L) = concentration of uncomplexed ligand.

Plot $k_s/(k_s - k_s')$ vs. $1/L_t$; when (L) can be approximated by L_t, then the plot will be linear with stability constant evaluated as

$$K_{11}' = \frac{\text{Y-intercept}}{\text{slope}} = -(\text{X-intercept}).$$

The value of q_{11} can be obtained from the Y-intercept value.

(b) Spectral method.

$$\frac{b}{\Delta A} = \frac{1}{K_{11} S_t \Delta a (L)} + \frac{1}{S_t \Delta a}$$

where,

b = cell path length

ΔA = difference in absorbances of substrate in the absence and presence of (L) moles/L of ligand

S_t = total substrate concentration

Δa = difference in molar absorptivity between the complex and the substrate and ligand; $\Delta a = a_{11} - a_s - a_L$

(L) = concentration of uncomplexed ligand.

Plot $b/\Delta A$ vs. $1/L_t$ (where the assumption is made that $(L) = L_t$; then,

$$K'_{11} = \frac{\text{Y-intercept}}{\text{slope}} = -(\text{x-intercept}).$$

(c) Solubility method.

$$S_t = \frac{K'_{11} S_o L_t}{1 + K'_{11} S_o} + S_o$$

where,

S_o = solubility of substrate in the absence of ligand

S_t = total solubility of substrate in the presence of ligand

L_t = total molar ligand concentration

k'_{11} = apparent 1:1 stability constant

Plot S_t vs. L_t; then,

$$k'_{11} = \frac{\text{slope}}{\text{intercept } (1 - \text{slope})}$$

I. Statistical Analysis of Data

To determine the best line through the experimental points, the method of least squares was applied. Kramer (10) has developed statistical methods of fitting these experimental data to linear relationships. The method of weighted least squares was applied to all double reciprocal (spectral and kinetic) plots with the slope and intercept defined by the following equations:

$$\text{Y-intercept} = \frac{\Sigma wx^2 \Sigma wy - \Sigma wx \Sigma wxy}{\Sigma w \Sigma wx^2 - (\Sigma wx)^2}$$

$$\text{slope} = \frac{\Sigma w \Sigma wxy - \Sigma wx \Sigma wy}{\Sigma w \Sigma wx^2 - (\Sigma wx)^2}$$

The computation of the weighting factor, w, was based on experience with each experimental system. Our experience with these systems indicated that as the value of the quantity plotted on the Y-axis increased, the accuracy of its determination decreased. Ideally w should be

based on actual variance of the dependent variable, but this information was not available from these experiments. It was observed, however, that the square of the dependent variable was proportional to its variance. Thus, the value of w was computed by the formula

$$w = \frac{d^2}{\Sigma d^2}$$

where d is the denominator of the fraction plotted on the Y-axis (ΔA), this denominator being the dependent variable.

Solubility data were fitted to an unweighted least-squares line with the slope and intercept defined by the following equations.

$$\text{slope} = \frac{n\Sigma xy - \Sigma x \Sigma y}{n\Sigma x^2 - (\Sigma x)^2}$$

$$\text{Y-intercept} = \frac{\Sigma y \Sigma x^2 - \Sigma x \Sigma xy}{n\Sigma x^2 - (\Sigma x)^2}$$

Both of these analyses were performed on a digital computer (Univac 1108).

J. Calculation of Unitary Standard Free Energy Change

The unitary free energy change is the portion of the free energy change that is characteristic of the chemical process taking place, the statistical effect of change in

number of particles (the cratic portion) being substracted out. The unitary standard molar free energy change is given by $-RT \ln K_N$, where K_N is the equilibrium constant on the mole fraction scale. Gurney (60) has described the concept of unitary quantities in detail.

A typical calculation of the unitary free energy change is as follows: For the system methyl trans-cinnamate and theophylline in 12.50% (w/w) CH_3CN-aqueous phosphate buffer (pH 6.6, μ = 0.3) mixture, the stability constant k_{11} is 5.0 M^{-1}.

d_4^{25} = density for the binary aqueous organic solvent mixture (for 12.50% (w/w) CH_3CN-H_2O mixture d_4^{25} = 0.9758 gm/ml)

k_{11} = 5.0 M^{-1} (the stability constant)

M^* = total moles of solvent in 1000 gm of the mixed solvent (for 12.50% (w/w) CH_3CN-H_2O mixed solvent,

$$M^* = \frac{125}{41.054} + \frac{875}{18.016} = 51.608);$$

then,

$$K_N = (d_4^{25} k_{11} M^*)$$

$$= 0.9758 \times 5.0 \times 51.608 = 2.52 \times 10^2;$$

therefore,

$$\Delta G_u^\circ = -RT \ln K_N$$

$$= -1364 \log K_N \text{ (for } T = 298.16^\circ \text{ K)}$$

$$= -1364 \times \log(2.52 \times 10^2)$$

$= -3.276 \times 10^{3}$ cal/mole

$= -5.44 \times 10^{-21}$ cal/molecule.

III. RESULTS

A. Methyl trans-Cinnamate and Theophylline

Previous work in our laboratories (4,11,28-30) found cinnamate substrates and xanthine ligands to be excellent systems for the study of complexing tendency, these systems demonstrating fairly large stability constants. Therefore, it was decided to continue using these systems to investigate the solvent effects on these types of molecular complexes. Methyl trans-cinnamate (hereafter referred to as methyl cinnamate) was used as the substrate, and theophylline as ligand. Theophylline appeared to be an excellent ligand for this study; it does not seem to dimerize extensively in aqueous solution (75); it ionizes in a pH range that makes it possible to utilize the ligand in both the free and the ionized forms; and it appears to interact with cinnamates in a 1:1 stoichiometry. The spectral method for the study of complex interactions was used.

(a) CH_3CN (Acetonitrile)-H_2O mixed solvents.

Since acetonitrile had usually been used to dissolve the substrate for the preparation of stock solutions in the study of molecular complexes in aqueous systems in the previous work in our laboratories (4,11,14,22), it was

decided to use it for the study of mixed solvent effects on molecular complexes.

A stability constant of 22.0 M^{-1} was obtained in 0.62% (w/w) acetonitrile aqueous phosphate buffer solution with ionic strength, μ = 0.3, pH = 6.6. The literature value (11) for this stability constant is 22 M^{-1} in 1% CH_3CN aqueous phosphate buffer solution. Stability constants were also measured at other percentages (w/w) of CH_3CN in aqueous phosphate buffer solutions. Some representative spectral data are shown in Table III and the spectral reciprocal plots are shown in Figures 1 through 3.

The stability constants, the standard unitary free energy changes[a], densities, M* values, and the surface tensions for these mixed solvents are shown in Table IV.

The surface tension measurements of the CH_3CN-H_2O mixed solvents were carried out at 25.0°. The values obtained were close to literature values (52), which were measured at 20.0°.

(b) Methanol-water mixed solvents. The methyl cinnamate-theophylline system was also studied by the spectral technique in methanol-water mixtures.

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[a]We are most grateful to Prof. P. Mukerjee, who called our attention to the importance of unitary quantities in systems such as these.

TABLE III

Absorbance of Methyl *trans*-Cinnamate in the Presence of Theophylline in Aqueous Acetonitrile Solutions[a]

Theophylline concentration ($M \times 10^3$)	A_{320} % (w/w) CH_3CN		
	3.11	5.62	6.89
0	0.700	0.700	0.693
5.95	0.873	0.835	0.810
8.93	0.957	0.898	0.870
11.90	1.029	0.952	0.920
17.85	1.156	1.081	1.010
23.80	1.290	1.168	1.118
29.75	1.362	1.257	1.196

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 2 cm cell, ester conc. = 1.02×10^{-3} M.

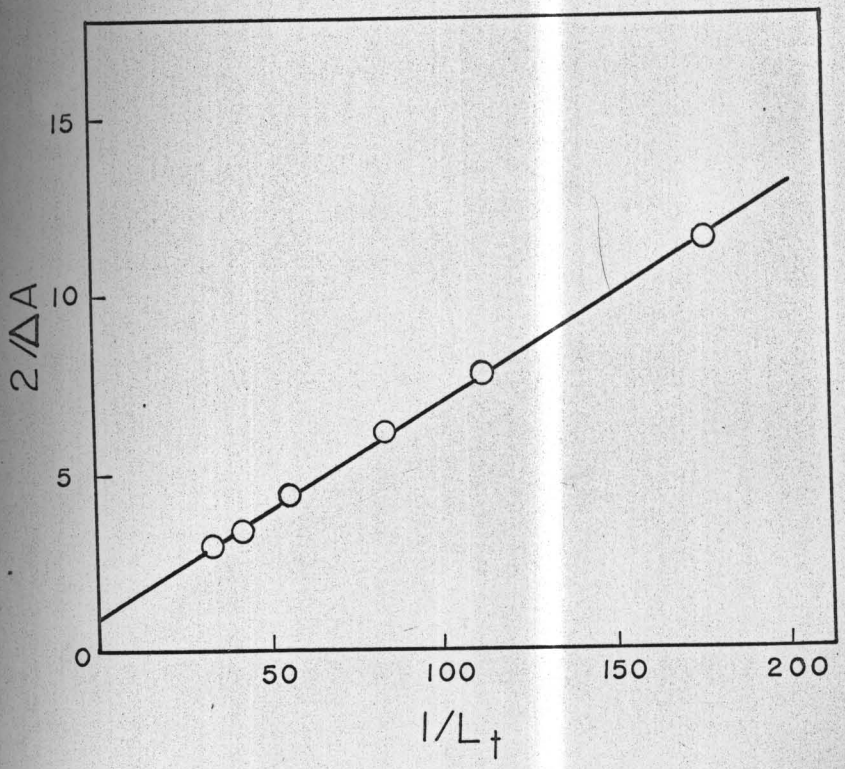


Figure 1. Spectral plot of methyl trans-cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 3.11% (w/w) CH_3CN; K_{11} = 15.76 M^{-1} (data given in Table III).

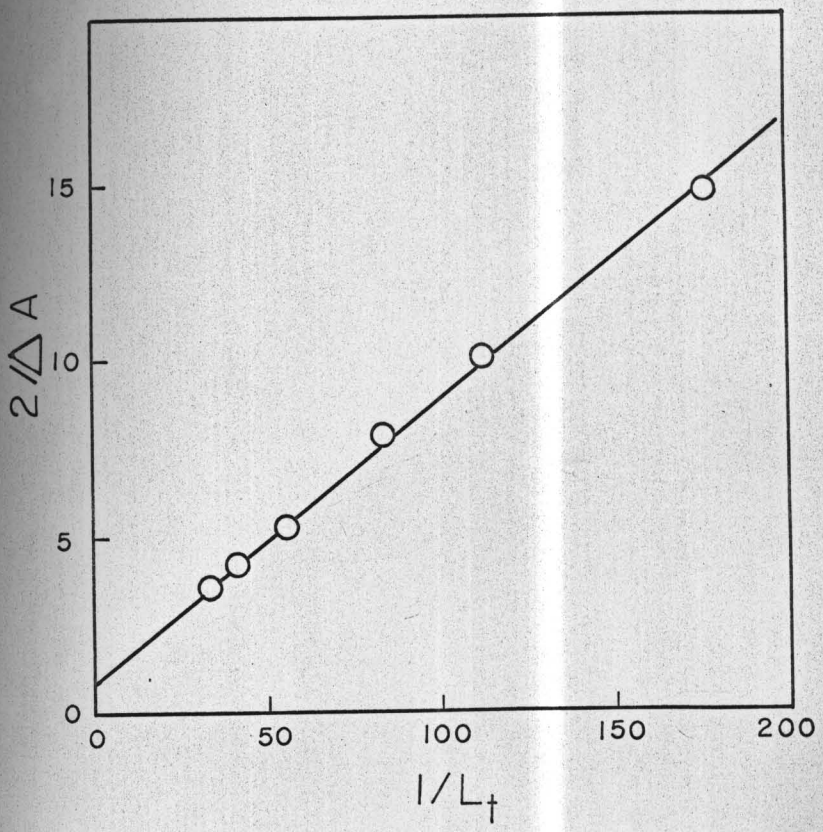


Figure 2. Spectral plot of methyl trans-cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 5.62% (w/w) CH_3CN; K_{11} = 10.93 M^{-1} (data given in Table III).

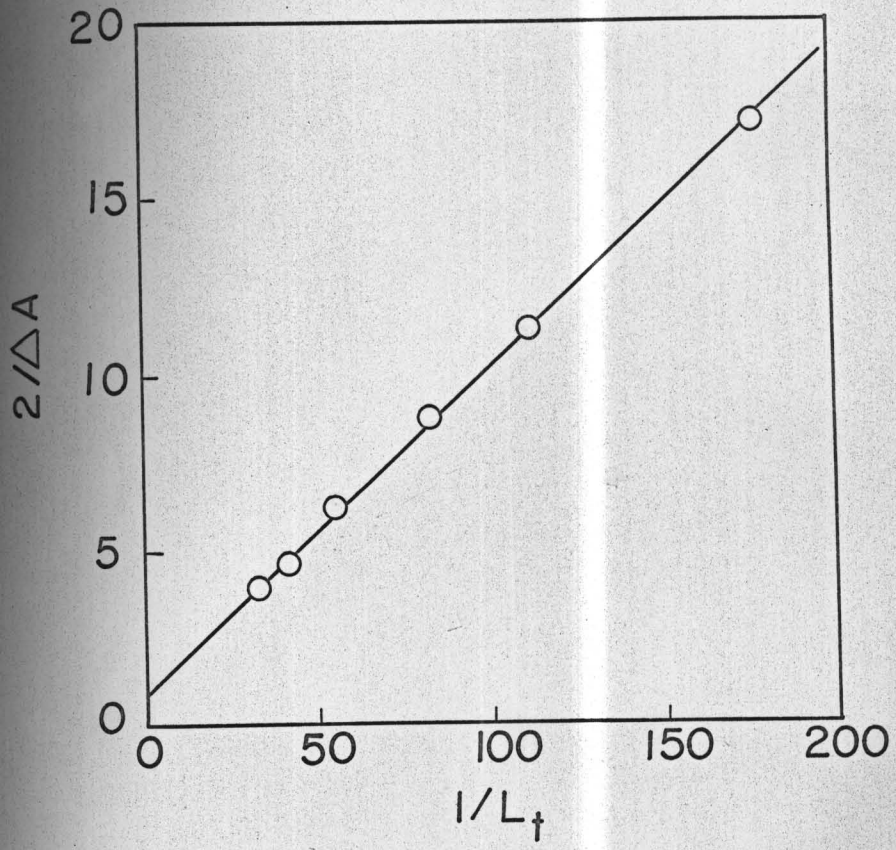


Figure 3. Spectral plot of methyl trans-cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 6.89% (w/w) CH_3CN; K_{11} = 9.51 M^{-1} (data given in Table III).

TABLE IV

Stability Constants, Unitary Standard Free Energy Changes, and Surface Tensions for Methyl *trans*-Cinnamate-Theophylline System in Aqueous Acetonitrile Solutions[a]

CH_3CN %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K'_{11} (M^{-1})	$-10^{21}\Delta G^{\circ}_u/N$ (cal/molecule)	$\gamma_{25.0^{\circ}}$ (dynes/cm)
0.62	55.307	0.9960	22.0	7.0	71.9
1.24	55.114	0.9948	16.9	6.7	69.2
3.11	54.532	0.9920	15.8	6.6	63.3
4.52	54.092	0.9895	14.7	6.6	59.0
5.62	53.750	0.9877	10.9	6.3	56.6
6.89	53.349	0.9855	9.5	6.1	54.1
8.48	52.860	0.9828	9.3	6.1	51.4
12.50	51.608	0.9758	5.0	5.4	46.0
16.57	50.340	0.9680	2.7	4.8	42.0

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

Stelmach (14) showed that as the concentration of methanol increases the stability constant for the system methyl cinnamate and theophylline drops sharply, but no systematic data were obtained.

The stability constants were measured in aqueous methanol phosphate buffer solutions (pH 6.6, μ = 0.3) at 25.0°. Some representative spectral data for these systems are shown in Table V, and the spectral reciprocal plots are shown in Figures 4 and 5. The stability constants, the standard unitary free energy changes and the surface tensions of these mixed solvents are shown in Table VI. The densities for the methanol-water mixtures at 25.0° were measured in this work; they are consistent with those values given in the Merck Index (27) and also with the values of Gallant (33).

(c) Dioxane-water mixed solvents. The same spectral technique was applied to study the system in dioxane-water mixtures at 25.0°. Table VII shows some of the spectral data and the spectral reciprocal plots are shown in Figures 6 and 7. In Table VIII, the stability constants, standard unitary free energy changes, densities, number of moles and surface tensions are included for this complex system in dioxane-H_2O mixtures. The densities and surface tensions for dioxane-water mixtures were measured at 25.0° and are shown in

TABLE V

Absorbance of Methyl trans-Cinnamate in the Presence of Theophylline in MeOH-H_2O Mixtures[a]

Theophylline concentration ($M \times 10^3$)	A_{320} %(w/w) MeOH	
	0.62	4.58
0	0.688	0.721
7.14	0.963	0.945
8.93	1.014	0.993
11.90	1.090	1.081
17.85	1.265	1.222
23.80	1.385	1.349
49.75	1.506	1.449

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 2 cm cell, ester conc. = 1.01×10^{-3} M.

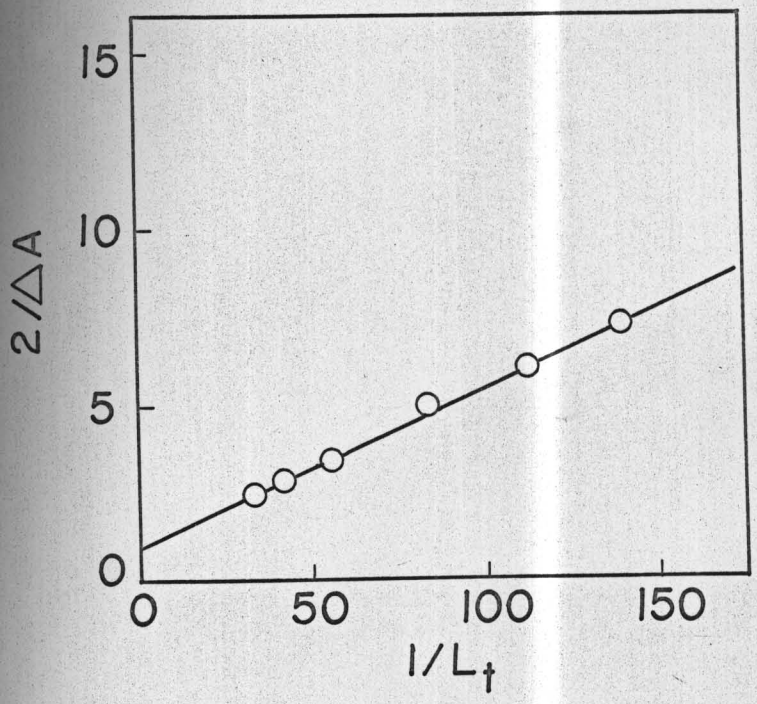


Figure 4. Spectral plots of methyl trans-cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 0.62%(w/w) MeOH; K_{11} = 19.46 M^{-1} (data given in Table V).

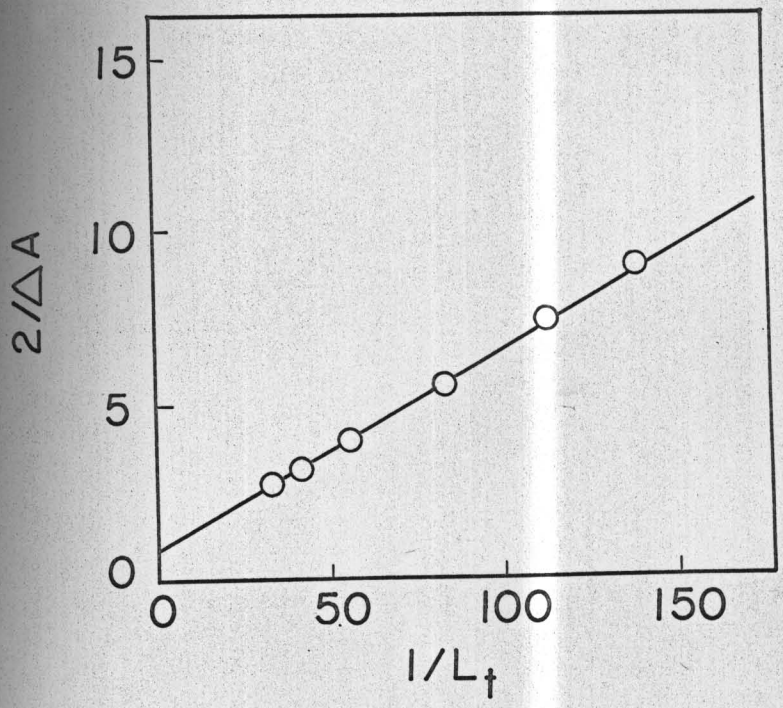


Figure 5. Spectral plot of methyl cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 4.58%(w/w) MeOH; K_{11} = 13.53 M^{-1} (data given in Table V).

TABLE VI

Stability Constants, Standard Unitary Free Energy Changes and Surface Tension for Methyl Cinnamate-Theophylline System in MeOH-H_2O Mixtures[a]

MeOH %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G^{\circ}u/N$ (cal/molecule)	$\gamma_{25.0^{\circ}}$ (dynes/cm)
0.62	55.350	0.9957	19.5	6.9	70.0
2.59	54.871	0.9920	18.4	6.8	66.0
4.58	54.387	0.9885	13.5	6.5	62.6
12.64	52.430	0.9757	9.1	6.0	52.4
33.81	47.287	0.9420	5.0	5.3	38.3

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

TABLE VII

Absorbance of Methyl Cinnamate in the Presence of Theophylline in Dioxane-H_2O Mixtures[a]

Theophylline concentration ($M \times 10^3$)	A_{320} %(w/w) Dioxane	
	0.81	40.47
0	0.598	0.458
8.93	0.880	0.481
11.90	0.960	0.488
17.85	1.075	0.503
23.80	1.209	0.513
29.75	1.303	0.528

[a] 25.0°, pH 6.6 phosphate buffer, $\mu = 0.3$, 2 cm cell, ester conc. = 9.15×10^{-4} M.

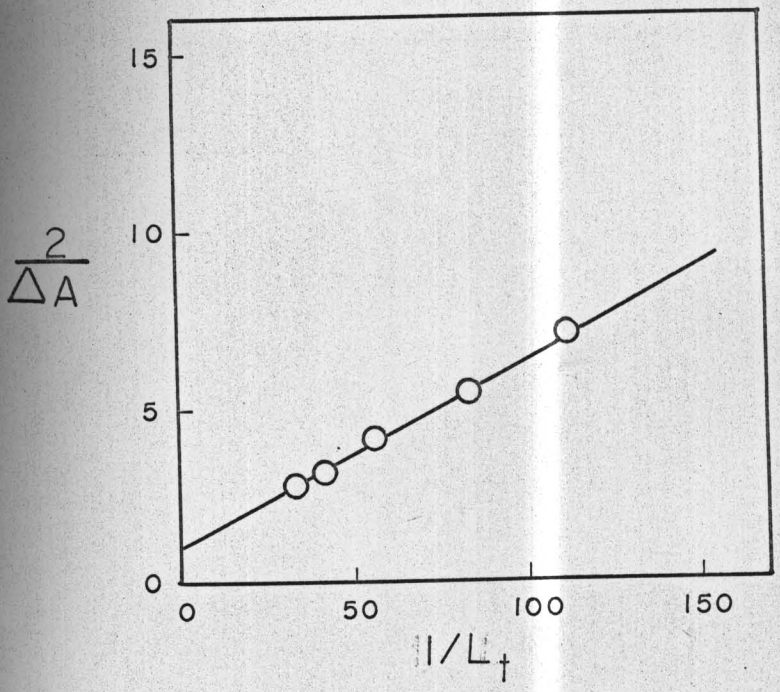


Figure 6. Spectral plot of methyl cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 0.81%(w/w) dioxane; k_{11} = 19.244 M^{-1} (data given in Table VII).

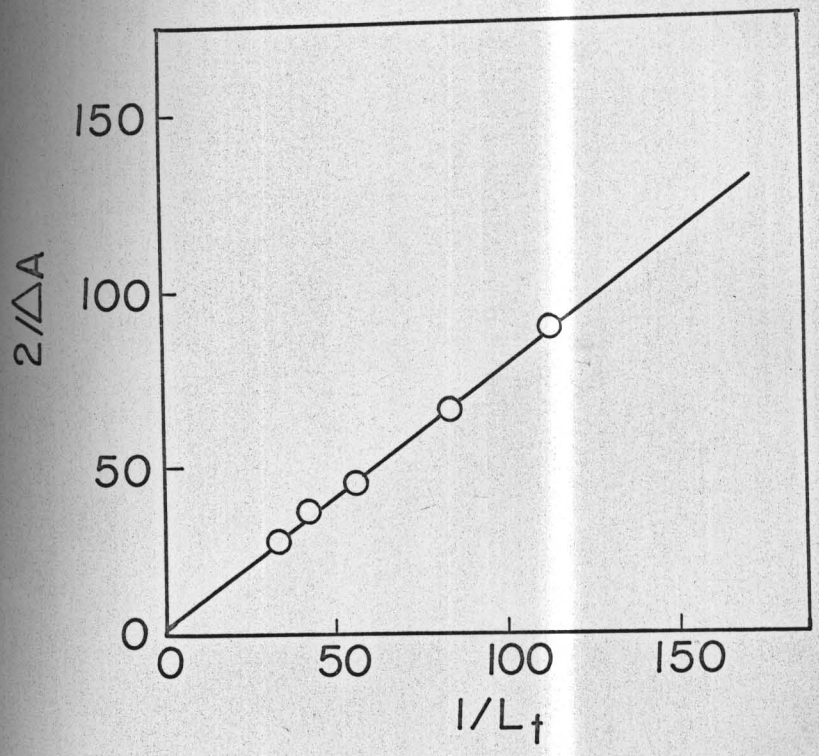


Figure 7. Spectral plot of methyl cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 40.47%(w/w) dioxane; K_{11} = 5.29 M^{-1} (data given in Table VII).

TABLE VIII

Stability Constants, Standard Unitary Free Energy Changes and Surface Tensions for Methyl Cinnamate-Theophylline System in Dioxane-H_2O Mixtures[a]

Dioxane %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^\circ/N$ (cal/molecule)	$\gamma_{25.0^\circ}$ (dynes/cm)
0.81	55.142	0.9976	19.2	6.9	70.2
3.36	54.016	1.0000	15.1	6.6	64.9
5.90	52.896	1.0023	14.2	6.5	62.1
10.94	50.670	1.0067	10.1	6.1	58.2
20.90	46.273	1.0220	8.4	5.9	51.0
40.47	37.632	1.0288	5.3	5.2	43.0

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

Table VIII; these are consistent with the values by Hovorka, Schaefer and Dreisbach (34).

(d) Ethylene glycol-water mixed solvents. As in the other mixed solvents studied earlier, the stability constants decreased as the concentration of organic solvent increased. The spectral data and the spectral reciprocal plots are shown in Table IX and in Figures 8 through 10, respectively.

The stability constants, the standard unitary free energy change and the surface tension are shown in Table X. Densities of the ethylene glycol-water mixtures were measured at 25.0°; they are consistent with the values reported by Fogg, Hixson and Thompson (37), Gallant (38), and Spangler and Davies (39). Surface tensions of the ethylene glycol-water mixtures at 25.0° were obtained from Gallant (38).

(e) DMSO-water mixed solvents. The stability constants were measured at 0.87, 3.58, 11.60, 22.02 and 32.12%(w/w) DMSO in aqueous phosphate buffer (μ = 0.3, pH = 6.6) at 25.0°. Some representative spectral data for these systems are shown in Table XI and the spectral reciprocal plots are shown in Figure 11. The stability constants, the standard unitary free energy changes and the surface tensions are shown in Table XII.

TABLE IX

Absorbance of Methyl Cinnamate in the Presence of Theophylline in Aqueous Ethylene Glycol Solutions[a]

Theophylline concentration ($M \times 10^3$)	A_{320} %(w/w) ethylene glycol			
	0.88[b]	6.35[c]	11.75[d]	17.09[c]
0	0.248	0.538	0.498	0.601
7.74	-	0.735	0.647	0.762
9.32	0.349	0.771	0.682	0.788
11.91	0.367	0.816	0.708	0.821
15.48	0.395	0.886	0.763	0.898
21.43	0.438	0.990	0.848	0.982
29.75	0.477	1.092	0.939	1.082

[a] 25.0°, pH 6.6 phosphate buffer, $\mu = 0.3$, 2 cm cell.

[b] ester conc. = 3.71×10^{-4} M.

[c] ester conc. = 8.10×10^{-4} M.

[d] ester conc. = 6.94×10^{-4} M.

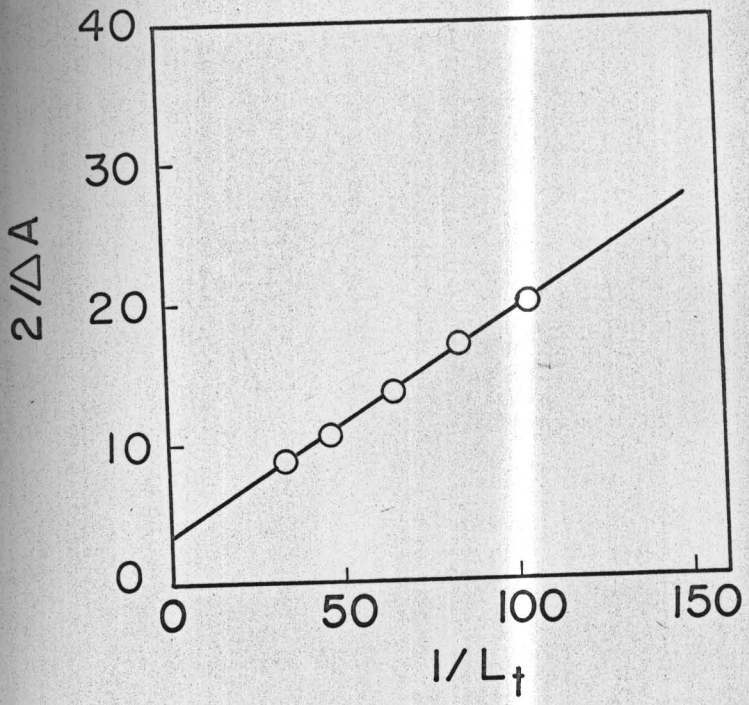


Figure 8. Spectral plot of methyl cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3; 0.88%(w/w) ethylene glycol; K_{11} = 21.20 M^{-1} (data given in Table IX).

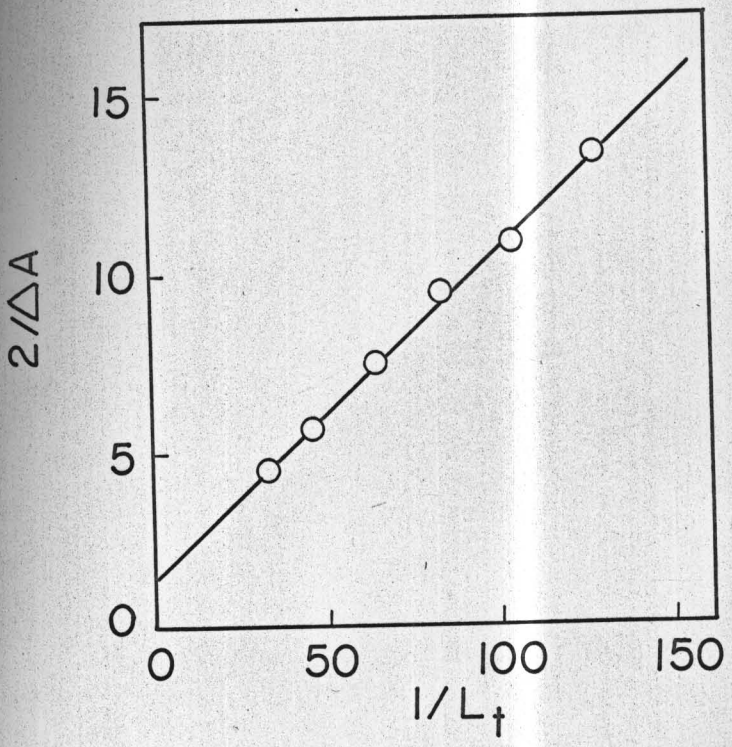


Figure 9. Spectral plot of methyl cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3; 11.75%(w/w) ethylene glycol; K_{11} = 15.41 M^{-1} (data given in Table IX).

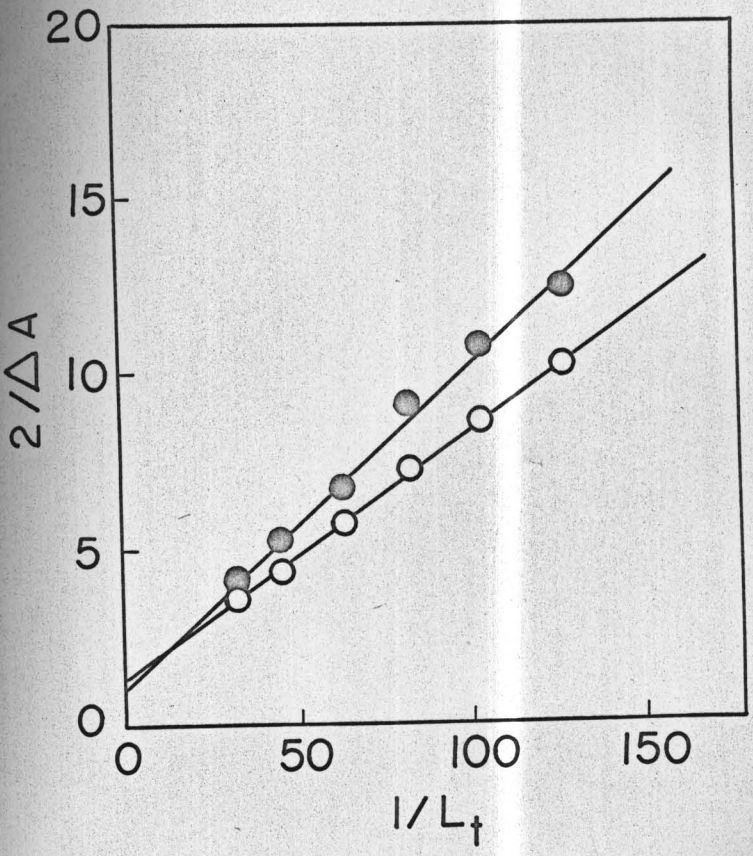


Figure 10. Spectral plot of methyl cinnamate-theophylline system; 25.0°, pH 6.6 phosphate buffer, μ = 0.3;
bottom line: 6.35%(w/w) ethylene glycol,
K_{11} = 17.86 M^{-1};
top line: 17.09%(w/w) ethylene glycol,
K_{11} = 12.26 M^{-1} (data given in Table IX).

TABLE X

Stability Constants, Standard Unitary Free Energy Changes and Surface Tensions for Methyl Cinnamate-Theophylline System in Aqueous Ethylene Glycol Solutions[a]

Ethylene glycol %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^\circ/N$ (cal/molecule)	$\gamma_{25.0^\circ}$[b] (dynes/cm)
0.88	55.160	0.9976	22.5	7.0	71.2
0.88	55.160	0.9976	21.2	6.9	71.2
3.53	54.116	1.0010	19.1	6.8	70.0
6.35	53.005	1.0047	17.9	6.8	68.8
8.97	51.972	1.0080	17.1	6.7	67.8
8.97	51.972	1.0080	14.4	6.5	67.8
11.75	50.877	1.0117	15.4	6.6	66.6
17.09	48.773	1.0188	12.3	6.3	64.7
22.33	46.710	1.0256	11.1	6.2	62.8

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

[b] From Gallant (38).

TABLE XI

Absorbance of Methyl Cinnamate in the Presence of Theophylline in DMSO-Water Mixed Solvents[a]

Theophylline concentration ($M \times 10^3$)	A_{320} %(w/w) DMSO		
	11.60	22.02	32.12
0	0.740	0.828	0.846
7.74	-	0.960	-
9.52	0.954	1.005	0.975
11.91	1.019	1.030	1.004
15.48	1.080	1.082	1.038
21.43	1.172	1.155	1.097
29.75	1.302	1.260	1.165

[a] 25.0°, pH 3.0 phosphate buffer, μ = 0.15, 2 cm cell, ester conc. = 1.02×10^{-3} M.

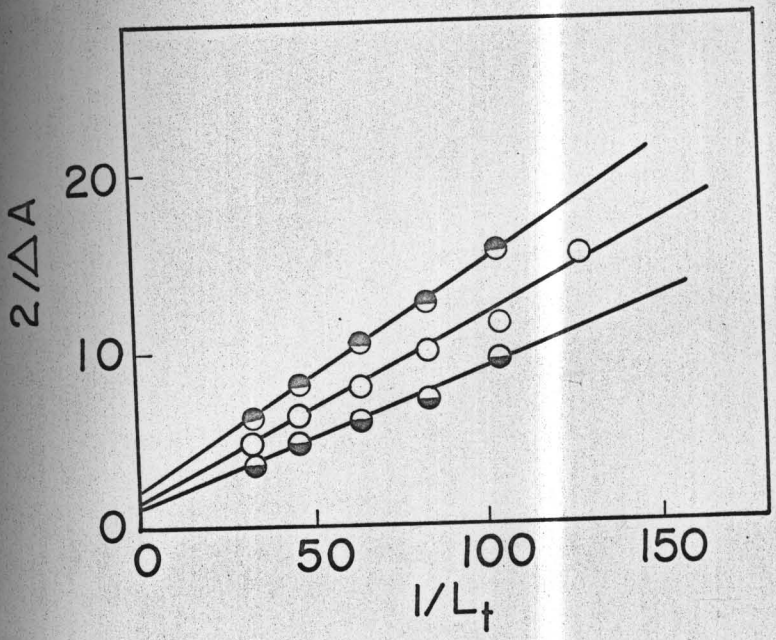


Figure 11. Spectral plot of methyl cinnamate-theophylline system; 25.0°, pH 5.0 phosphate buffer, μ = 0.15; from bottom to top:
11.60%(w/w) DMSO, K_{11} = 13.14 M^{-1};
22.02%(w/w) DMSO, K_{11} = 12.02 M^{-1};
32.12%(w/w) DMSO, K_{11} = 15.14 M^{-1};
(data given in Table XI).

TABLE XII

Stability Constants, Standard Unitary Free Energy Changes and Surface Tensions for Methyl Cinnamate-Theophylline System in DMSO-Water Mixed Solvent[a]

DMSO %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^{\circ}/N$ (cal/molecule)	$\gamma_{25.0^{\circ}}$ (dynes/cm)
0.87[b]	55.128	0.9976	19.3	6.9	71.5
3.58[b]	53.971	1.0010	17.2	6.7	70.4
11.60[c]	50.553	1.0116	13.1	6.4	67.5
22.02[c]	46.102	1.0263	12.0	6.2	64.4
32.12[c]	41.789	1.0420	15.1	6.4	61.8

[a] 25.0°.

[b] pH 6.6 phosphate buffer, μ = 0.3.

[c] pH 5.0 phosphate buffer, μ = 0.15.

Densities for the DMSO-water mixtures were measured at 25.0°. They are consistent with those values given by Cowie and Toporowski (40). Surface tensions for the DMSO-water mixtures were measured at 25.0° and are consistent with those values given by Tommila and Pajunen (41).

(f) Other solvents. Since the stability constants decrease with the decrease of the surface tensions of the mixed solvents, studies of the system in solvents which had higher surface tensions than that of water were carried out. Aqueous sodium chloride or lithium chloride solutions have higher surface tensions than water, therefore stability constants were measured in 4.94, 10.0, 12.99 and 15.98%(w/w) LiCl aqueous solutions at 25.0°. The spectral data are shown in Table XIII and the spectral reciprocal plots in Figure 12. Stability constants also were measured in 4.76 and 13.04%(w/w) NaCl aqueous phosphate buffer solution (μ = 0.3, pH 6.6) at 25.0°C. The spectral data are shown in Table XIV and the spectral reciprocal plots in Figures 13 and 14. Densities of aqueous lithium chloride and sodium chloride solution were measured at 25.0° and are shown in Table XVI.

Aqueous glycerine solution has little change in surface tension from that of pure water; therefore, the

TABLE XIII

Absorbance of Methyl Cinnamate in the Presence of Theophylline in Aqueous LiCl Solution[a]

Theophylline concentration ($M \times 10^3$)	A_{320} %(w/w) LiCl		
	4.94	12.99	15.98
0	0.330	0.300	0.335
9.52	0.484	0.565	0.644
11.91	0.502	0.630	0.677
15.48	0.565	0.705	0.713
21.43	0.606	-	0.850
29.75	0.658	0.870	0.948

[a] 25.0°C, aqueous lithium chloride solution, 0.62%(w/w) CH_3CN, 2 cm cell, ester conc. = 4.36×10^{-4} M.

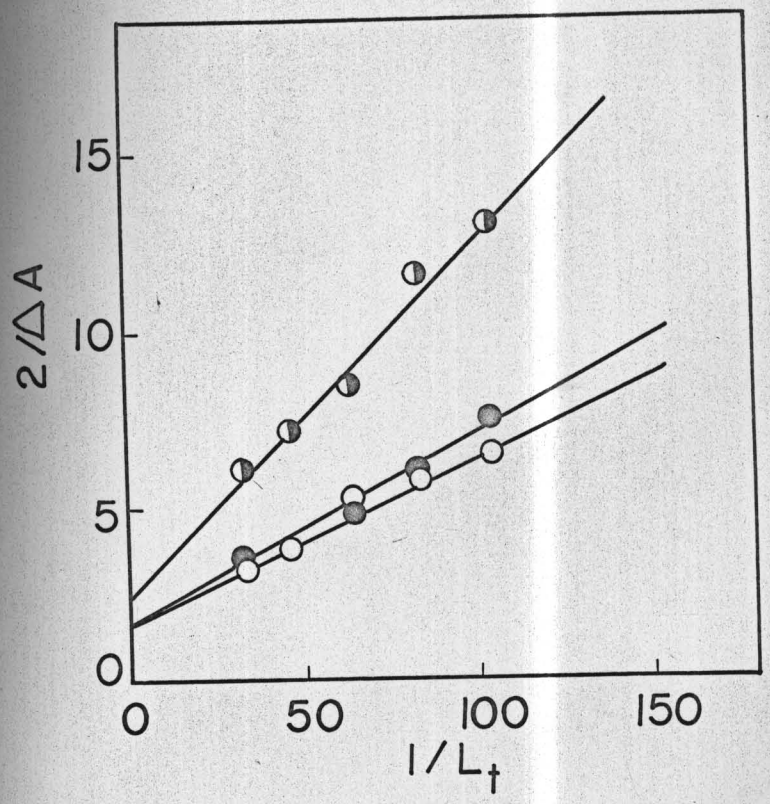


Figure 12. Spectral plot of methyl cinnamate-theophylline system; 25.0°, aqueous lithium chloride solution, 0.62%(w/w) CH_3CN (data given in Table XIII); the lines from top to bottom are for the following %(w/w) of LiCl: 4.94, 12.99 and 15.98%(w/w) LiCl.

TABLE XIV

Absorbance of Methyl Cinnamate in the Presence of Theophylline in Aqueous NaCl Solutions[a]

Theophylline concentration ($M \times 10^3$)	A_{320} %(w/w) NaCl	
	4.76[b]	13.04[c]
0	0.445	0.520
9.52	0.711	0.762
11.91	0.758	0.808
15.48	0.819	0.861
21.43	0.929	0.964
29.75	1.044	1.038

[a] 25.0°, pH 6.6 phosphate buffer ($\mu = 0.3$), 2 cm cell, 0.62%(w/w) MeOH.

[b] ester conc. = 6.79×10^{-4} M.

[c] ester conc. = 5.89×10^{-4} M.

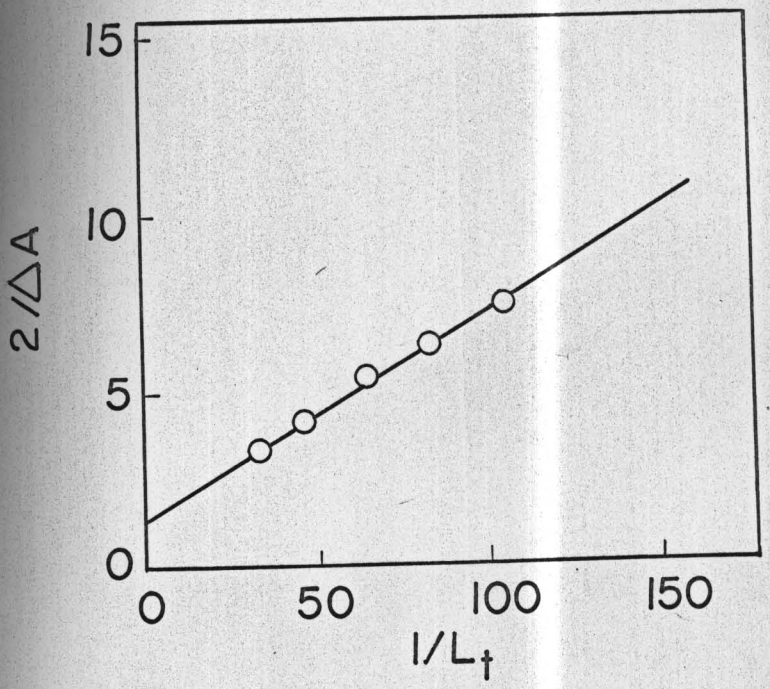


Figure 13. Spectral plot of methyl cinnamate-theophylline system; 25.0°, phosphate buffer solution (μ = 0.3, pH = 6.6), 0.62%(w/w) MeOH; 4.76%(w/w) NaCl; K_{11} = 22.56 M^{-1} (data given in Table XIV).

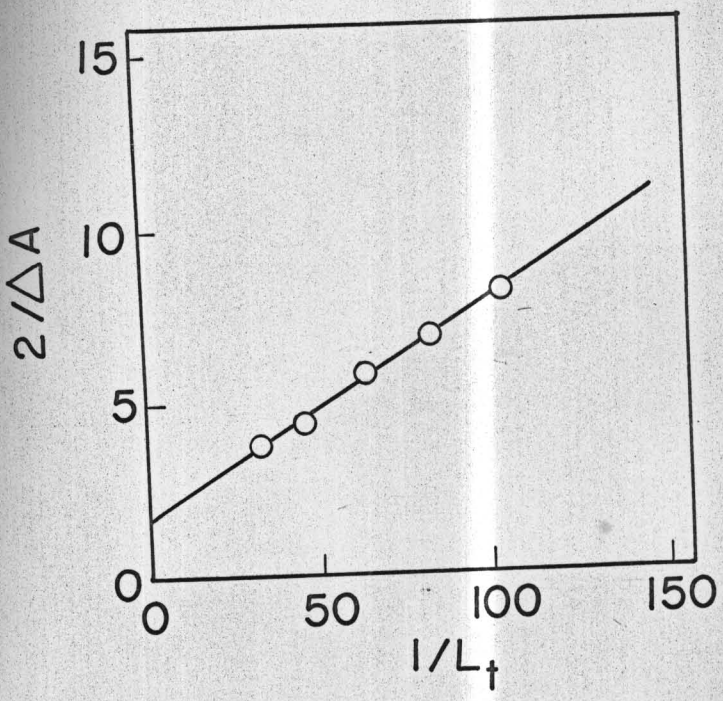


Figure 14. Spectral plot of methyl cinnamate-theophylline system; 25.0°, 0.62%(w/w) MeOH, aqueous phosphate buffer solution (μ = 0.3, pH = 6.6), 13.04%(w/w) NaCl; k_{11} = 27.45 M^{-1} (data given in Table XIV).

stability constant was measured at 10.1%(w/w) glycerine-aqueous phosphate buffer solution (pH = 6.6, μ = 0.3) at 25.0°, and it was found that it was almost the same as that in pure water. The spectral data and spectral plot are shown in Table XV and Figure 15. The stability constants, the standard unitary free energy changes and the surface tensions for methyl cinnamate-theophylline system in aqueous lithium chloride, sodium chloride and glycerine solutions are shown in Table XVI.

The plot of the stability constants vs. various percentage (w/w) of organic or inorganic solvent is shown in Figure 16, in which the CH_3CN, MeOH, dioxane, ethylene glycol, glycerine, NaCl and LiCl solvents are seen to generate separate smooth curves, while the DMSO solvent produces some scattering of points. The reason for dispersion in DMSO solvents is not known. If we plot the stability constants vs. the surface tensions of the reaction solvents, the points for all the solvent systems fit on a single smooth curve; this is shown in Figure 17.

It can be seen from the above studies for the methyl cinnamate-theophylline system in various solvents, that a correlation between the complex stability (the standard unitary free energy changes) and the surface tension is found for all the solvents studied. If we plot the standard unitary free energy changes vs. the

TABLE XV

Absorbance of Methyl Cinnamate in the Presence of Theophylline in Aqueous Glycerine Solution[a]

Theophylline concentration ($M \times 10^3$)	A_{320}
0	0.710
7.74	0.950
9.52	0.969
11.91	1.014
15.48	1.130
21.43	1.206
29.75	1.328

[a] 25.0°, pH 6.6 phosphate buffer, $\mu = 0.3$, 0.62%(w/w) MeOH, 10.1%(w/w) glycerine, 2 cm cell, ester conc. = 1.01×10^{-3} M.

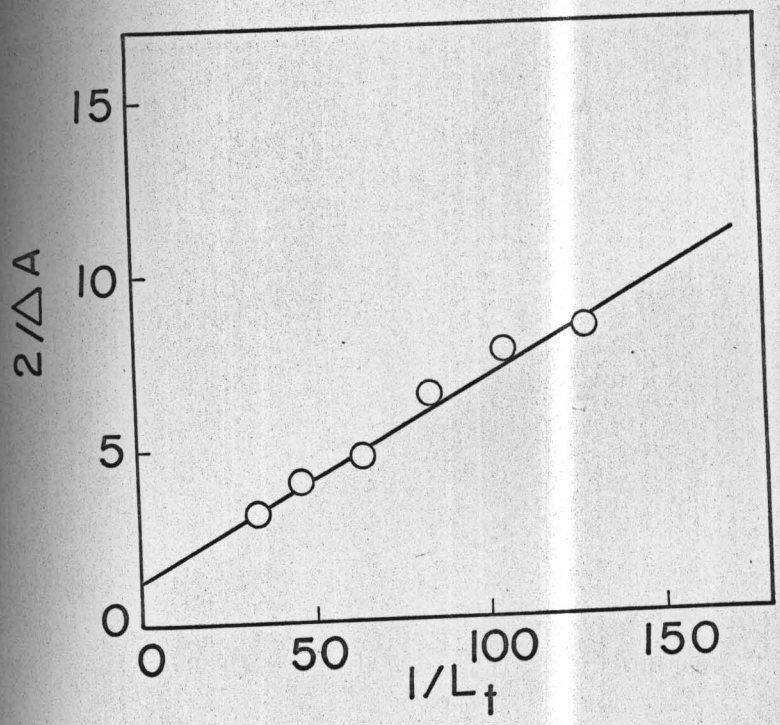


Figure 15. Spectral plot of methyl cinnamate-theophylline system; 25.0°, 0.62%(w/w) MeOH, 10.1%(w/w) glycerine-aqueous phosphate buffer solution (pH = 6.6, μ = 0.3); K_{11} = 22.53 M^{-1} (data given in Table XV).

TABLE XVI

Stability Constants, Standard Unitary Free Energy Changes and Surface Tensions for Methyl Cinnamate-Theophylline System

	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^\circ/N$ (cal/molecule)	$\gamma_{25.0^\circ}$ (dynes/cm)
4.94 %(w/w) LiCl[a]	52.758	1.0252	27.1	7.2	74.0[c]
10.0	49.950	1.0541	28.2	7.2	76.2[c]
12.99	48.291	1.0706	30.9	7.3	77.6[c]
15.98	46.631	1.0879	34.6	7.3	79.0[c]
4.76 %(w/w) NaCl[b]	52.864	1.0320	22.6	7.0	73.5[d]
13.04	48.268	1.0919	27.5	7.2	76.6[d]
10.1 %(w/w) glycerine[b]	51.098	1.0220[e]	22.5	6.9	71.5[e]

[a] 25.0°, 0.62%(w/w) CH_3CN.

[b] 25.0°, pH 6.6 phosphate buffer, μ = 0.3, 0.62%(w/w) MeOH.

[c] lit. (52).

[d] lit. (43).

[e] lit. (44).

Figure 16. Stability constants *vs.* %(w/w) organic or inorganic solvents for methyl cinnamate-theophylline system; 25.0°C.

Data given in Table

IV for CH_3CN ..... ○

VI for MeOH ..... ⊖

VIII for dioxane ..... ◑

X for ethylene glycol.. ⊗

XII for DMSO ..... ⊖

XVI for LiCl ..... △

NaCl ..... □

glycerine ..... ⬯

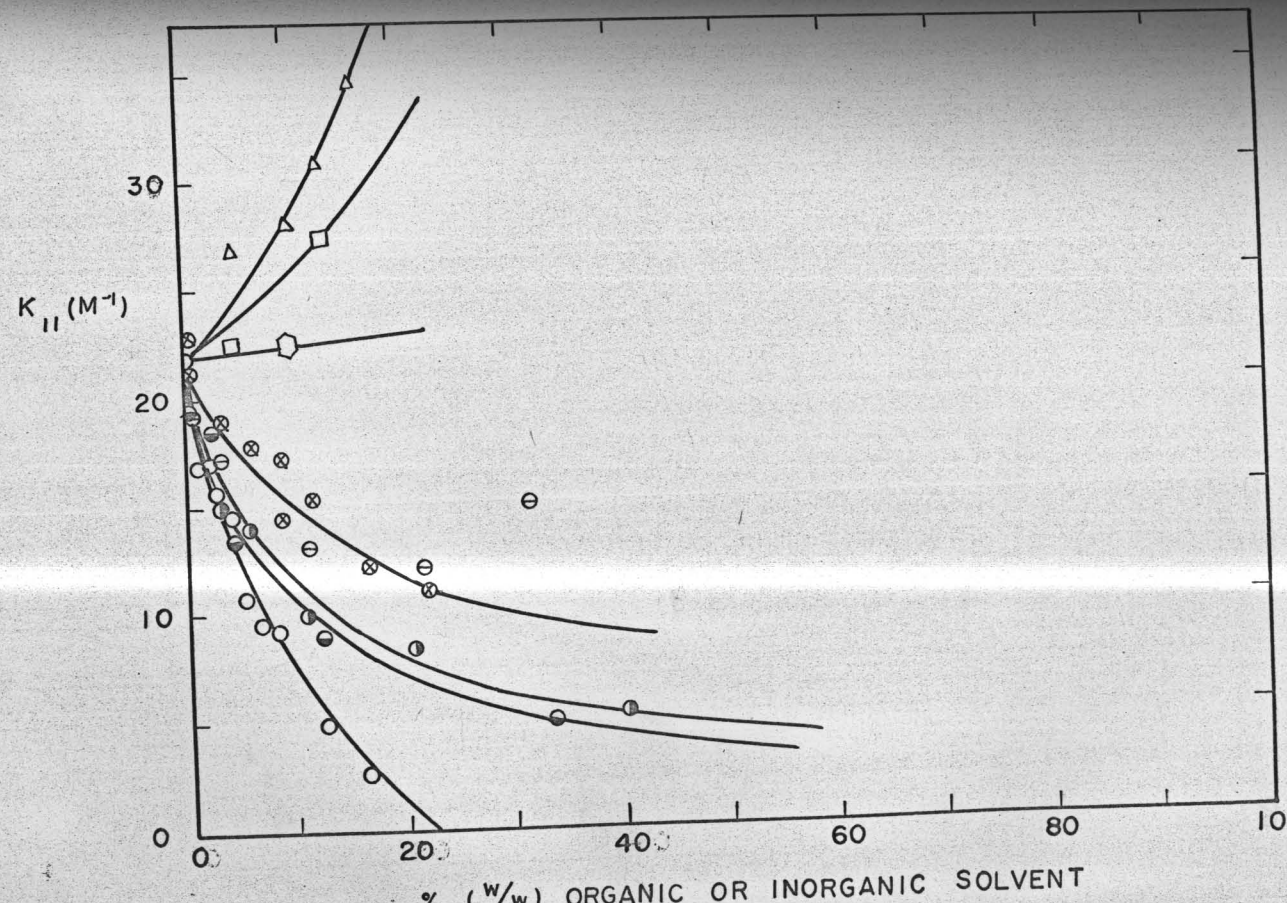

K_{11} (M^{-1})
30
20
10
0
0
20
40
60
80
100
% ($^{w}/_{w}$) ORGANIC OR INORGANIC SOLVENT

Figure 17. Stability constants *vs.* surface tensions for methyl cinnamate-theophylline system; 25.0°, data given in Table:

IV for CH_3CN

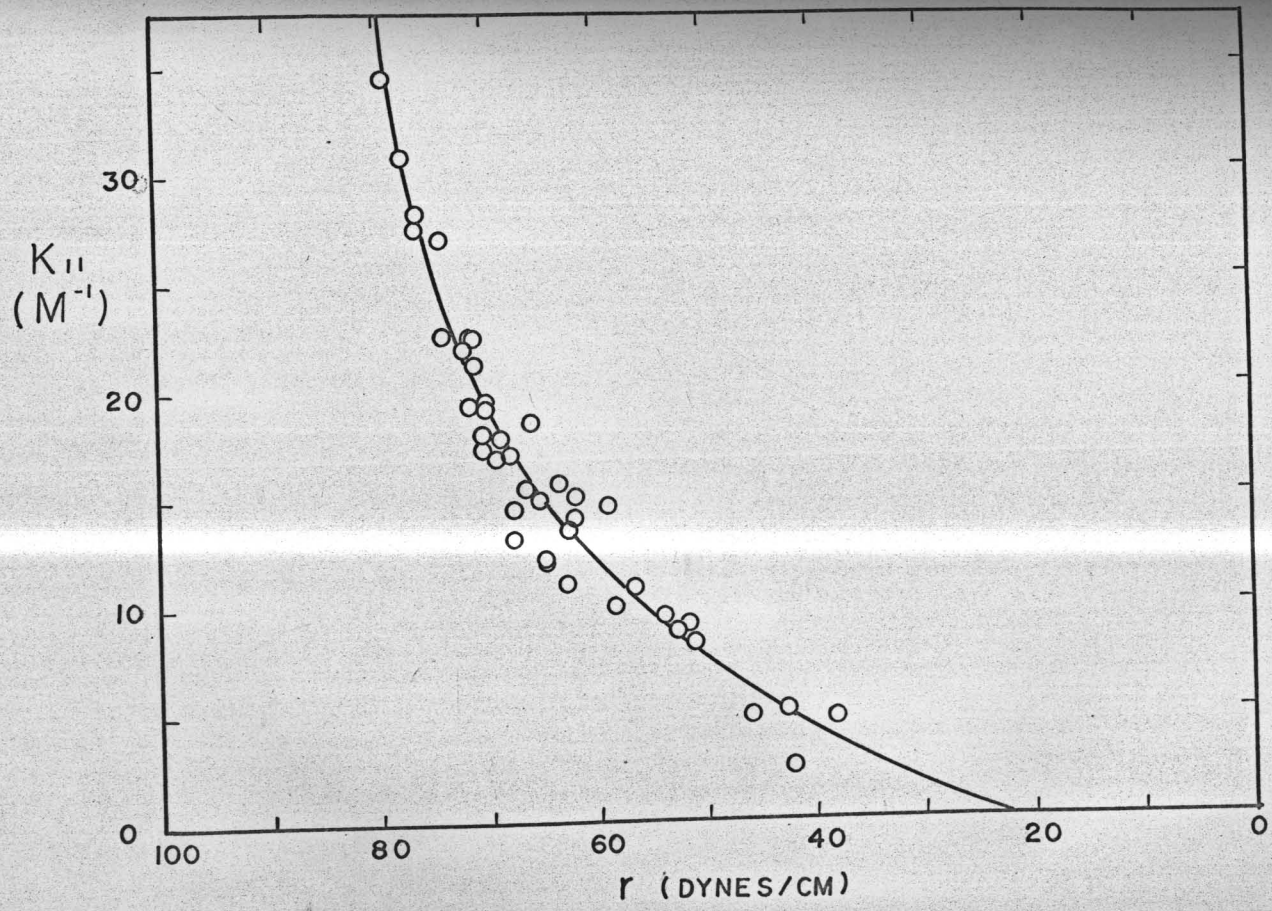
VI for MeOH

VIII for dioxane

X for ethylene glycol

XII for DMSO

XVI for LiCl
NaCl
glycerine


K_{II}
(M^{-1})
30
20
10
0
100
80
60
40
20
0
Γ (DYNES/CM)

surface tensions for various solvents, which are shown in Figure 18, a reasonably straight line is obtained; that is, the standard unitary free energy change appears to be a linear function of the surface tension of the reaction solvent.

B. Methyl Cinnamate-Theophylline Anion System

The stability constant for the system between methyl cinnamate and theophylline anion (hereafter called theophyllinate) had been studied elsewhere (4,12). Theophyllinate gives a much smaller stability constant with methyl cinnamate than does theophylline. Mixed solvent studies were carried out by the spectral technique to compare the solvent effects on neutral and anionic complexes. The stability constants were measured in the mixed solvents of CH_3CN- and MeOH-aqueous bicarbonate buffer solutions (pH = 10.7, μ = 0.3) at 25.0°. The spectral data and the spectral reciprocal plots are shown in Tables XVII and XVIII and Figures 19 and 20, respectively.

Table XIX shows the stability constants, densities, M* values, the standard unitary free energy changes, and the surface tensions for methyl cinnamate-theophyllinate system.

Since, from the studies for the methyl cinnamate-theophylline system described earlier, a correlation

Figure 18. Standard unitary free energy changes vs. surface tensions for methyl cinnamate-theophylline system; 25.0°; data given in Table:

IV for CH_3CN

VI for MeOH

VIII for dioxane

X for ethylene glycol

XII for DMSO

XVI for LiCl
NaCl
glycerine

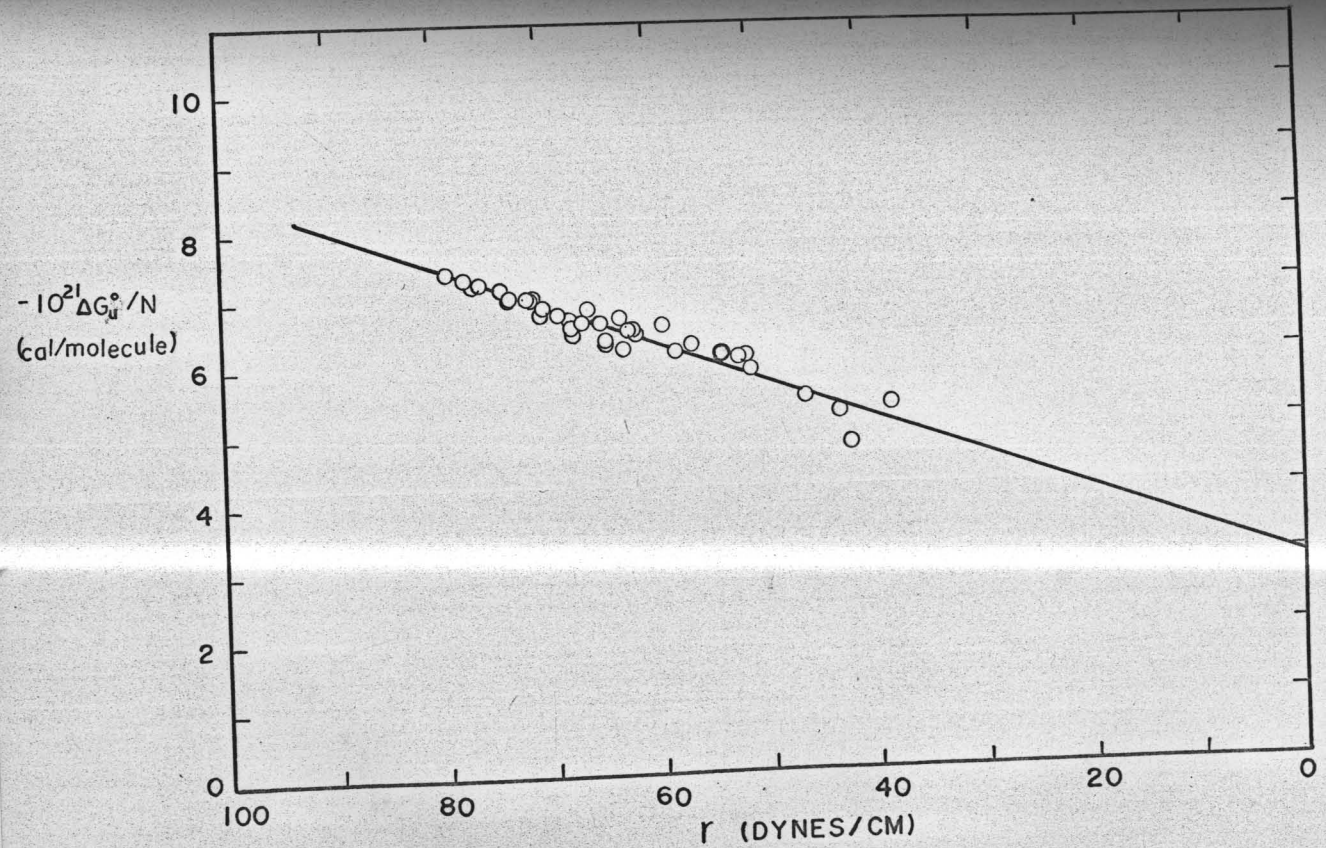

10
8
6
4
2
0
$-10^{21}\Delta G_u^\circ/N$
(cal/molecule)
100
80
60
40
20
0
r (DYNES/CM)

TABLE XVII

Absorbance of Methyl Cinnamate in the Presence of Theophyllinate in Aqueous Acetonitrile Solutions[a]

Theophyllinate concentration ($M \times 10^2$)	A_{320} %(w/w) CH_3CN			
	0.62	4.52	8.48	12.50
0	0.320	0.350	0.342	0.316
2.32	0.584	-	0.471	0.404
2.86	0.610	0.576	-	0.428
3.57	0.675	0.616	0.538	0.449
4.64	0.731	0.680	0.598	0.479
6.43	0.844	0.770	0.654	0.553
8.93	0.934	0.892	0.755	0.626

[a] 25.0°, pH 10.7 bicarbonate buffer, $\mu = 0.3$; 2 cm cell, ester conc. = 5.06×10^{-4} M.

TABLE XVIII

Absorbance of Methyl Cinnamate in the Presence of Theophyllinate in Aqueous Methanol Solutions[a]

Theophyllinate concentration ($M \times 10^2$)	A_{320} %(w/w) MeOH			
	4.58	8.58	16.74	25.15
0	0.335	0.342	0.355	0.332
2.32	0.554	0.538	-	-
2.86	0.604	0.583	0.505	0.442
3.57	0.647	0.631	0.542	0.477
4.64	0.708	0.685	0.593	0.513
6.43	0.831	0.783	0.654	0.566
8.93	0.925	0.885	0.745	0.643

[a] 25.0°, pH 10.7 bicarbonate buffer, μ = 0.3, 2 cm cell, ester conc. = 5.08×10^{-4} M.

Figure 19. Spectral plots of methyl cinnamate-theophyllinate system; 25.0°, pH 10.7 bicarbonate buffer, μ = 0.3; the lines from top to bottom are for the following percentages of CH_3CN:

12.50 %(w/w); K_{11} = 1.55 M^{-1}

8.48 %(w/w); K_{11} = 3.76 M^{-1}

4.52 %(w/w); K_{11} = 5.69 M^{-1}

0.62 %(w/w); K_{11} = 14.66 M^{-1}

(data given in Table XVII).

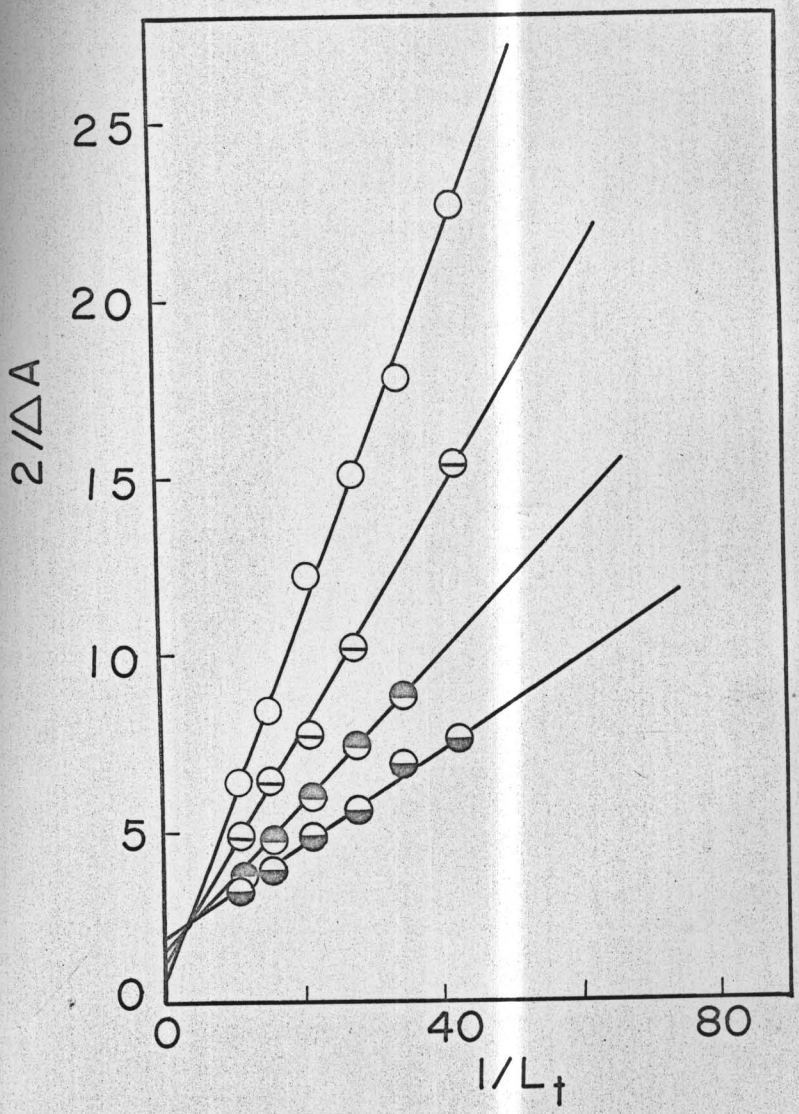

25
20
15
10
5
0
2/ΔA
0
40
80
1/L_t

Figure 20. Spectral plots of methyl cinnamate-theophyllinate system; 25.0°, pH 10.7 bicarbonate buffer, μ = 0.3; the lines from top to bottom are for the following percentages of MeOH:

25.15 %(w/w); k_{11} = 2.58 M^{-1}

16.74 %(w/w); k_{11} = 4.10 M^{-1}

8.58 %(w/w); k_{11} = 7.28 M^{-1}

4.58 %(w/w); k_{11} = 7.65 M^{-1}

(data given in Table XVIII).

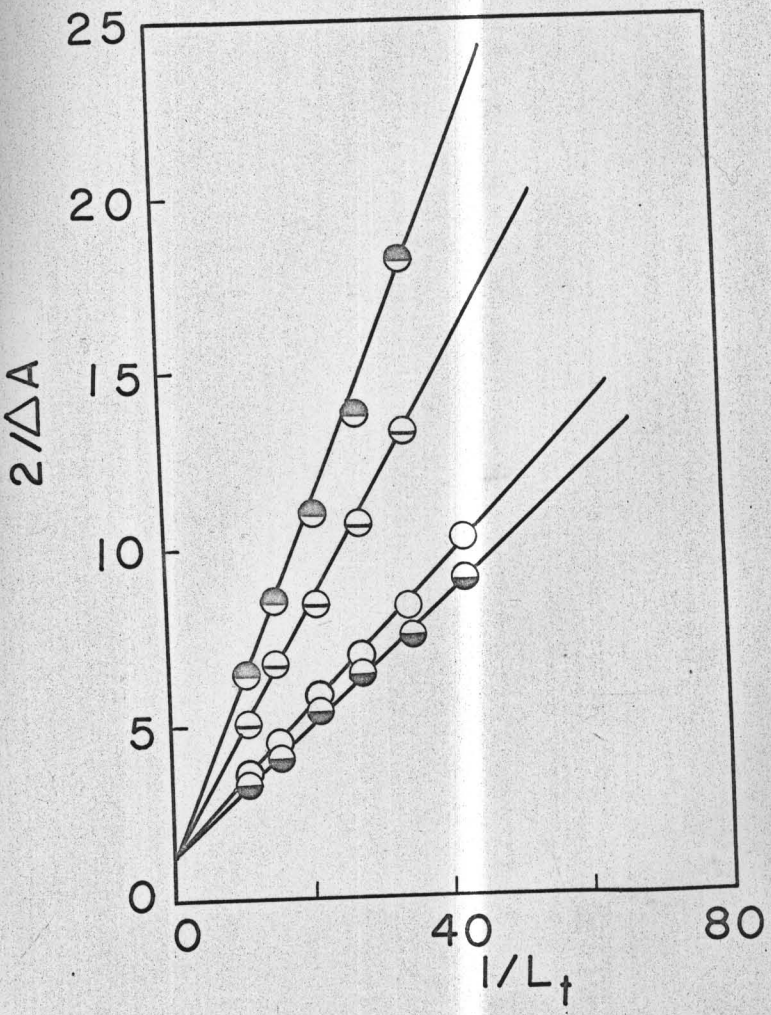

25
20
15
10
5
0
2/ΔA
0
40
80
1/L_t

TABLE XIX

Stability Constants, Standard Unitary Free Energy Changes, and Surface Tensions for Methyl Cinnamate-Theophyllinate System in Aqueous Organic Solvent Mixtures[a]

Organic solvent %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^\circ/N$ (cal/molecule)	$\gamma_{25.0^\circ}$ (dynes/cm)
0.62, CH_3CN	55.307	0.9960	14.7	6.6	71.9
4.52	54.092	0.9895	5.7	5.6	59.0
8.48	52.860	0.9828	3.8	5.2	51.4
12.50	51.608	0.9758	1.6	4.3	46.2
4.58, MeOH	54.387	0.9885	7.7	5.9	62.6
8.58	53.416	0.9820	7.3	5.9	57.0
16.74	51.440	0.9695	4.1	5.2	48.6
25.15	49.396	0.9560	2.6	4.7	42.8

[a] 25.0°, pH 10.7 bicarbonate buffer, μ = 0.3.

between the standard unitary free energy change and the surface tension was found, a similar plot for methyl cinnamate-theophyllinate is shown in Figure 21. A reasonable relationship was also obtained. The slope of this straight line is steeper and the extrapolated value $(- 10^{21}\Delta G^{\circ}_{u}/N)_{r \longrightarrow o}$ of the plot is smaller than that of methyl cinnamate-theophylline system. The interpretations of these will be discussed in the Discussion section.

C. Methyl Cinnamate-8-Chlorotheophylline Anion

Because 8-chlorotheophylline anion (8-chloro-theophyllinate) gave a large stability constant with methyl cinnamate relative to theophyllinate (11), it was decided to continue the mixed solvent studies for this system to examine the interaction between the substrate and the ligand. The stability constants were measured in the aqueous methanol and acetonitrile borate buffer solutions (pH 9.2, μ = 0.3) at 25.0° by the spectral technique. Some spectral data and spectral reciprocal plots are shown in Tables XX and XXI and Figures 22 and 23. The system was also studied at 15.5, 25.0, 40.0 and 50.0° in aqueous acetonitrile borate buffer solutions. The thermodynamic data are shown in Table XXV, in which the unitary entropy changes, ΔS_u, was calculated by

Figure 21. Plot of standard unitary free energy change *vs.* surface tension for methyl cinnamate-theophyllinate system; 25.0°, (data given in Table XIX).

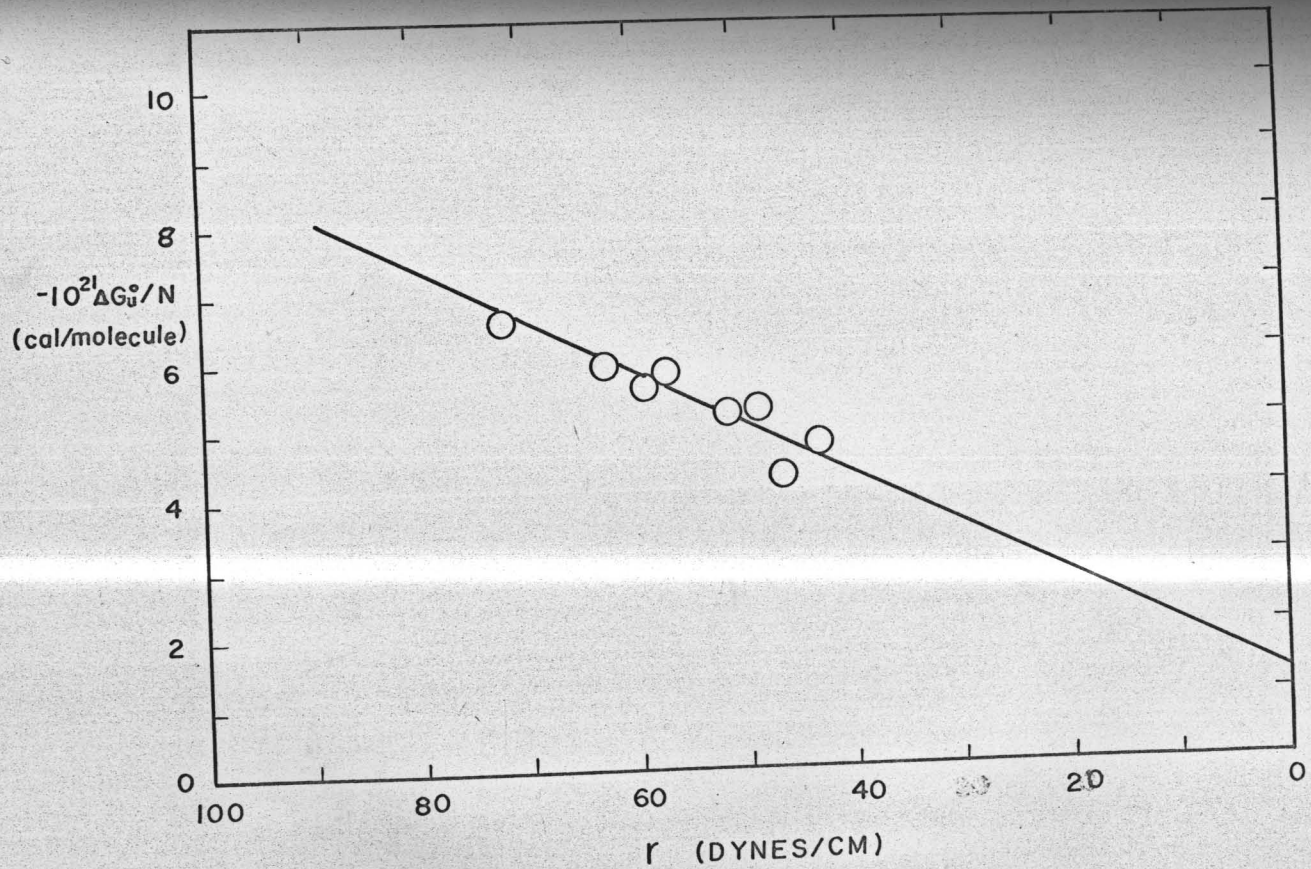

10
8
$-10^{21}\Delta G_{ii}^{\circ}/N$
(cal/molecule)
6
4
2
0
100
80
60
40
20
0
r (DYNES/CM)

TABLE XX

Absorbance of Methyl Cinnamate in the Presence of 8-Chlorotheophyllinate in Aqueous Acetonitrile Solutions[a]

8-Chlorotheophyllinate concentration ($M \times 10^2$)	A_{320} %(w/w) CH_3CN	
	4.52	8.58
0	0.250	0.248
1.59	0.393	-
2.38	-	0.388
3.18	0.494	0.424
4.76	0.576	0.491
6.35	0.637	0.532
7.94	0.691	0.584

[a] 25.0°, pH 9.2 borate buffer, $\mu = 0.2$; 1 cm cell, ester conc. = 7.38×10^{-4} M.

TABLE XXI

Absorbance of Methyl Cinnamate in the Presence of 8-Chlorotheophyllinate in MeOH-Water Mixed Solvents[a]

8-Chlorotheophyllinate concentration ($M \times 10^2$)	A_{320}					
	%(w/w) MeOH					
	4.58	8.58	12.64	16.74	25.15	33.81
0	0.250	0.238	0.242	0.259	0.243	0.212
2.06	0.463	0.436	0.408	0.400	0.340	-
2.54	0.493	0.459	0.436	0.413	0.355	0.292
3.18	0.537	0.486	0.467	0.454	0.381	0.310
4.13	0.584	0.552	0.508	0.491	0.414	0.323
5.71	0.658	0.622	0.587	0.554	0.455	0.374
7.94	0.731	0.708	0.664	0.630	0.525	0.420

[a] 25.0°, pH 9.2 borate buffer, $\mu = 0.2$; 1 cm cell; ester conc. = 7.32×10^{-4} M.

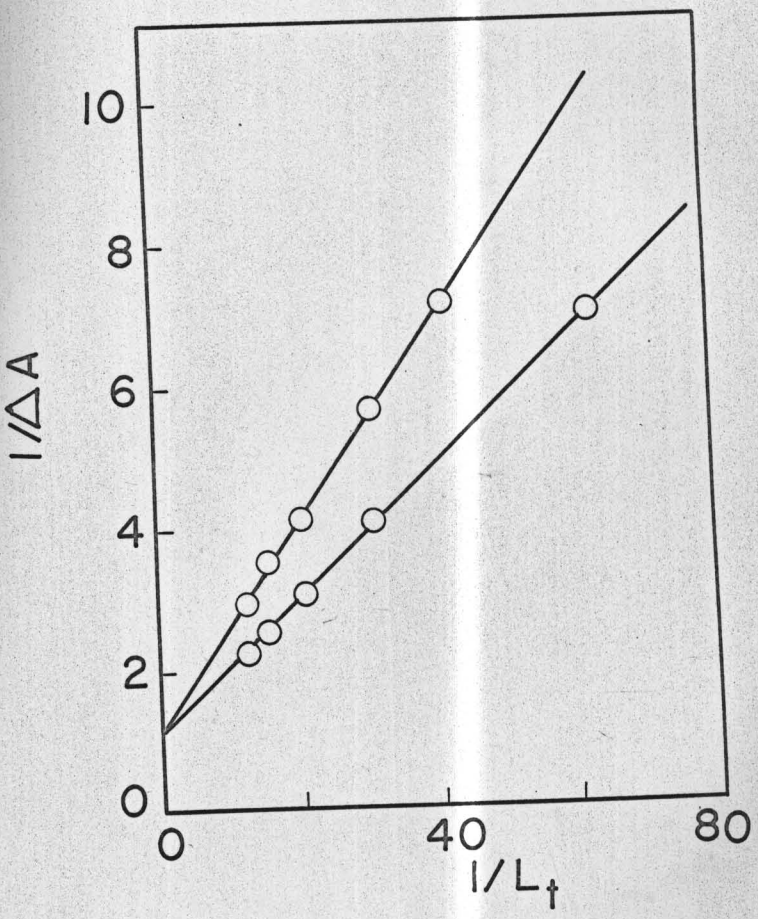


Figure 22. Spectral plot of methyl cinnamate-8-chloro-theophyllinate system; 25.0°, pH 9.2 borate buffer, μ = 0.2; (data given in Table XX). Lines from top to bottom are for 8.48 and 4.52%(w/w) CH_3CN, respectively.

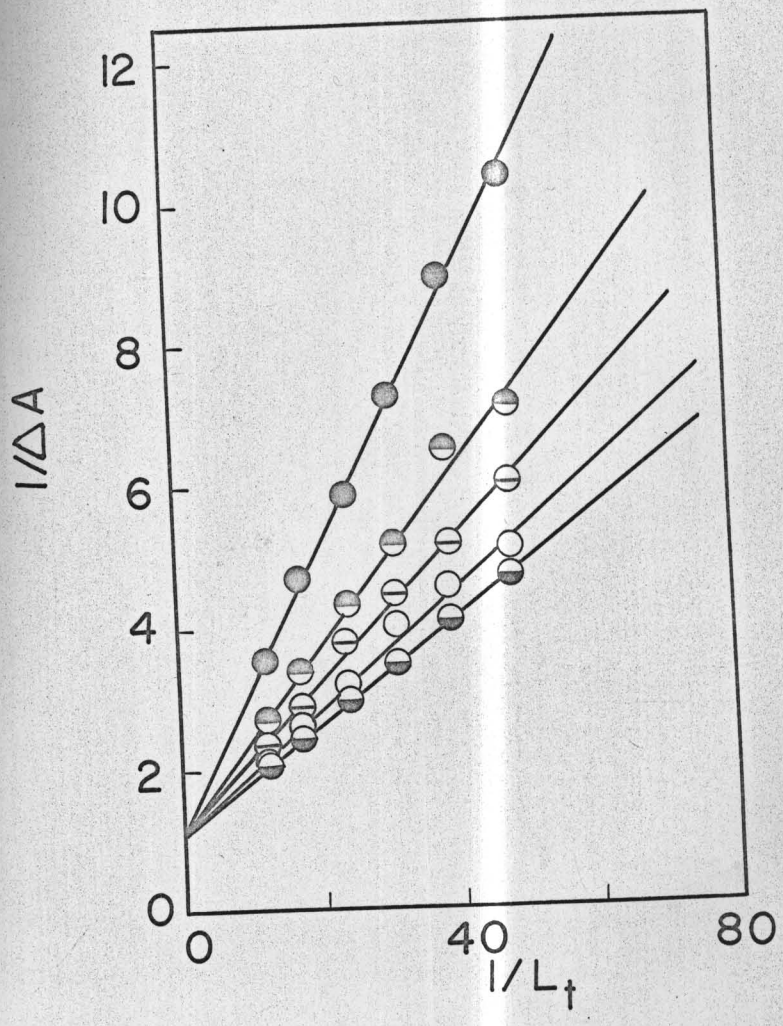


Figure 23. Spectral plots of methyl cinnamate-8-chlorotheophyllinate system; 25.0°, pH 9.2 borate buffer, μ = 0.2; (data given in Table XXI). The lines from top to bottom are for the following percentage (w/w) of MeOH: 25.15, 16.74, 12.64, 8.58 and 4.58%(w/w) MeOH.

$$\Delta Su = \Delta S + R \ln (M^* d_4^t) \quad (60)$$

where M^* is the number of moles in 1000 gm of solvent and d_4^t is the density at t°C.

A study of the methyl cinnamate-8-chlorotheophyllinate system was carried out in deuterium oxide solvent by both spectral and kinetic techniques. It was found that the stability constants for this system were nearly the same in water and in deuterium oxide solution. The stability constant in aqueous phosphate buffer solution (0.65%(w/w) CH_3CN, pH 6.6, μ = 0.3) at 25.0° was found to be 20.9 M^{-1} by the spectral technique and 22.5 M^{-1} (lit. (11), 22.0 M^{-1}) by the kinetic method. The stability constant in deuterium oxide solution (pD 9.66 deuteroxide-chloride buffer, μ = 0.2, 0.99%(v/v) CD_3CN) at 25.0° was found to be 18.7 M^{-1} by the spectral technique and 21.3 M^{-1} by the kinetic method. A q_{11} value of 1.0 was found in the deuterium oxide solution, indicating that the complex of methyl cinnamate-8-chlorotheophyllinate is essentially unreactive to the reagent.

The spectral data and the spectral reciprocal plot in the deuterium oxide solvent for this system are shown in Table XXII and Figure 24, respectively. Table XXIII presents the observed pseudo-first-order rate constants for the alkaline hydrolysis of methyl cinnamate in the presence of 8-chlorotheophyllinate in deuterium oxide

TABLE XXII

Absorbance of Methyl Cinnamate in the Presence of 8-Chlorotheophyllinate in D_2O[a]

8-Chlorotheophyllinate concentration ($M \times 10^2$)	A_{320}
0	0.128
2.57	0.326
3.09	0.351
3.86	0.378
5.15	0.428

[a] 25.0°, pD 9.66 deuteroxide-chloride buffer, μ = 0.2, 0.99%(v/v) CD_3CN; 1 cm cell.

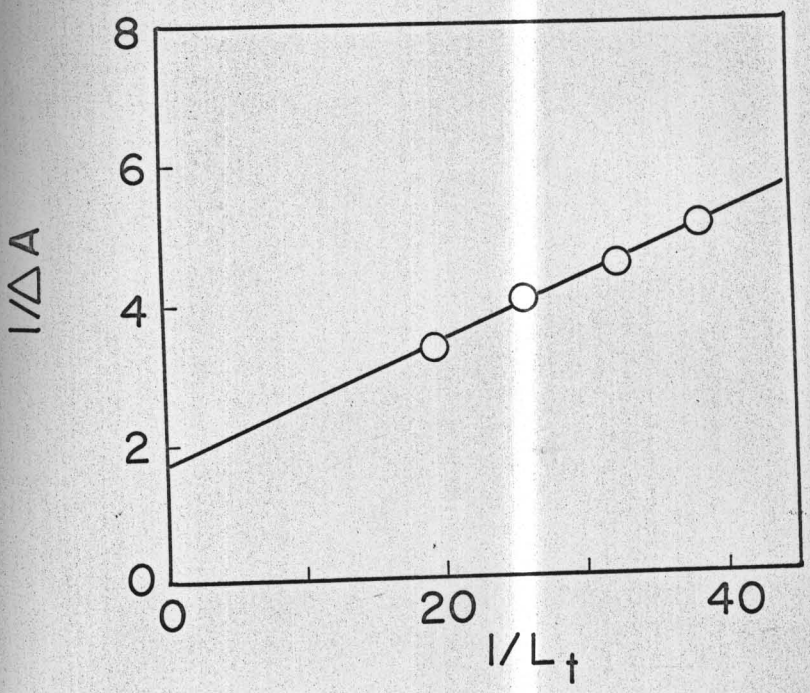


Figure 24. Spectral plot of methyl cinnamate-8-chlorotheophyllinate system; 25.0°, pD 9.66 deuteroxide-chloride buffer, μ = 0.2; 0.99%(v/v) CD_3CN; K_{11} = 18.70 M^{-1} (data given in Table XXII).

TABLE XXIII

Observed Pseudo-First-Order Rate Constants for the Alkaline Hydrolysis of Methyl Cinnamate in the Presence of 8-Chlorotheophyllinate in D_2O[a]

8-Chlorotheophyllinate (M)	$10^3 K_{obs}$ (sec^{-1})
0.0000	8.35
0.0186	6.42
0.0224	6.25
0.0276	6.08
0.0321	6.03
0.0373	5.79
0.0447	5.51
0.0693	5.16

[a] 25.0°, pD 13.57, deuteroxide-chloride buffer, μ = 0.2, 0.99%(v/v) CD_3CN.

solvent. Figures 25 and 26 show typical first-order plots from which such constants were obtained, and the kinetic reciprocal plot is shown in Figure 27.

The stability constants, standard unitary free energy changes and surface tensions are shown in Tables XXIV and XXV. The density for deuterium oxide is $d_4^{25} = 1.1044$ (45,46,52). The surface tension for deuterium oxide was measured at 25.0° and found to be 71.2 dynes/cm (lit. (45,47), 71.4 dynes/cm); the surface tension for 0.99%(v/v) CD_3CN-deuterium oxide solution was found to be 67.6 dynes/cm at 25.0°.

The plot of stability constants and standard unitary free energy changes *vs.* surface tensions are shown in Figures 28 and 29, respectively.

The second-order rate constant for the alkaline hydrolysis of methyl cinnamate in pH 12.7 hydroxide-chloride buffer (μ = 0.3, 1.64%(v/v) CH_3CN) at 25.0° was found to be 8.29×10^{-2} M^{-1} sec^{-1} (lit. (11), $8.26 \pm 0.20 \times 10^{-2}$ M^{-1} sec^{-1}), while in pD = 13.57 (uncertain) deuteroxide-chloride buffer (μ = 0.2, 0.99%(v/v) CD_3CN) at 25.0° was found to be 2.25×10^{-2} M^{-1} sec^{-1}. This indicated the reaction exhibits a kinetic deuterium solvent isotope effect with k_H/k_D of about 3.7.

Attempts were made to carry out the similar solvent study for the system between methyl cinnamate and

Figure 25. Pseudo-first-order plots for the alkaline hydrolysis of methyl cinnamate in the presence of 8-chlorotheophyllinate in D_2O, 25.0°, pD 13.57, deuteroxide-chloride buffer, μ = 0.2, 0.99%(v/v) CD_3CN; 8-chlorotheophyllinate concentration (M) bottom to top: 0.0186; 0.0321; 0.0693.

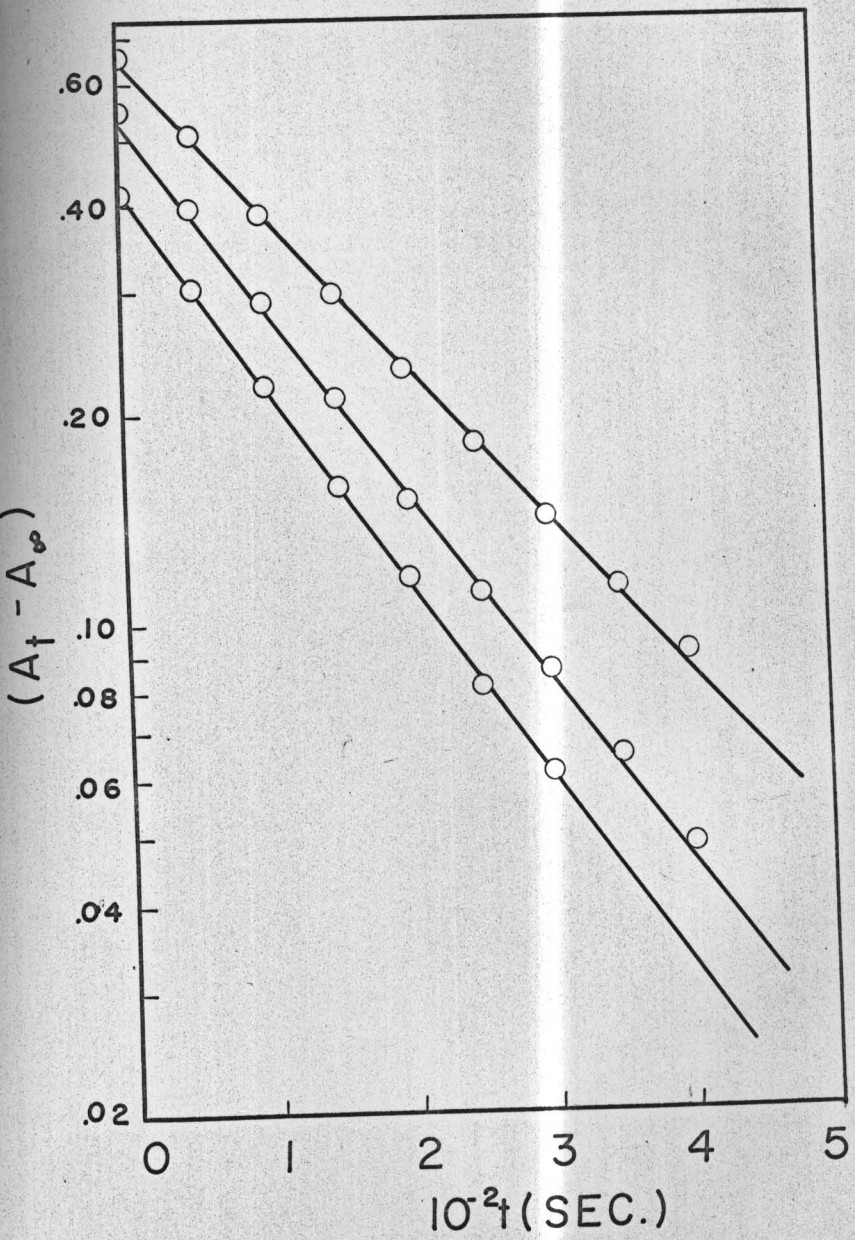
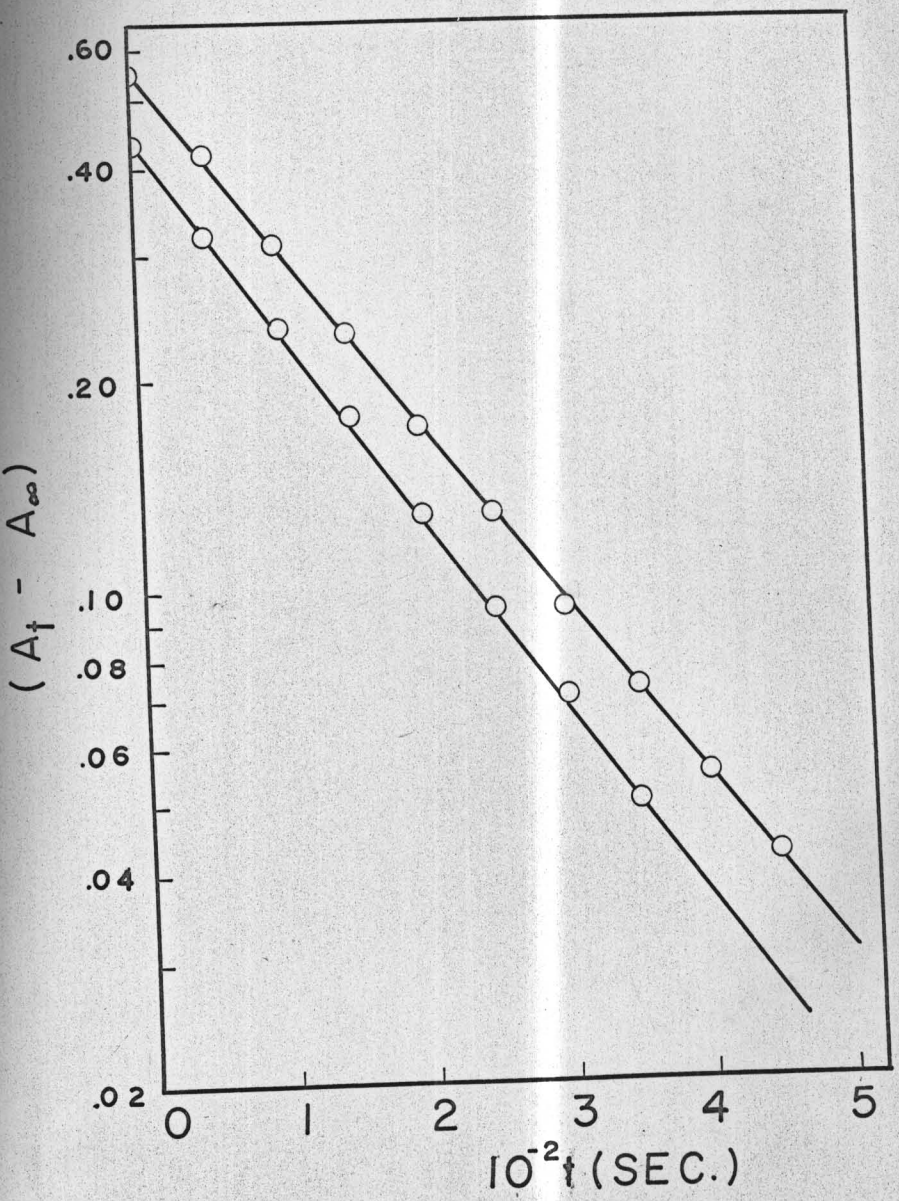

.60
.40
.20
.10
.08
.06
.04
.02
$(A_t - A_\infty)$
0
1
2
3
4
5
$10^{-2}t$ (SEC.)

Figure 26. Pseudo-first-order plots for the alkaline hydrolysis of methyl cinnamate in the presence of 8-chlorotheophyllinate in D_2O; 25.0°, pD 13.57 deuteroxide-chloride buffer, μ = 0.2, 0.99%(v/v) CD_3CN; 8-chlorotheophyllinate concentration (M) bottom to top: 0.0224; 0.0373.


.60
.40
.20
.10
.08
.06
.04
.02
$(A_t - A_\infty)$
0
1
2
3
4
5
$10^{-2}t$ (SEC.)

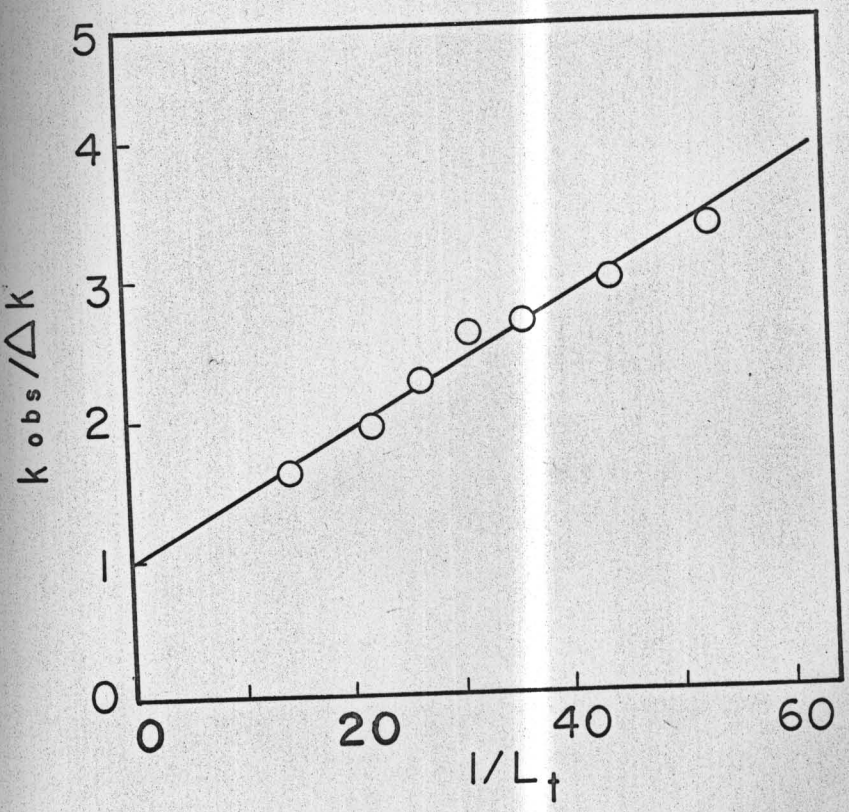


Figure 27. Kinetic plot of methyl cinnamate-8-chloro-theophyllinate system (data given in Table XXIII).

TABLE XXIV

Stability Constants, Standard Unitary Free Energy Changes and Surface Tensions for Methyl Cinnamate-8-Chlorotheophylline System in Different Solvents

Solvent	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^\circ/N$ (cal/molecule)	$\gamma_{25.0^\circ}$ (dynes/cm)
0.62%(w/w) CH_3CN[a]	55.313	0.9960	20.9	6.9	71.9
4.52	54.092	0.9884	11.5	6.3	59.0
8.48	52.860	0.9828	8.7	6.0	51.4
16.57	50.340	0.9680	2.1	4.6	42.0
4.58%(w/w) MeOH[b]	54.387	0.9885	15.5	6.6	62.6
8.58	53.416	0.9820	12.4	6.4	57.0
12.64	52.430	0.9757	10.4	6.2	52.4
16.74	51.440	0.9695	8.6	6.0	48.6
25.15	49.396	0.9560	6.2	5.6	42.8
33.81	47.287	0.9420	3.8	5.0	38.3
D_2O[c]	50.000	1.1044	18.7	6.8	67.6

[a] 25.0°, aqueous acetonitrile borate buffer (pH 9.20, μ = 0.2).

[b] 25.0°, aqueous methanol borate buffer (pH 9.20, μ = 0.2).

[c] 25.0°, pD 9.66 deuteroxide-chloride buffer, μ = 0.2; 0.99%(v/v) CD_3CN.

TABLE XXV

Thermodynamic Data for Methyl Cinnamate-8-Chlorotheophyllinate System in Aqueous Acetonitrile Solution[a]

Temperature (°C)	CH_3CN %(w/w)	K_{11} (M^{-1})	M* (moles/1000 g)	d_4^t	ΔS (e.u.)	ΔSu (e.u.)	-ΔH (cal/mole)
15.5	0.62	25.8	55.313	0.9980			
25.0	0.62	20.9	55.313	0.9960	-5	3	3300
40.0	0.61	18.4	55.313	0.9912			
50.0	0.61	13.1	55.313	0.9867			
15.5	4.55	15.8	53.086	0.9925			
25.0	4.52	11.5	53.101	0.9884	-14	-6	5700
40.0	4.46	7.1	53.134	0.9842			
50.0	4.43	5.6	53.169	0.9791			
15.5	8.51	11.6	50.984	0.9870			
25.0	8.48	8.7	51.001	0.9828			
40.0	8.36	5.9	51.064	0.9769	-17	-9	6300
50.0	8.32	3.4	51.085	0.9714			

[a]Aqueous acetonitrile borate buffer (pH 9.2, μ = 0.2).

Figure 28. Stability constant-surface tension plot for methyl cinnamate-8-chlorotheophyllinate system; 25.0° (data given in Table XXIV).

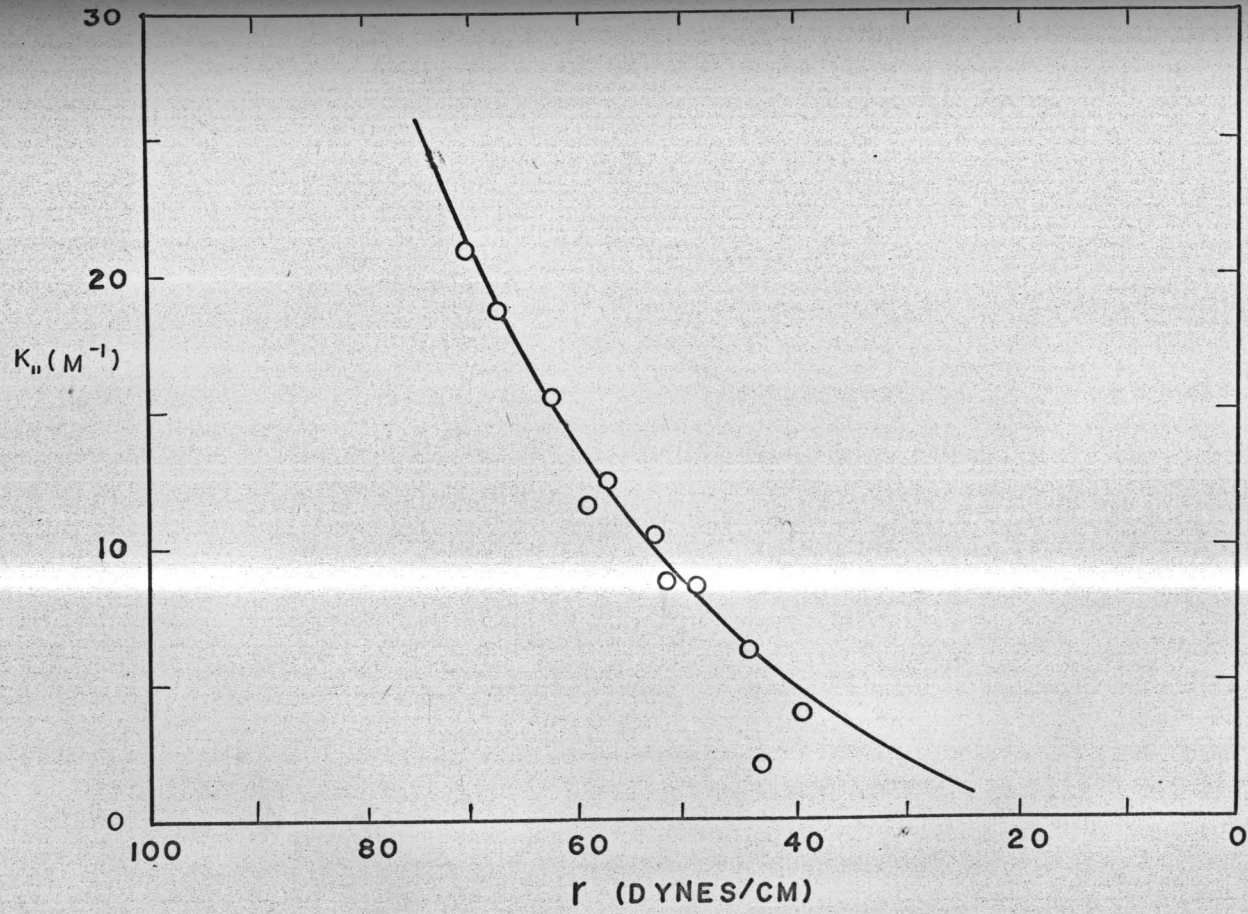
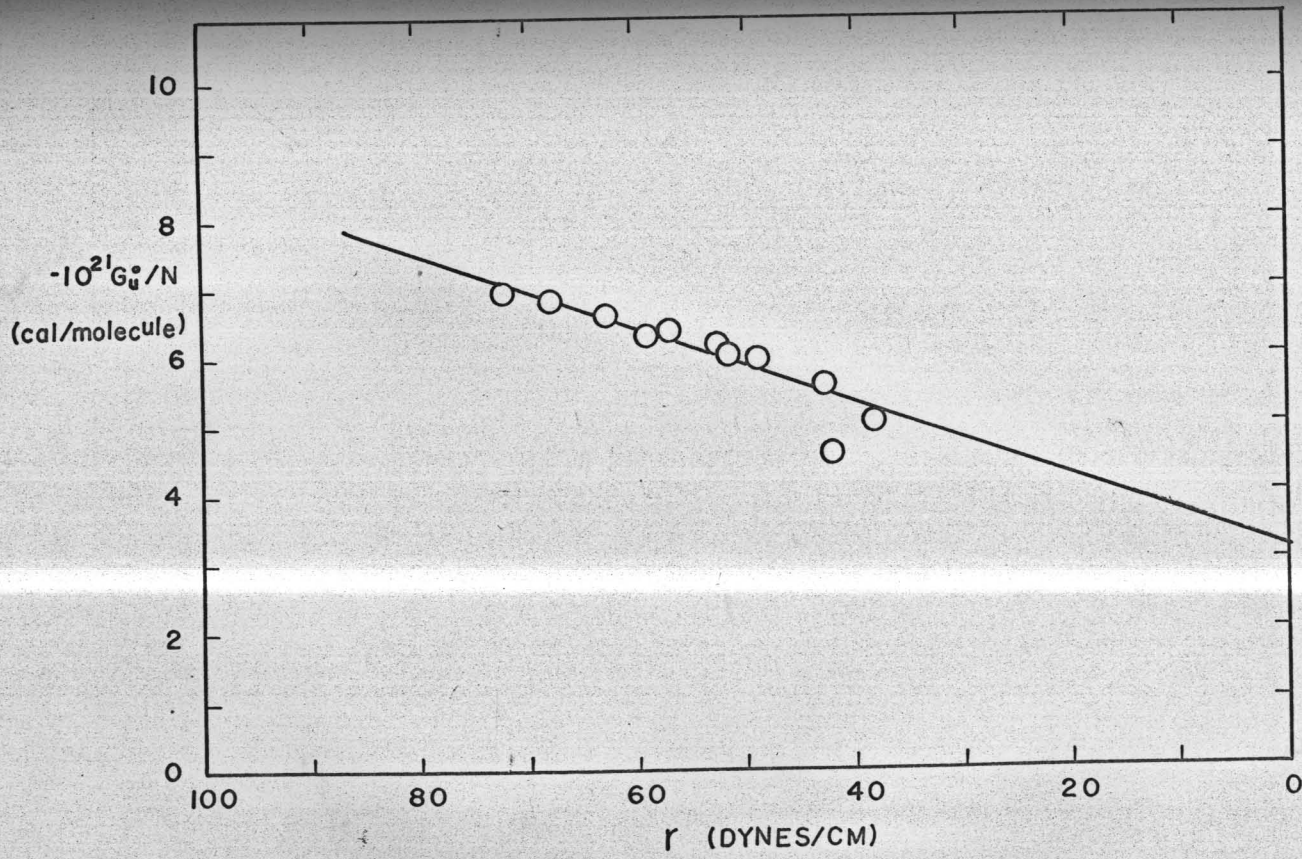

30
20
10
0
K_{II} (M^{-1})
100
80
60
40
20
0
r (DYNES/CM)

Figure 29. Plot of standard unitary free energy charge against surface tension for methyl cinnamate-8-chlorotheophyllinate system; 25.0°C (data given in Table XXIV).


10
8
$-10^{21}G_u^s/N$
(cal/molecule)
6
4
2
0
100
80
60
40
20
0
r (DYNES/CM)

8-bromotheophylline anion. Reproducible results were hard to achieve. A possible explanation for this is that the ease of 8-substituted theophyllines to undergo either S_N reaction with the attack of hydroxide ion will be in the order of $I^- > Br^- > Cl^-$ (49).

D. Naphthalene and Theophylline

In the standard unitary free energy-maximal overlap area plot in Cohen's dissertation (4), the naphthalene-theophylline complex showed a large positive deviation from the line. Attempts were made to examine this deviation by a solvent-effect study to see if this deviation correlates with the extrapolated value, $(-10^{21}\Delta\overset{\circ}{G}u/N)_{r \rightarrow o}$. Since the ultraviolet spectrum of naphthalene was not amenable to an examination of spectral shifts with the ligands used, it was studied by the solubility method. Solubility studies were carried out at 0, 3.97, 8.0, 16.22, 24.66 and 33.37%(w/w) MeOH-aqueous phosphate buffer solution (pH 6.6, μ = 0.3) at 25.0°, the solubility data are shown in Table XXVI and in Figures 30 through 33. The stability constants, standard unitary free energy changes and surface tensions for this system in aqueous methanol solutions are shown in Table XXVII and the free energy-surface tension plot is shown in Figure 34.

TABLE XXVI

Solubility of Naphthalene in the Presence of Theophylline in Aqueous Methanol Solutions[a]

Theophylline concentration	Naphthalene (10^4 M)					
	%(w/w) MeOH					
(M x 10^2)	0.0	3.97	8.00	16.22	24.66	33.37
0.0	1.96	2.56	3.20	5.65	10.5	25.1
0.0	-	2.62	-			
0.3	2.51	3.08	3.69	-	11.4	27.7
0.6	2.62	3.41	-	6.63	12.4	29.9
0.9	3.29	3.62	4.66	7.07	13.6	-
1.2	3.65	4.24	5.25	-	14.7	31.2
1.5	3.96	4.59	5.48	8.45	15.3	33.0
1.8	4.29	5.10	6.15	8.92	15.8	-
2.1	4.68	5.51	6.77	-	16.4	-
2.4	5.25	6.03	7.12	10.03	17.5	-
2.7	5.59	6.46	7.89	-	18.1	37.9
3.0	5.97	6.73	-	11.08	18.7	39.8

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

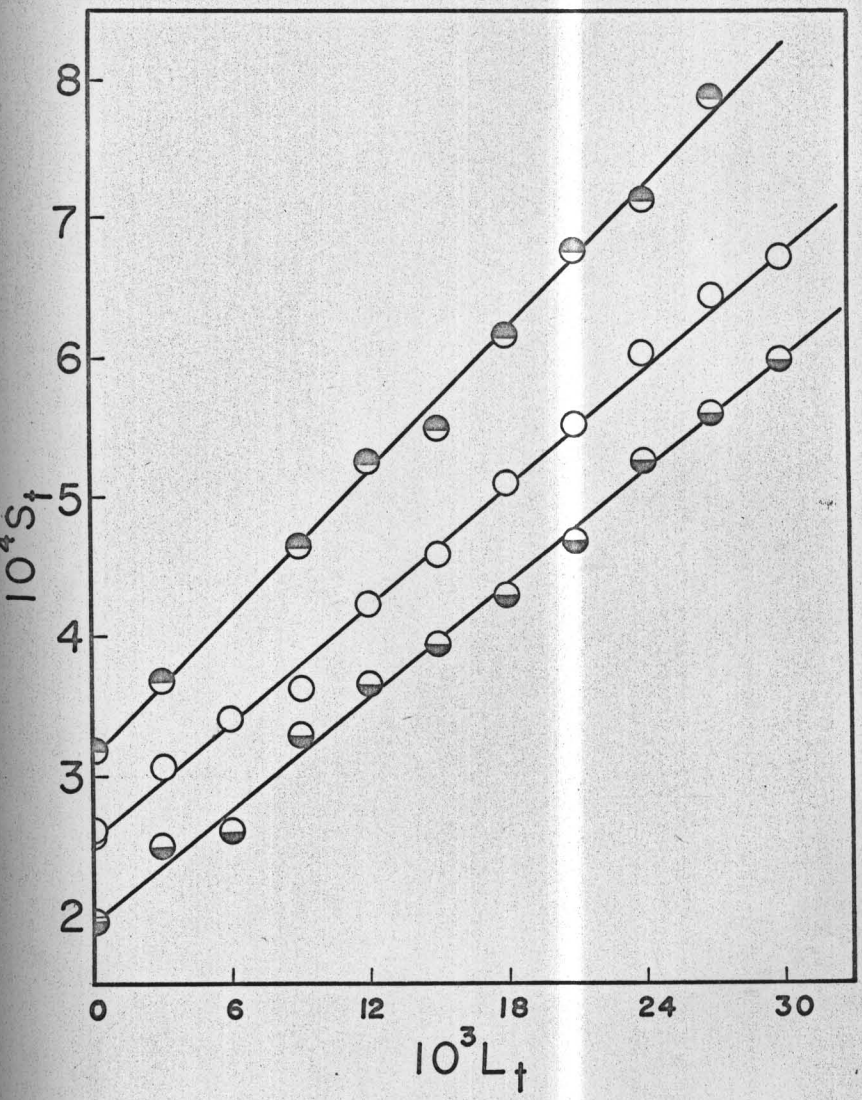


Figure 30. Solubility of naphthalene in the presence of theophylline (data given in Table XXVI). The lines from bottom to top are for the following percentage(w/w) of MeOH: 0.0, 3.97 and 8.00% (w/w) MeOH.

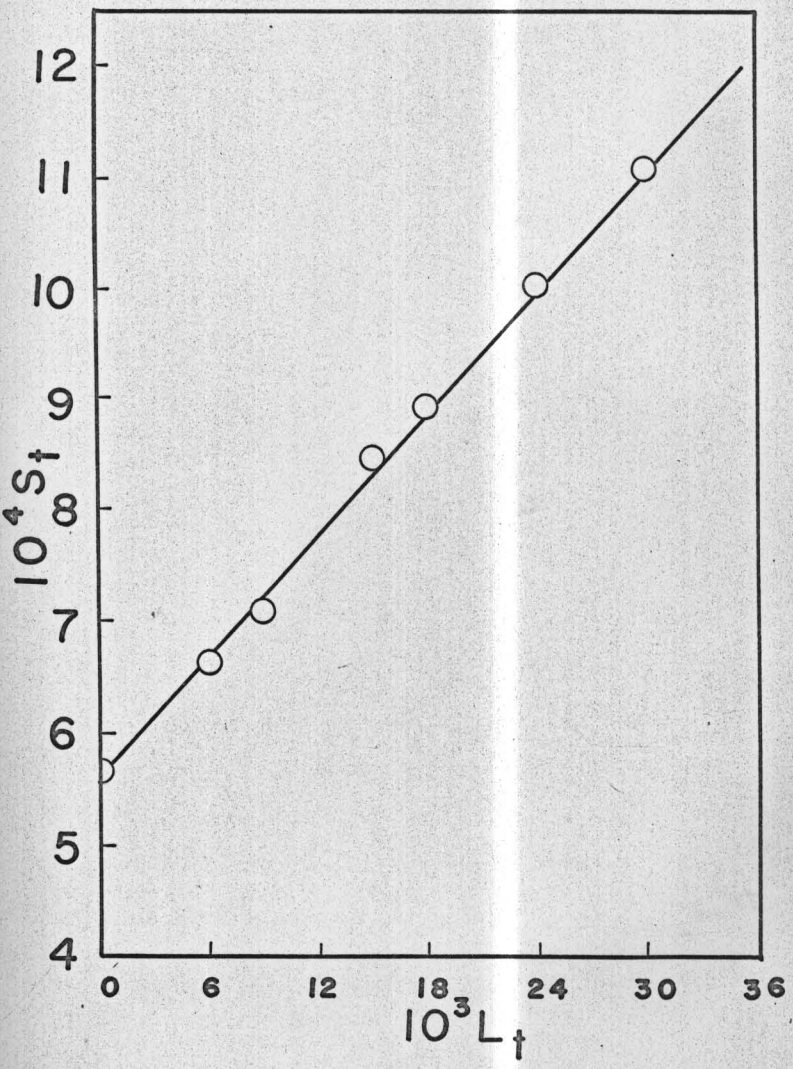


Figure 31. Solubility of naphthalene in the presence of theophylline; 25.0°, pH 6.6 phosphate buffer, μ = 0.3; 16.22%(w/w) MeOH; K_{11} = 33.81 M^{-1} (data given in Table XXVI).

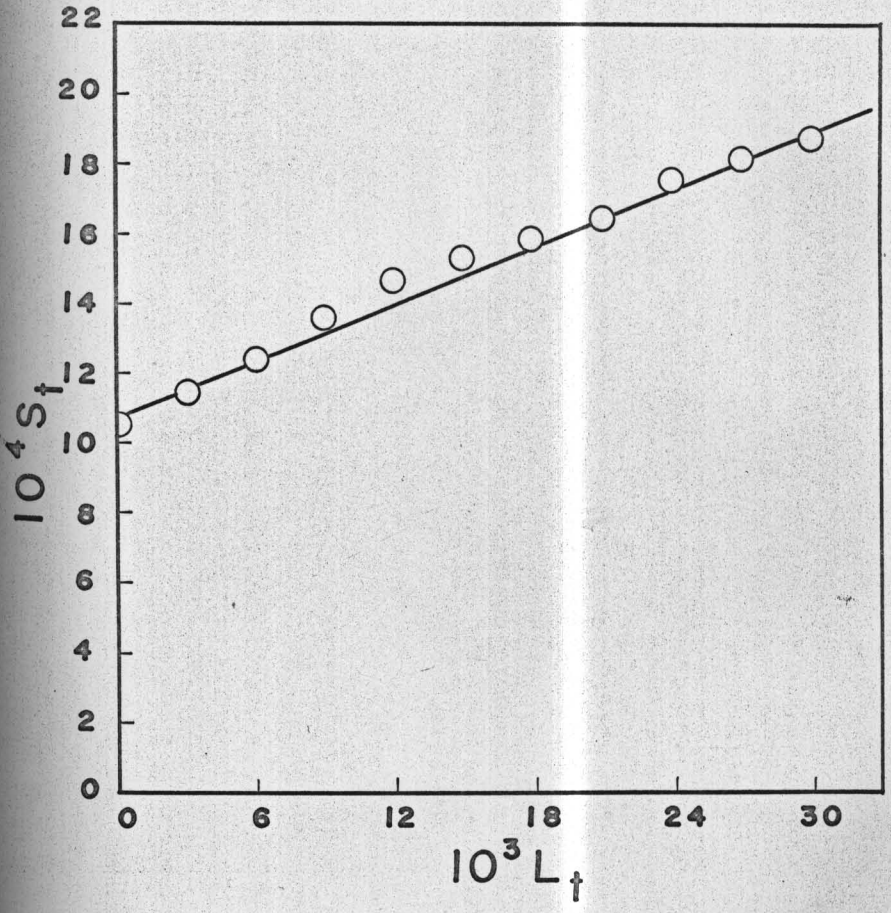


Figure 32. Solubility of naphthalene in the presence of theophylline; 25.0°, pH 6.6 phosphate buffer, μ = 0.3; 24.66%(w/w) MeOH; K_{11} = 25.75 M-1 (data given in Table XXVI).

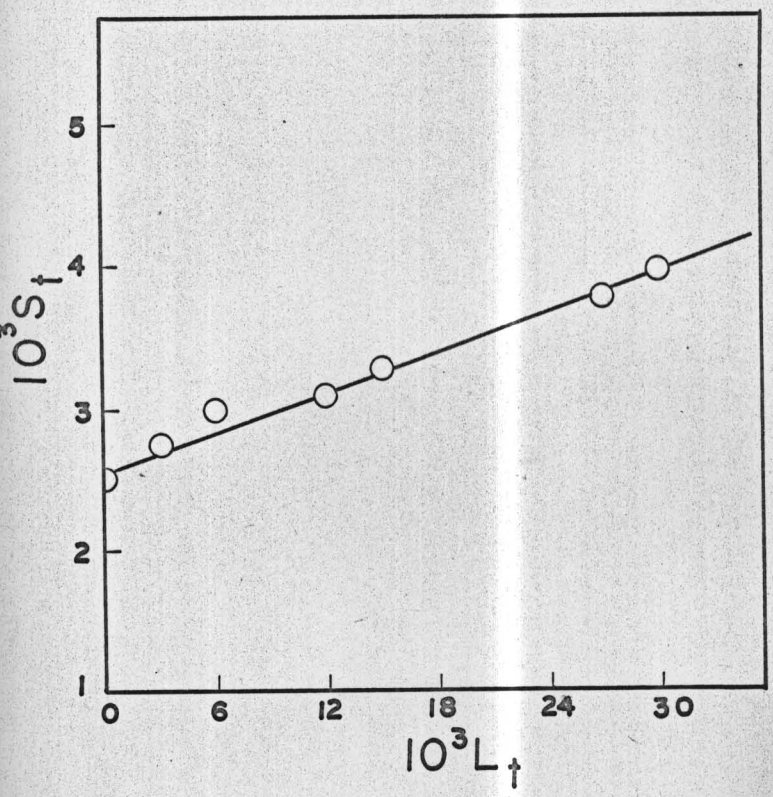


Figure 33. Solubility of naphthalene in the presence of theophylline; 25.0°, pH 6.6 phosphate buffer, μ = 0.3; 33.37%(w/w) MeOH; K_{11} = 18.17 M^{-1} (data given in Table XXVI).

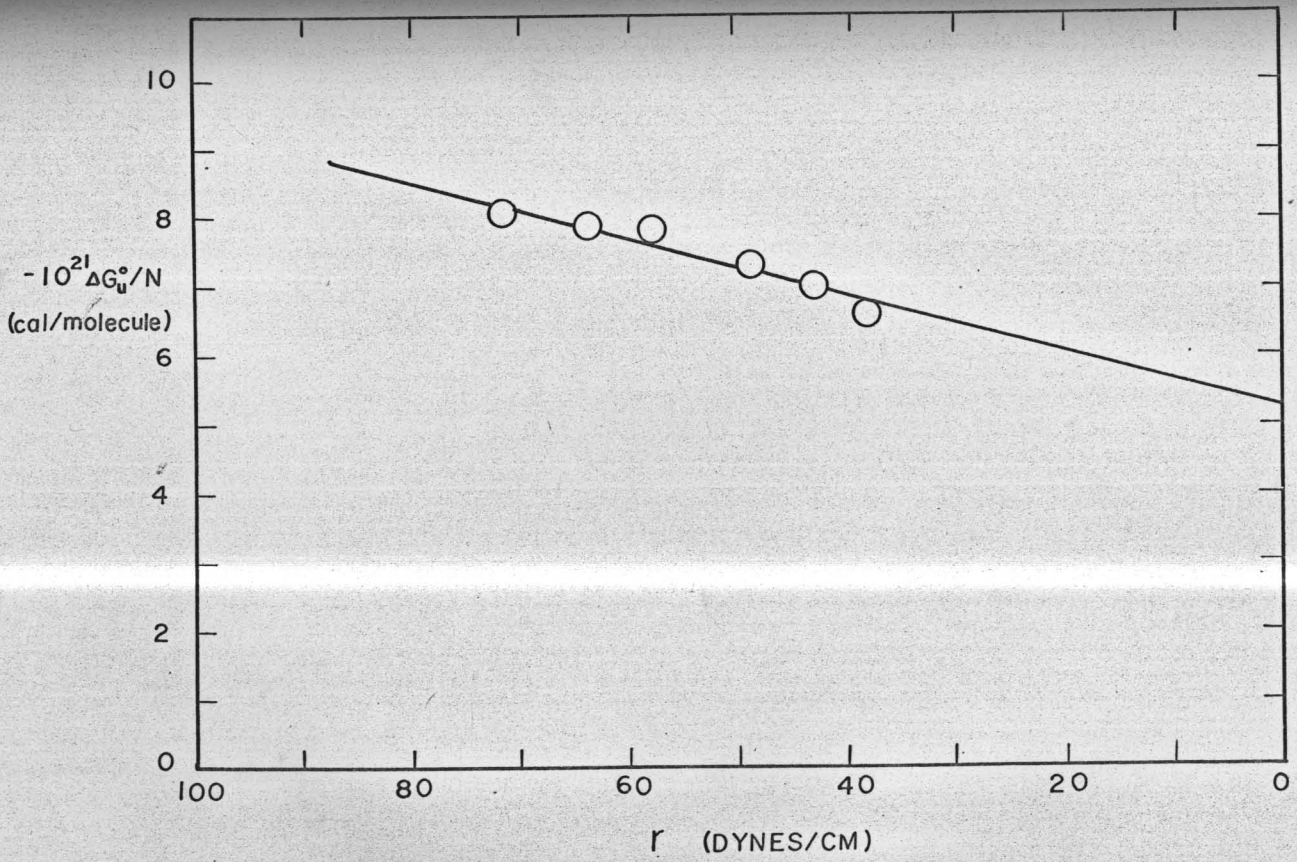
TABLE XXVII

Stability Constants, Standard Unitary Free Energy Changes and Surface Tensions for Naphthalene-Theophylline System in Aqueous Methanol Solution[a]

MeOH %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G^{\circ}_u/N$ (cal/molecule)	$\gamma_{25.0^{\circ}}$ (dynes/cm)
0	55.51	0.9971	67.3	8.1	71.7
3.97	54.542	0.9900	56.0	7.9	64.0
8.00	53.563	0.9830	54.8	7.8	58.0
16.22	51.565	0.9702	33.8	7.3	49.0
24.66	49.515	0.9569	25.8	7.0	42.9
33.37	47.399	0.9429	18.2	6.6	38.3

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

Figure 34. Plot of standard unitary free energy change against surface tension for naphthalene-theophylline system in aqueous methanol solutions (data given in Table XXVII).


10
8
6
4
2
0
$-10^{21}\Delta G_u^\circ/N$
(cal/molecule)
100
80
60
40
20
0
r (DYNES/CM)

E. Methyl 2-Naphthoate and 8-Nitrotheophylline Anion

The methyl 2-naphthoate-8-nitrotheophylline anion (8-nitrotheophyllinate) system has been studied elsewhere (4). Cohen (4) found that the stability constant was 230 M^{-1}, which was the largest constant obtained for systems of this type in aqueous solution in his studies. The nitro group is sufficiently different electronically from the other types of compounds we have studied to question whether or not it is the reason for the largest positive deviation from the line of the free energy-maximal overlap area plot in Cohen's dissertation (4). Therefore, solvent effect studies were carried out as before. Solubility studies were made in aqueous methanol phosphate buffer solution (pH 7.70, μ = 0.3) at 25.0°. The solubility data are shown in Table XXVIII and in Figure 35. The stability constants, standard unitary free energy changes and surface tension for this system are shown in Table XXIX and the free energy-surface tension plot is shown in Figure 36.

The summary plots of free energy change against surface tension for all the systems studied are shown in Figure 37.

TABLE XXVIII

Solubility of Methyl 2-Naphthoate in the Presence of 8-Nitrotheophyllinate in Aqueous Methanol Solutions[a]

8-Nitrotheophyllinate concentration ($M \times 10^2$)	Methyl 2-Naphthoate (10^4 M) %(w/w) MeOH			
	0.00	3.97	8.00	16.22
0.00	0.84	1.11	1.39	2.58
0.14	1.15	1.32	1.72	2.91
0.28	1.32	1.69	1.91	3.11
0.42	1.56	1.85	2.20	3.34
0.56	1.92	2.06	2.54	3.45
0.70	2.06	2.35	2.77	3.87
0.84	2.49	2.64	3.19	4.07
0.98	2.66	2.86	3.42	4.26
1.12	2.88	3.16	3.67	4.55
1.26	3.29	3.45	3.98	4.84
1.40	3.54	3.70	4.38	4.97

[a] 25.0°, pH 7.70 phosphate buffer, $\mu = 0.3$.

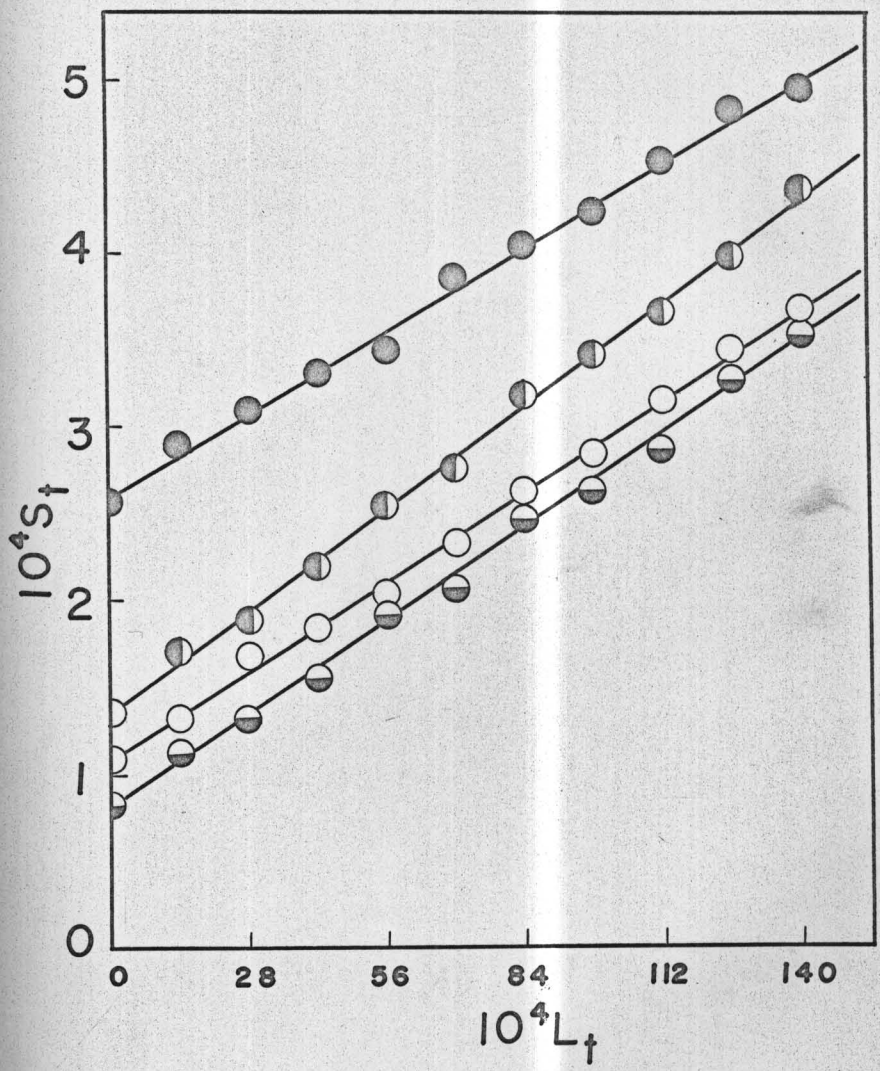


Figure 35. Solubility of methyl 2-naphthoate in the presence of 8-nitrotheophyllinate; 25.0°, pH 7.70 phosphate buffer, μ = 0.3 (data given in Table XXVIII). The lines from bottom to top are for the following percentage of MeOH: 0.00, 3.97, 8.00 and 16.22%(w/w) MeOH.

TABLE XXIX

Stability Constants, Standard Unitary Free Energy Changes and Surface Tensions for Methyl 2-Naphthoate-8-Nitrotheophyllinate System in Aqueous Methanol Solutions[a]

MeOH %(w/w)	M* (moles/1000 g)	d_4^{25} (gm/ml)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^\circ/N$ (cal/molecule)	$\gamma_{25.0^\circ}$ (dynes/cm)
0.00	55.51	0.9971	240.5	9.3	71.7
3.97	54.542	0.9900	173.4	9.0	64.0
8.00	53.563	0.9830	157.5	8.9	58.0
16.22	51.565	0.9702	101.6	8.4	49.0

[a] 25.0°, pH 7.70 phosphate buffer, μ = 0.3.

Figure 36. Plot of standard unitary free energy change against surface tension for methyl 2-naphthoate-8-nitrotheophyllinate system in aqueous methanol solutions; 25.0°, pH 7.70 phosphate buffer, μ = 0.3 (data given in Table XXIX).

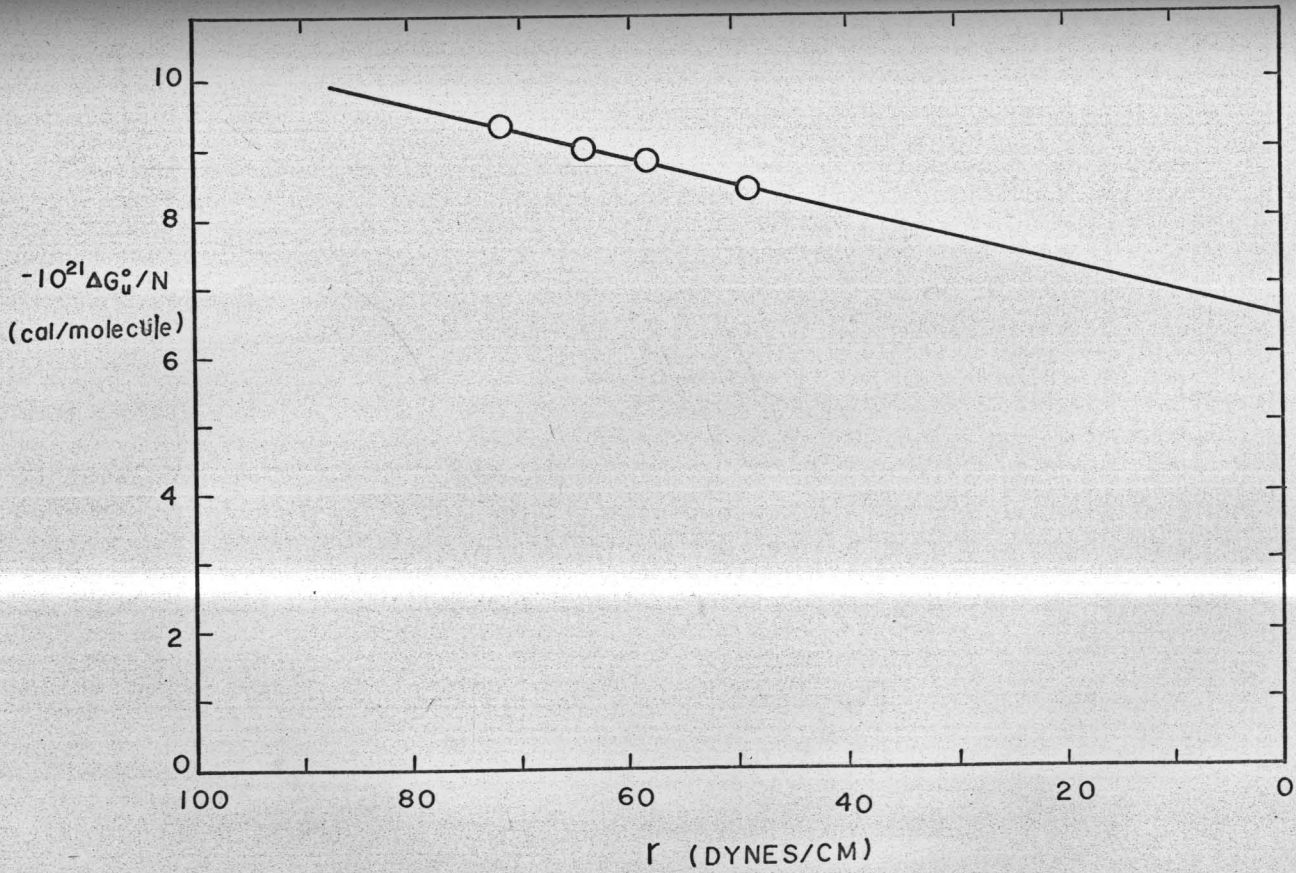
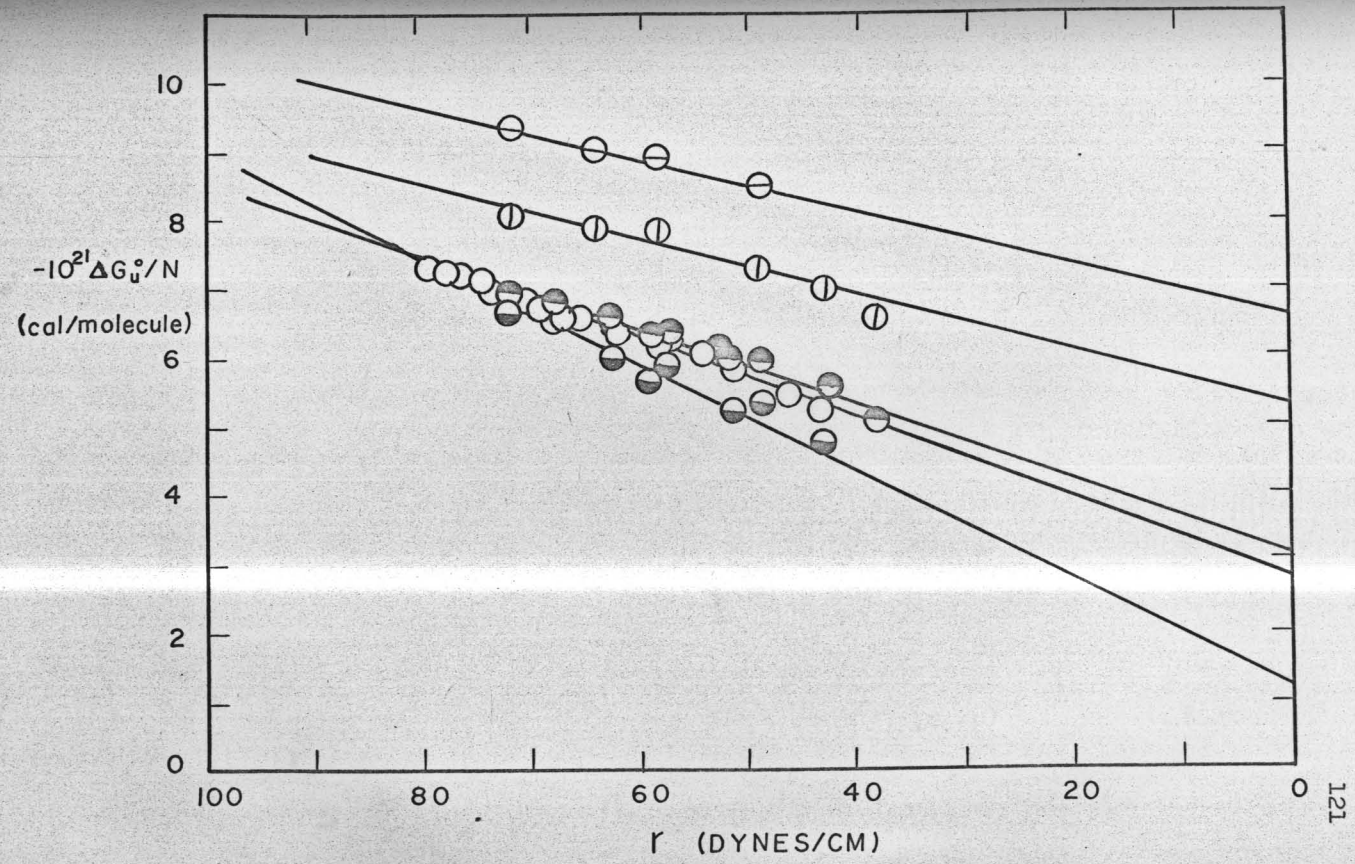

10
8
6
4
2
0
$-10^{21}\Delta G_u^\circ/N$
(cal/molecule)
100
80
60
40
20
0
r (DYNES/CM)

Figure 37. Plots of standard unitary free energy change against surface tension for all the systems studied; the lines from bottom to top are for the following complex systems: methyl cinnamate-theophyllinate, methyl cinnamate-theophylline, methyl cinnamate-8-chlorotheophyllinate, naphthalene-theophylline, and methyl 2-naphthoate-8-nitrotheophyllinate. (Plots are from Figures 16, 21, 29, 34 and 36).


10
8
$-10^{21}\Delta G_u^\circ/N$
(cal/molecule)
6
4
2
0
100
80
60
40
20
0
r (DYNES/CM)

IV. DISCUSSION

A. Correlation of Complex Stability with Surface Tension

Much of our earlier work (4,12,22,29), which sought correlations of complex stability with interactant structure, led to the development of a model for complex formation. This model assumes plane-to-plane interaction of substrate (S) and ligand (L) in medium (M). The maximal overlap area correlation can be developed from this model and is represented by the equation

$$\Delta G^{\circ} = A\,(G^{\circ}_{SL} - G^{\circ}_{MS} - G^{\circ}_{ML})$$

where

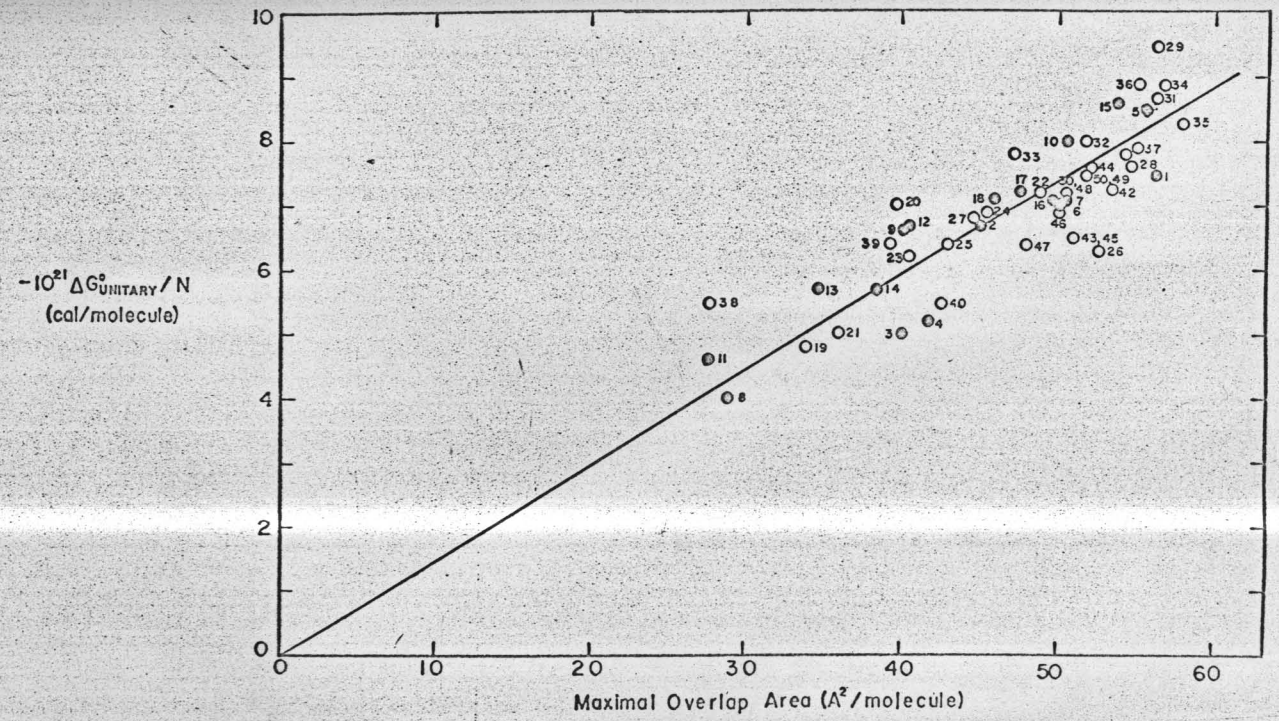
ΔG° = the standard unitary free energy change for complex formation

G°_{IJ} = the standard free energy of interaction per unit area between the "surfaces" designated by the subscripts

A = the maximal molecular overlap area between substrate and ligand.

A plot of standard unitary free energy change against maximal overlap area (Figure 38) gave a linear correlation (with extensive scatter) for 50 complex systems. The resulting line, which was determined by a least squares analysis, gave a value of 0.1 (in units of $-10^{21}\Delta G^{\circ}_{u}/N$) for the ordinate intercept, indicating

Figure 38. Plot of standard unitary free energy change against maximal overlap area; ○ = ionic complexes, ● = neutral complexes (data from Dr. Cohen (4)).


$-10^{21}\Delta G^{\circ}_{UNITARY}/N$
(cal/molecule)
Maximal Overlap Area (A^2/molecule)
0
2
4
6
8
10
0
10
20
30
40
50
60
29
36
34
31
15
5
35
10
32
37
33
14
28
1
17
22
30
49
50
48
42
16
7
6
18
24
27
2
46
20
12
9
39
23
25
47
43,45
26
38
13
14
40
21
30
4
19
11
8

that the line passes essentially through the origin, in agreement with the model equation. This treatment has revealed that, in the first-order approximation, all of the 50 systems behave similarly, and, on the average, there is no difference between neutral and ionic complexes. However, the dispersion seen in the plot may be considered a second-order effect, possibly correlatable with specific structural features in the substrate and ligand (since most of the deviations from the line are too large to be ascribed to mis-estimates of maximal overlap areas). Therefore, the present studies were carried out by choosing complex systems which represented various behavior with respect to Cohen's (4) line (see Figure 38). Moreover, the units and magnitude of the slope of the area correlation naturally suggest a possible relationship of stability with a solvent property such as surface tension, so it was considered appropriate to study (finally, in this laboratory) the effects of medium on stability, perhaps as a tool for elucidating the second-order effects referred to above.

From the standard unitary free energy-surface tension plots shown in Figure 37 for the five complex systems (No. 16, methyl trans-cinnamate-theophylline; No. 43, methyl trans-cinnamate-theophylline anion; No. 30, methyl trans-cinnamate-8-chlorotheophylline

anion; No. 10, naphthalene-theophylline; and No. 29, methyl 2-naphthoate-8-nitrotheophylline anion system -- the numbers used here being the same as in Cohen's (4) plot shown in Figure 38), straight lines were obtained for these systems; that is, the stability of the molecular complexes appears to be a reasonably linear function of the surface tension of the solvents used.

The organic solvents used in the binary aqueous organic solvent mixtures in which the solvent effect studies were carried out were acetonitrile, methanol, ethylene glycol, dimethylsulfoxide, glycerine, and dioxane. These organic solvents represented alcohols (methanol, ethylene glycol and glycerine), a sulfoxide (dimethylsulfoxide), a nitrile (acetonitrile), and an ether (dioxane). Aqueous inorganic salt solutions, lithium chloride and sodium chloride solutions, were also used, as was deuterium oxide. From these solvent studies, the stability of a given complex system was found to be solely a function of the surface tension of the solvent at a given temperature, no matter what the chemical nature of the organic solvent.

The dielectric constant is a physical property of the solvent often suggested as an important parameter in solution equilibria. Rose and Labes (93) studied the effect of increased ethanol concentration on the complexing of naphthalene with 1,3,5-trinitrobenzene in

ethanol-chloroform mixtures, and found that as the dielectric constant increased, the stability constant decreased. They attribute this to preferential stabilization by solvation of either naphthalene or 1,3,5-trinitrobenzene rather than of the complex. Similar solvent effects were investigated using proton magnetic resonance (94). But in our complex systems, the dielectric constant did not correlate uniquely with the stability of the molecular complex. This can be seen from the values of dielectric constants and stability constant or the standard unitary free energy change for the methyl trans-cinnamate-theophylline system in binary aqueous methanol, ethylene glycol, dioxane, acetonitrile, lithium chloride and sodium chloride solutions at 25.0°. These values are shown in Tables XXX through XXXIV. Although the stability of the complex decreased when the dielectric constant decreased for individual binary aqueous organic solvent mixtures, the stability of the complex was not a unique function of the dielectric constant for all the solvents studied, as seen from the plot of the unitary standard free energy change against the dielectric constant in Figure 39. Moreover, the stability of the complex increased as the dielectric constant decreased in the aqueous sodium chloride and lithium chloride solutions at 25.0°. Therefore, it is apparent that the

TABLE XXX

Stability Constants, Standard Unitary Free Energy Changes and Dielectric Constants for Methyl trans-Cinnamate-Theophylline System in CH_3CN-H_2O Mixtures[a]

CH_3CN %(w/w)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^o/N$ (cal/molecule)	Dielectric constant[b] (ϵ)
0.62	22.04	6.99	78.4
1.24	16.85	6.72	78.2
3.11	15.76	6.64	77.3
4.52	14.67	6.56	76.7
5.62	10.93	6.26	76.3
6.89	9.51	6.11	75.9
8.48	9.28	6.08	75.0
12.50	5.00	5.44	73.2
16.57	2.66	4.79	71.9

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

[b] lit. (92).

TABLE XXXI

Stability Constants, Standard Unitary Free Energy Changes and Dielectric Constants for Methyl trans-Cinnamate-Theophylline System in Aqueous Methanol Solutions[a]

MeOH %(w/w)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^o/N$ (cal/molecule)	Dielectric constant[b] (ϵ)
0.62	19.46	6.86	78.0
2.59	18.40	6.80	77.1
4.58	13.53	6.48	76.1
12.64	9.08	6.04	72.4
33.81	4.96	5.31	62.5

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

[b] lit. (95-96).

TABLE XXXII

Stability Constants, Standard Unitary Free Energy Changes and Dielectric Constants for Methyl trans-Cinnamate-Theophylline System in Aqueous Dioxane Solution[a]

Dioxane %(w/w)	K_{11} (M^{-1})	$-10^{21}\Delta G_u^o/N$ (cal/molecule)	Dielectric constant[b] (ϵ)
0.81	19.24	6.86	77.4
3.36	15.05	6.59	75.3
5.90	14.17	6.51	73.0
10.94	10.07	6.14	68.8
20.90	8.36	5.88	59.8
40.47	5.29	5.24	42.4

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

[b] lit. (95).

TABLE XXXIII

Stability Constants, Standard Unitary Free Energy Changes and Dielectric Constants for Methyl trans-Cinnamate-Theophylline System in Ethylene Glycol-Water Mixtures[a]

Ethylene glycol %(w/w)	K'_{11} (M^{-1})	$-10^{21}\Delta G^{o}_{u}/N$ (cal/molecule)	Dielectric constant[b] (ϵ)
0.88	22.51	7.01	78.1
0.88	21.20	6.94	78.1
3.53	19.08	6.83	77.3
6.35	17.86	6.75	76.4
8.97	17.14	6.69	75.6
8.97	14.35	6.52	75.6
11.75	15.41	6.57	75.0
17.09	12.26	6.31	73.5
22.33	11.09	6.17	72.0

[a] 25.0°, pH 6.6 phosphate buffer, μ = 0.3.

[b] lit. (95).

TABLE XXXIV

Stability Constants, Standard Unitary Free Energy Changes and Dielectric Constants for Methyl trans-Cinnamate-Theophylline System in Aqueous Inorganic Solutions

	K_{11} (M^{-1})	$-10^{21}\Delta G_u^o/N$ (cal/molecule)	Dielectric constant (ϵ)
4.94%(w/w) LiCl[a]	27.06	7.16	60.5[c]
10.00	28.15	7.18	43.0[c]
12.99	30.85	7.25	32.4[c]
15.98	34.64	7.34	22.0[c]
4.76%(w/w) NaCl[b]	22.56	7.00	62.6[d]
13.04	27.45	7.15	34.0[d]

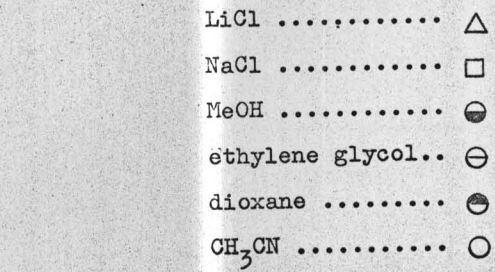
[a] 25.0°, 0.62%(w/w) CH_3CN.

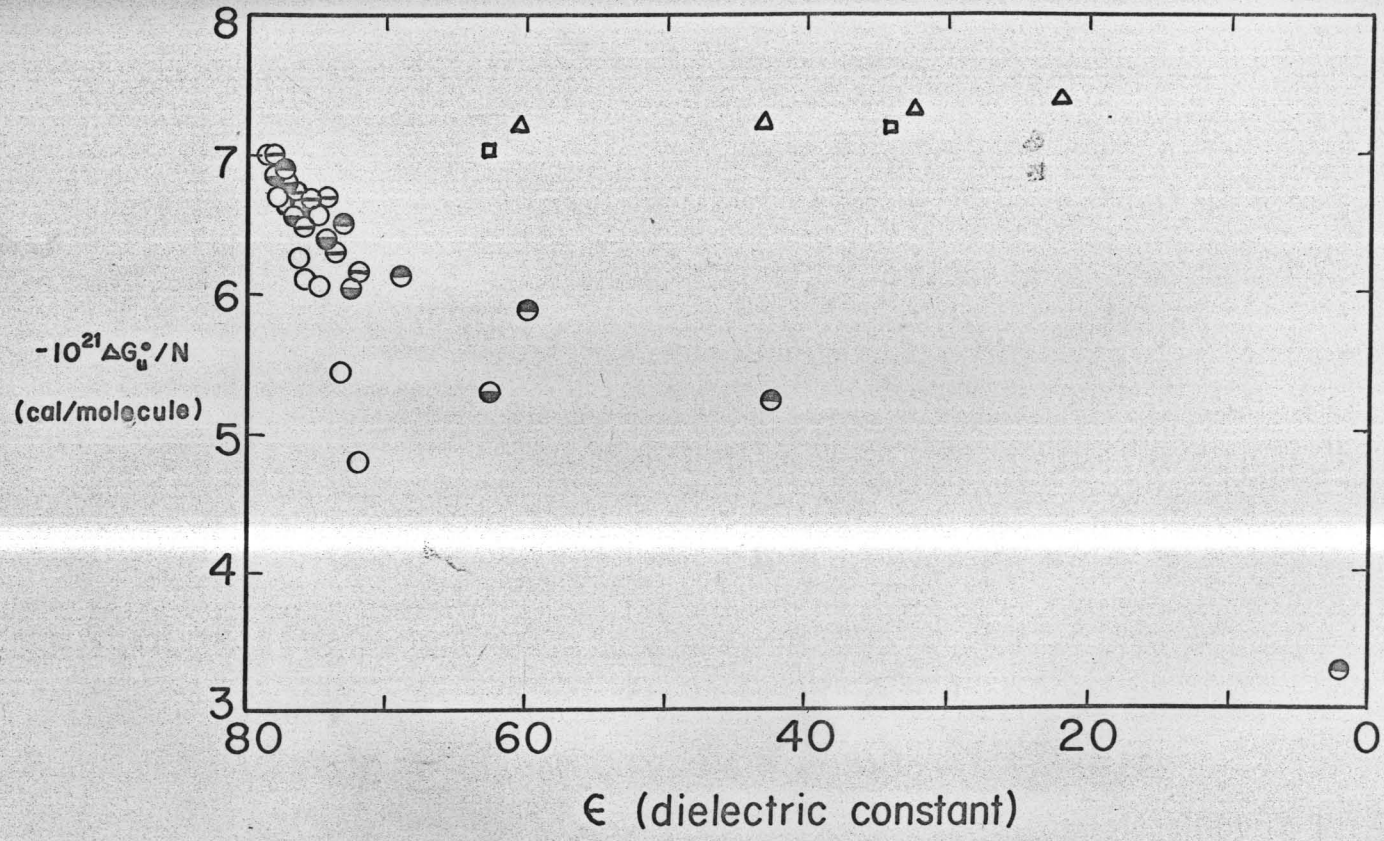
[b] 25.0°, pH 6.6 phosphate buffer (μ = 0.3), 0.62%(w/w) MeOH.

[c] Lit. (97).

[d] Lit. (15).

Figure 39. Plot of standard unitary free energy change against dielectric constant for methyl trans-Cinnamate-Theophylline system; 25.0°, (see data given in Tables XXX through XXXIV).




8
7
6
5
4
3
80
60
40
20
0
$-10^{21}\Delta G_t^\circ/N$
(cal/molecule)
ϵ (dielectric constant)

stability of this complex system is not solely a function of the dielectric constant.

Although surface tension was employed as the independent variable in these correlations, it seemed that interfacial tensions between the mixed aqueous-organic solvent and an immiscible organic liquid might also be an appropriate parameter. Of course, such data are less readily available, both in the literature and experimentally. One difficulty is that the organic component of the binary solvent may partition into the organic phase. Despite this ambiguity, interfacial tensions were measured for the following two-phase systems: benzene-water-methanol; carbon tetrachloride-water-methanol; isooctane-water-methanol; isooctane-water-dioxane; and isooctane-water-acetonitrile. Table XXXV shows some representative data of the standard unitary free energy changes and the interfacial tensions. These interfacial tensions were between the two-liquid-phase ternary systems, benzene-H_2O-MeOH (53), CCl_4-H_2O-MeOH (54), and isooctane-H_2O-MeOH. A plot of the standard unitary free energy change against the interfacial tension gives a straight line for each complex system; moreover, the extrapolated values of the standard unitary free energy change at zero interfacial tension, $(-10^{21}\Delta G_u^o/N)_{r \rightarrow o}$, were all the same in benzene-H_2O-MeOH, CCl_4-H_2O-MeOH and isooctane-H_2O-MeOH for each

TABLE XXXV

Standard Unitary Free Energy Changes and Interfacial Tensions for Complex Systems at 25.0°[a]

MeOH %(w/w)	Interfacial Tension (dynes/cm) Benzene-H_2O-MeOH[b]	CCl_4-H_2O-MeOH[c]	Iso8-H_2O-MeOH	Complex System	$-10^{21}\Delta G_u^o/N$ (cal/molecule)
0	34.2	43.0	50.0	Methyl 2-naphthoate-8-nitro-theophyllinate system	9.34
3.97	30.2	36.8	43.2		9.00
8.00	26.5	32.0	38.0		8.88
16.22	20.2	24.7	31.0		8.40
0	34.2	43.0	50.0	Methyl cinnamate-theophyllinate system	6.59
4.58	29.6	35.8	42.3		5.92
8.58	26.0	31.4	37.5		5.85
16.74	19.9	24.3	30.2		5.24
25.15	15.0	19.6	24.2		4.73
0.62	33.5	42.0	49.0	Methyl cinnamate-theophylline system	6.86
2.59	31.5	38.9	45.4		6.80
4.58	29.7	35.8	42.3		6.48
12.64	23.0	27.5	33.8		6.04
33.81	11.5	16.3	19.7		5.31

(continued)

TABLE XXXV - Cont.

MeOH %(w/w)	Interfacial Tension (dynes/cm) Benzene-H_2O-MeOH[b]	CCl_4-H_2O-MeOH[c]	Iso8-H_2O-MeOH	Complex System	$-10^{21}\Delta G^{o}_{u}/N$ (cal/molecule)
0.0	34.2	43.0	50.0	Naphthalene-theophylline system	8.09
3.97	30.2	36.8	43.2		7.89
8.00	26.5	32.0	38.0		7.84
16.22	20.2	24.7	31.0		7.31
24.66	15.4	19.9	24.8		6.99
33.37	11.6	16.5	20.0		6.59
0.0	34.2	43.0	50.0	Methyl cinnamate-8-chloro-theophyllinate system	6.94
4.58	29.6	35.8	42.3		6.62
8.58	26.0	31.4	37.5		6.38
12.64	22.7	27.5	33.8		6.18
16.74	19.9	24.3	30.2		5.96
25.15	15.0	19.6	24.2		5.59
33.81	11.5	16.3	19.7		5.04

[a] pH 6.6 phosphate buffer, μ = 0.3.

[b] Lit. (53).

[c] Lit. (54).

complex system. Some typical standard unitary free energy-interfacial tension plots are shown in Figures 40 and 41.

To test the surface tension as an unique determiner of the stability for the complex system, standard unitary free energy change was plotted against surface tension for a system in a variety of solvents, (e.g., aqueous alcohol, nitrile, sulfoxide, ether, and inorganic solutions); a linear correlation was observed for a given complex system at given temperature no matter what the chemical nature of the organic solvent, as described earlier. In the same way, the interfacial tension function was tested. Table XXXVI shows the standard unitary free energy changes and the interfacial tensions for the two-phase systems (isooctane-water-dioxane; isooctane-water-methanol; and isooctane-water-acetonitrile) for the complex system between methyl trans-cinnamate and theophylline in aqueous dioxane, methanol and acetonitrile solutions at 25.0°. If we plotted the standard unitary free energy changes against these interfacial tensions, as shown in Figure 42, a poor line was observed.

This indicates that interfacial tension is not as good a master variable as is surface tension; but obviously some correlation exists. One possibility is

Figure 40. Plots of standard unitary free energy changes against interfacial tension of the two-liquid-phase ternary system (benzene-H_2O-MeOH) for the complex systems at 25.0°. Lines from bottom to top are for the following complex systems:

methyl cinnamate-theophyllinate

methyl cinnamate-theophylline

methyl cinnamate-8-chlorotheophyllinate

naphthalene-theophylline

methyl 2-naphthoate-8-nitrotheophyllinate.

(Data given in Table XXXV).

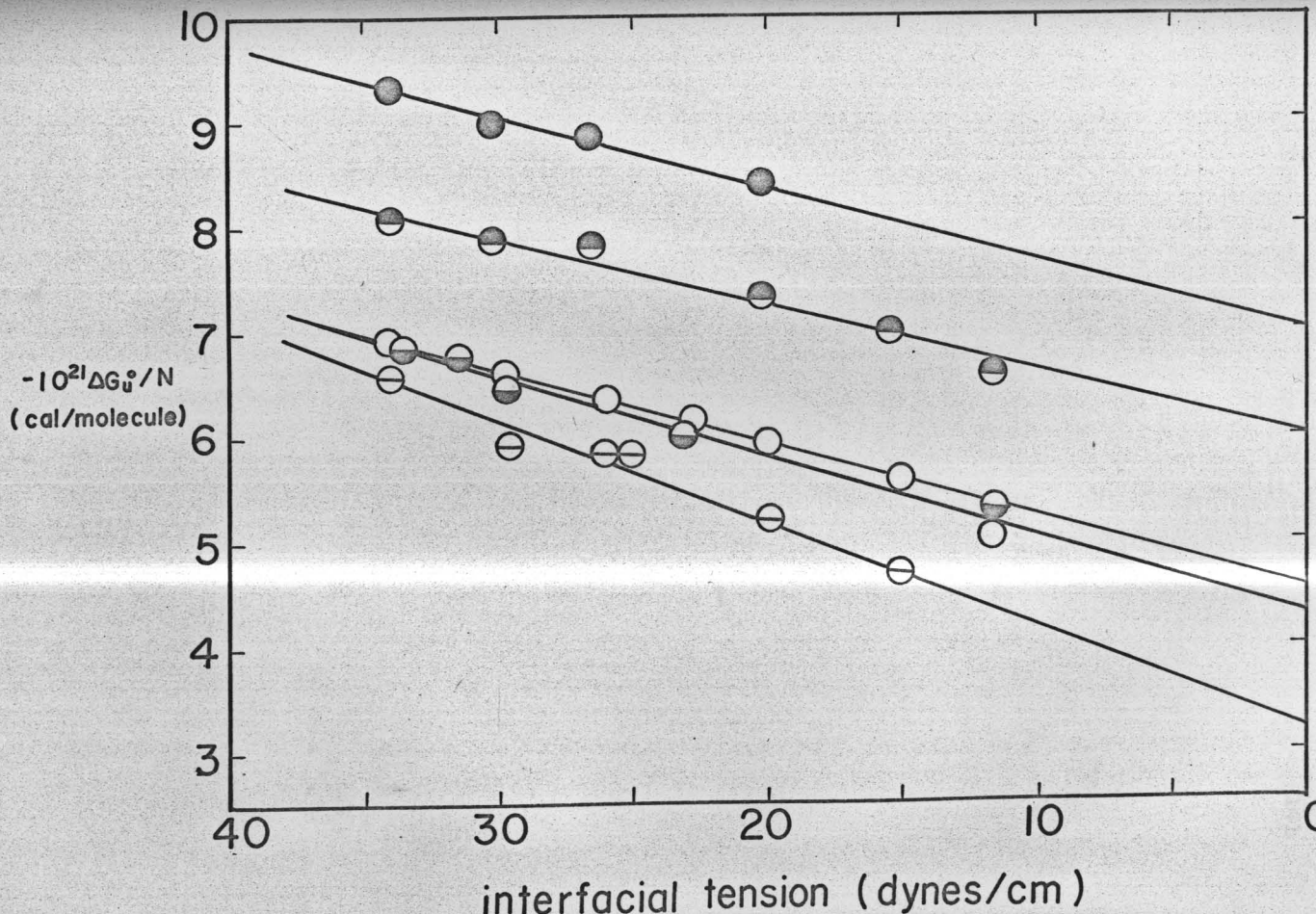

10
9
8
7
6
5
4
3
$-10^{21}\Delta G_{ii}^{\circ}/N$
(cal/molecule)
40
30
20
10
0
interfacial tension (dynes/cm)

Figure 41. Plots of standard unitary free energy change against interfacial tension of the two-liquid-phase binary system (CCl_4-H_2O-MeOH) for the complex systems at 25.0°. Lines from top to bottom are for the following complex systems:

methyl 2-naphthoate-8-nitrotheophyllinate
naphthalene-theophylline
methyl cinnamate-8-chlorotheophyllinate
methyl cinnamate-theophylline
methyl cinnamate-theophyllinate.

(Data given in Table XXXV).

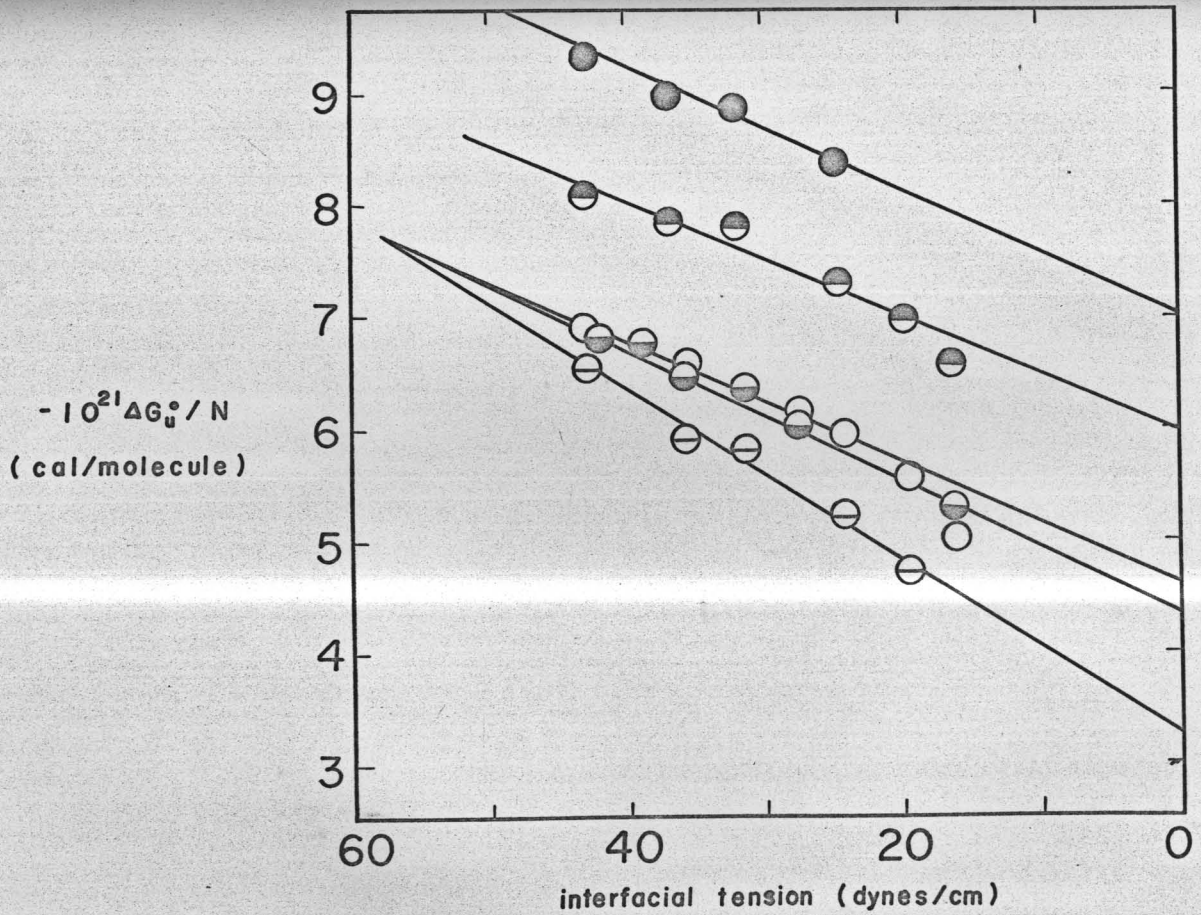

$-10^{21}\Delta G_u^\circ / N$
(cal/molecule)
9
8
7
6
5
4
3
60
40
20
0
interfacial tension (dynes/cm)

TABLE XXXVI

Standard Unitary Free Energy Change and Interfacial Tension for Methyl trans-Cinnamate-Theophylline System[a]

Dioxane %(w/w)	$-10^{21}\Delta G_u^o/N$ (cal/molecule)	Interfacial Tension (dynes/cm) Isooctane-H_2O-Dioxane
0.81	6.86	46.2
3.36	6.59	39.2
5.90	6.51	34.6
10.94	6.14	28.0
20.90	5.88	21.9
40.47	5.24	13.2

MeOH %(w/w)	$-10^{21}\Delta G_u^o/N$ (cal/molecule)	Interfacial Tension (dynes/cm) Isooctane-H_2O-MeOH
0.62	6.86	49.0
2.59	6.80	45.4
4.58	6.48	42.3
12.64	6.04	33.8
33.81	5.31	19.7

(continued)

TABLE XXXVI - Cont.

CH_3CN %(w/w)	$-10^{21}\Delta G^{o}_{u}/N$ (cal/molecule)	Interfacial Tension (dynes/cm) Isooctane-H_2O-CH_3CN
0.62	6.99	49.0
1.24	6.72	47.0
3.11	6.64	41.0
4.52	6.56	37.0
5.62	6.26	35.0
6.89	6.11	32.5
8.48	6.08	29.7
12.50	5.44	24.0
16.57	4.79	19.7

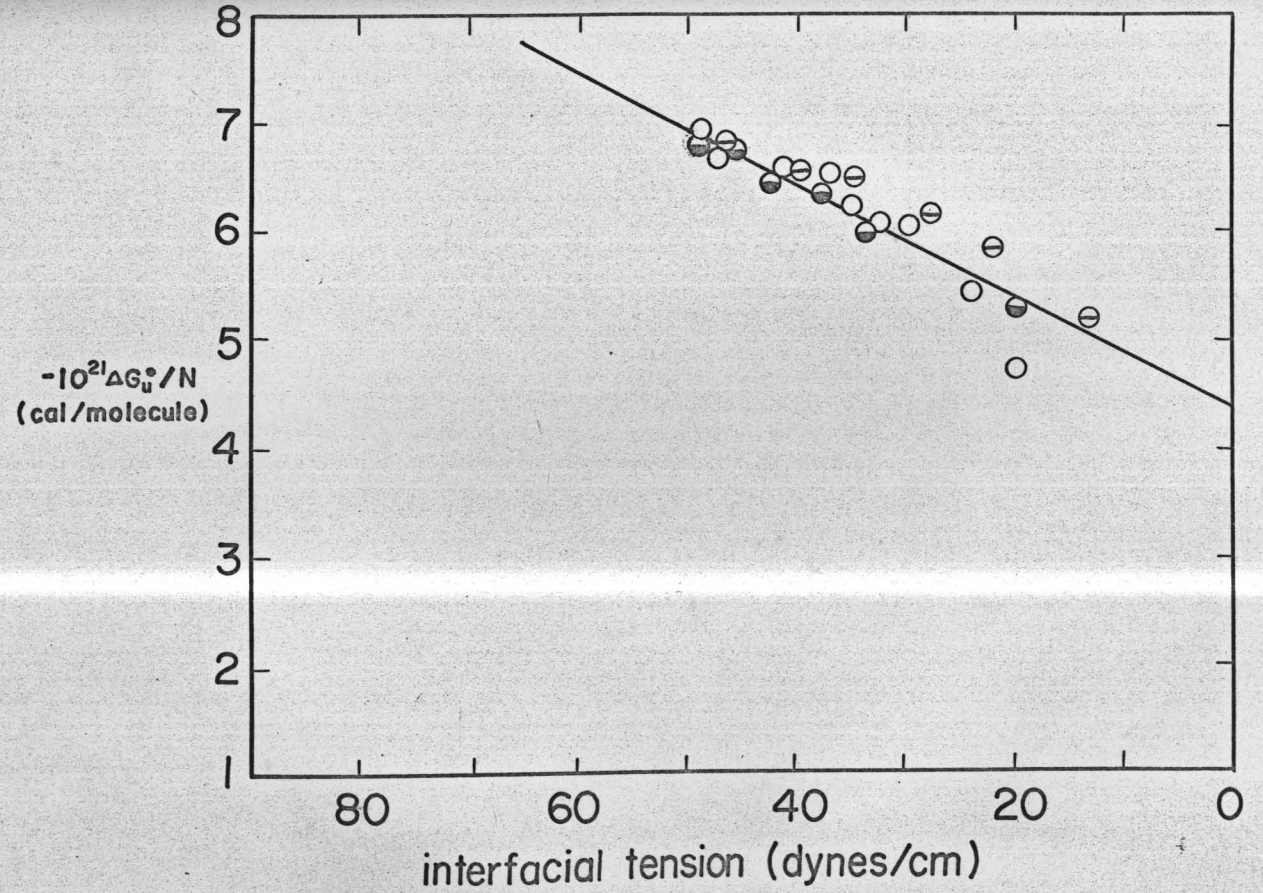
[a]pH 6.6 phosphate buffer, μ = 0.3, 25.0°.

Figure 42. Plot of the standard unitary free energy change against the interfacial tension for methyl trans-cinnamate-theophylline system; (data given in Table XXXVI). The interfacial tension between:

Isooctane-H_2O-MeOH ..... ◒

Isooctane-H_2O-Dioxane .... ⊖

Isooctane-H_2O-CH_3CN ..... ○


8
7
6
5
4
3
2
1
$-10^{21}\Delta G_u^\circ/N$
(cal/molecule)
80
60
40
20
0
interfacial tension (dynes/cm)

that the interfacial tension measurement is not accurate because the binary solvent may partition into the organic phase. If this ambiguity does exist, that is, the interfacial tension thus measured does not represent the real binary aqueous environment, then the difference in the extrapolated value of

$(-10^{21}\Delta G_u^{\circ}/N)_{\text{interfacial tension}} \rightarrow 0$ and

$(-10^{21}\Delta G_u^{\circ}/N)_{\text{surface tension}} \rightarrow 0$ in our free energy change- interfacial or surface tension plots may be due to this ambiguity.

It is presently concluded from the above observations that the surface tension of the solvent yields the best empirical correlation between a physical property of a mixed aqueous solvent and the extent of molecular complexation. Sinanoglu and Abdulnur (98) have undertaken theoretical studies on the effect of solvents on the denaturation of deoxyribonucleic acid. 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 tension of water and concluded that the standard free energy change may be affected mainly by the surface tension of the solvent. Subsequently, Cassidy and coworkers (91) examined the extent of quinhydrone formation between hydroquinone and p-benzoquinone in 20 different pure and aqueous solvents, and indicated a rough correlation between extent of

quinhydrone formation, as judged by the magnitude of the association constant and of the related standard free energy change, and the surface tension of the solvent. When, however, they plotted the standard free energy change of quinhydrone formation in highly aqueous acetic acid, glycol, tetrahydrofuran, and methanol *vs.* surface tension of the solvent mixture, linear correlations were not observed. They ascribed the deviations from the straight line to changes in the intrinsic solvating properties of the solvent and in the net change in surface area. When they plotted the standard free energy change of quinhydrone formation in pure organic solvents *vs.* surface tension of the solvent, a rough linear correlation was seen. Higuchi and coworkers (90) also have studied the influence of various organic solvents in mixed solvent systems on the extent of complex formation between TMPPT (1,3,7,9-tetramethylpyrimido-(5,4,g)pteridine-2,4,6,8(1H,3H,7H,9H)-tetrone) and DMCA (N,N-dimethylcinnamide) and on the menadione-caffeine complex; although they failed to quantitatively correlate the extent of interaction of the complex formation with the surface tension of the media, they indicated that surface tension probably 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 (51,89). But it may be far from secure at this stage to correlate the observed binding with the behavior of large molecules in solution. In Higuchi, et al.'s (90) work, the complex systems were studied in 10%(v/v) organic solvent in water at 25.0°, the organic solvents used were glycerine, methanol, acetonitrile, acetone and dioxane with an aqueous sucrose solution also being used. If we plot their log K_{11} against surface tension for their systems, a rough linear correlation is observed, though more scatter is seen than in the present work. Moreover, for the TMPPT-DMCA complex system, they reported both 1:1 and 1:2 complexes, and this complication may contribute to the scatter. In Cassidy, et al.'s(91) work, carried out on quinhydrone complex formation, more varieties of solvents were used (including aqueous acetic acid, tetrahydrofuran, methanol, glycol, hydrochloric acid and n-butyl alcohol mixed solvents); the pH values and ionic strengths were not reported. The pH will affect the complex by controlling the extent of ionization, probably with an effect on the stability constant. Stelmach (14) examined the effect of ionic strength on molecular complex formation and found minor effects on stability; for example, if the ionic strength is changed from 0.088 to 1.500, the stability constant for the naphthalene-theophylline complex system changes from 55.6 M^{-1} to

63.6 M^{-1} at 25.0°. Also it is known that the ionic strength will change the surface tension (42,53). Therefore, these may be reasons that Cassidy and coworkers (91) failed to get a satisfactory linear correlation between the standard free energy change and surface tension.

B. Nature of Molecular Complex Interaction

Our basic model equation for complex formation is shown by equation (1),

$$\Delta G^\circ = A\,(G^\circ_{SL} - G^\circ_{MS} - G^\circ_{ML}) \qquad (1)$$

As was pointed out earlier, the standard free energy change appears to be a linear function of the surface tension for a given complex; this suggests approximating the solvent interaction terms with surface tension terms to give

$$\Delta G^\circ = A\,(G^\circ_{SL} - k_S\gamma - k_L\gamma) \qquad (2)$$

where k_S and k_L are proportionality constants. Rearranging equation (2), we get equation (3)

$$\Delta G^\circ = -A\,(k_S + k_L)\,\gamma + AG^\circ_{SL} \qquad (3)$$

This is consistent with the empirical correlation, since if we plot ΔG° against γ, we will get a straight

line with negative slope. The parameters of the line are given by equations (4) and (5).

$$\text{ordinate intercept} = A\,G^{\circ}_{SL} \qquad (4)$$

$$\text{slope} = -A\,(k_S + k_L) \qquad (5)$$

In the earlier section, we plotted the standard unitary free energy change against the surface tension for the five complex systems; for each of them we obtained a straight-line relationship (see Figures 16, 21, 29, 34 and 36). The extrapolated values of the standard unitary free energy change at zero surface tension, $(-10^{21}\Delta G^{\circ}_u/N)_{r \rightarrow 0}$ is the intercept value of equation (4), that is, AG°_{SL}. G°_{SL} values obtained in this way may be measures of the S - L interaction per unit area. The slope yields the quantity $(k_S + k_L)$. From the free energy-surface tension plots (Figure 37), we can see that the slope is not the same for all of these five complex systems. Unfortunately, there is no simple way to separate $(k_S + k_L)$ into individual contributions. (One way would be to study a dimer, in which $k_S = k_L$, but we have not included such a species.) The slopes and the intercepts of the standard unitary free energy change-surface tension plots are shown in Table XXXVII.

The vertical deviations of the points from the line in Cohen's (4) free energy-maximal overlap area plot

TABLE XXXVII

Slope and Intercept Values of the Standard Unitary Free Energy-Surface Tension Plot for the Complex System

Complex System	Area[f] (A^2/molecule)	Intercept ($-AG^{\circ}_{SL}$) (x 10^{21} cal/molecule)	$-G^{\circ}_{SL}$ (x 10^{23} cal/A^2)	(Slope)[g]
Me Cinn-T[a]	49.0	2.90	5.90	- 5.57
Me Cinn-8CT[b]	47.8	3.15	6.60	- 5.50
Naph-T[c]	49.8	5.30	10.60	- 4.00
Me Cinn-T[d]	50.2	1.25	2.49	- 7.71
Me 2-Naph-8NT[e]	55.6	6.57	11.84	- 3.79

[a]Methyl cinnamate-theophylline complex system

[b]Methyl cinnamate-8-chlorotheophyllinate complex system

[c]Naphthalene-theophylline complex system

[d]Methyl cinnamate-theophyllinate complex system

[e]Methyl 2-naphthoate-8-nitrotheophyllinate complex system

[f]maximal overlap area

[g]in units of 10^{23} (cal/molecule) (dynes^{-1} cm).

(shown in Figure 38) are given in Table XXXVII. If we plot these deviations from Cohen's (4) free energy-maximal overlap area line against G°_{SL} (which is obtained by dividing the intercept value, AG°_{SL}, by the maximal overlap area, A), a straight line is obtained. This plot is shown in Figure 43. The equation of the line in Figure 43 is as follows:

$$\text{"Deviation from line"} = m\, G^{\circ}_{SL} + b$$

where m is found to be +20.6 A^2/molecule and b is found to be $+1.5 \times 10^{-21}$ cal/molecule. This relationship appears to indicate that the line in Cohen's (4) free energy-maximal overlap area plot is a very good average of all effects, since if we change the slope of his plot by $\pm 5\%$ (without changing the zero ordinate intercept) a scattering of points is obtained.

Since this "deviation"-G°_{SL} plot covers the range from -2.49 to -11.84 ($\times 10^{-23}$ cal/A^2) in G°_{SL}, it seems acceptable to use this plot to obtain by interpolation the substrate-ligand interaction forces, G°_{SL}, for other complex systems in this region. Some of the S - L interaction values of the complex systems obtained from this plot are shown in Table XXXIX. These figures lead to an interpretation that is in conflict with a conclusion of Cohen (4). Consider No. 16 (methyl cinnamate-theophylline) and No. 43 (methyl cinnamate-theophyllinate)

Figure 43. Plot of the vertical deviations of Cohen's (4) free energy-maximal overlap area plot against G°_{SL} (data given in Tables XXXVII and XXXVIII).

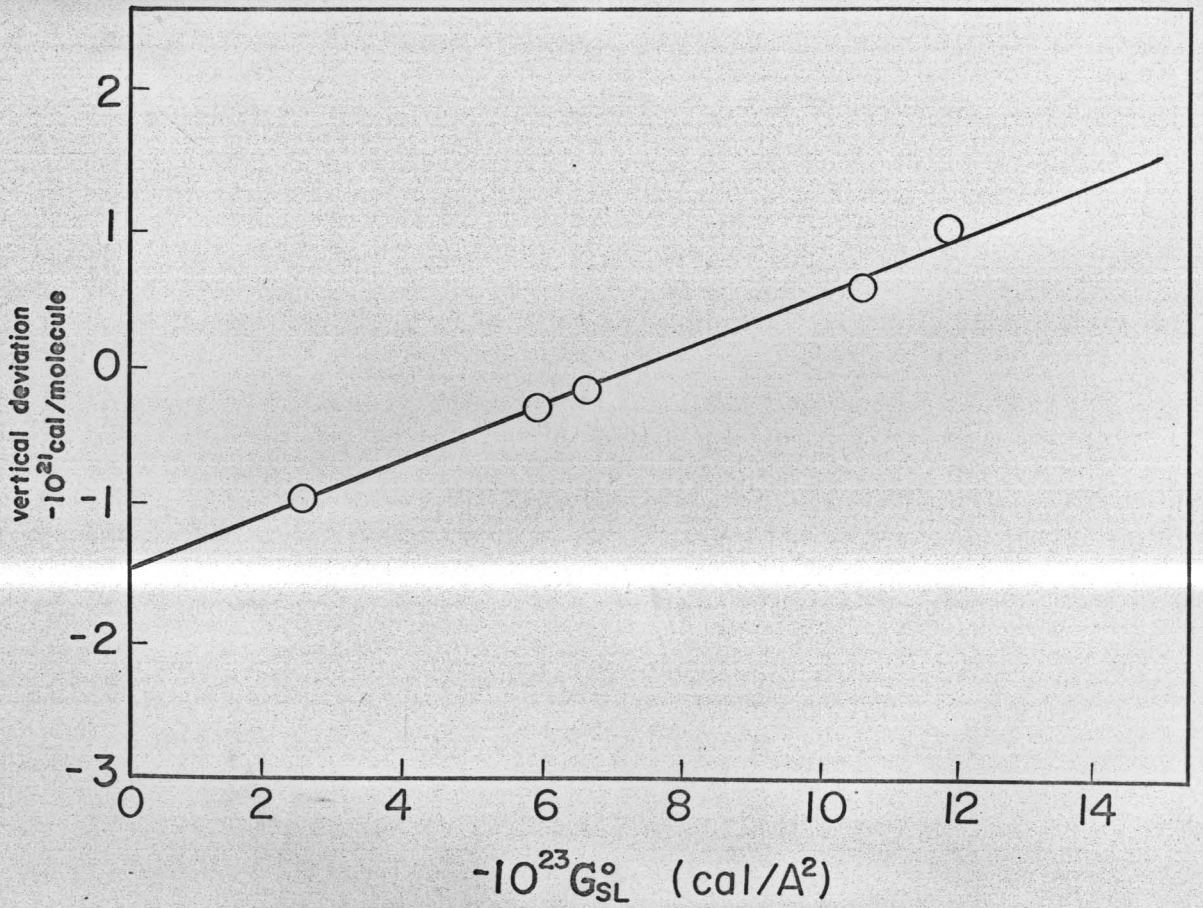

vertical deviation
-10^{21} cal/molecule
2
1
0
-1
-2
-3
0
2
4
6
8
10
12
14
$-10^{23} G^{\circ}_{SL}$ (cal/A^2)

TABLE XXXVIII

Vertical Deviations from Cohen's (4) Free Energy-Maximal Overlap Area Plot

No.[a]	Complex System	$(-10^{21}\ \Delta\mathring{G}u/N)$[g] Observed	Calculated[h]	Deviation
16	Me Cinn-T[b]	7.00	7.30	- 0.30
30	Me Cinn-8CT-[c]	6.94	7.12	- 0.18
10	Naph-T[d]	8.00	7.42	+ 0.58
43	Me Cinn-T-[e]	6.50	7.48	- 0.98
29	Me 2-Naph-8NT-[f]	9.30	8.28	+ 1.02

[a]Complex system No. in Cohen's (4) plot (see Figure 38)

[b]Methyl cinnamate-theophylline complex system

[c]Methyl cinnamate-8-chlorotheophyllinate complex system

[d]Naphthalene-theophylline complex system

[e]Methyl cinnamate-theophyllinate complex system

[f]Methyl 2-naphthoate-8-nitrotheophyllinate complex system

[g]in units of (cal/molecule)

[h]calculated with the equation of Cohen's average line (see Figure 38)

TABLE XXXIX

Substrate-Ligand Interaction Forces for Some of the Complex Systems at 25.0°

		$-G^{\circ}_{SL}$ ($\times 10^{23}$ cal/A^2)	
Substrate	Ligand	Neutral form	Anionic form
Methyl cinnamate	theophylline	5.90[a]	2.49[b]
Naphthalene	theophylline	10.60[c]	5.35[d]
Methyl 1-naphthoate	theophylline	9.05[c]	4.05[f]
Methyl 2-naphthoate	theophylline	10.75[g]	6.20[h]

In Cohen's (4) free energy-maximal overlap plot in Figure 38, the numbers for these complex systems are as follows:

[a]No. 16

[b]No. 43

[c]No. 10

[d]No. 46

[e]No. 5

[f]No. 42

[g]No. 15

[h]No. 41

complex systems. The difference in overall stability is $-(7.0 - 6.5) \times 10^{-21} = -0.5 \times 10^{-21}$ (cal/molecule) (refer to Table XXXVIII). But the S - L contribution, calculated as the difference in ΔG^{o}_{SL}, decreases by $-(2.9 - 1.25) \times 10^{-21} = -1.65 \times 10^{-21}$ (cal/molecule) (refer to Table XXXVII), or -1.15×10^{-21} cal/molecule more than the overall decrease. The difference of 1.15×10^{-21} then must be offset by an increase in the solvent contribution, i.e., the combined solvent repulsive forces appear to increase upon ionization of the ligand. This is the opposite conclusion to the one Cohen (4) came to, in which he attributed the decrease in K_{11} to increased solvation of L upon ionization. These effects also can be seen for the other systems with a common ligand, theophylline, both in neutral and anionic forms, and the substrates such as naphthalene, methyl 1-naphthoate, and methyl 2-naphthoate (Table XXXIX). These all show a large decrease (the mean value of the ratio of the rate constants being 2.14 ± 0.35) in the S - L interaction upon ionization of the ligand.

It is interesting to note that if the vertical deviation in Figure 43 is more than -1.5 units, then apparently the S - L interaction is repulsive, but there is no system with this feature in Cohen's (4) plot for 50 complex systems.

It must be pointed out that this quantitative separation of the overall free energy change for complex formation into contributions from the S - L interaction and from solvent interaction forces is very speculative, and the preceding paragraph merely illustrates a potential application of such a partitioning of effects. Weaknesses of the approach are obvious. For example, the assumption that the solvent effect in determining complex stability is eliminated by linear extrapolation to zero surface tension may not be satisfactory (in fact, extrapolations to zero interfacial-tension give different quantitative results). In order to buttress this approach, it will be necessary to discover independent relationships between (for example) the quantitative G°_{SL} isolated by this procedure and other quantities that measure intermolecular interactions. Nevertheless, the method has some interest as a potential means for isolating the solvent contribution from the overall interaction energy in complex formation equilibria.

C. Summary

Earlier work in this laboratory (4,12,22,29) led to an empirical linear relationship between the standard unitary free energy change for complex formation and the

maximal area of plane-to-plane overlap between substrate S and ligand L in aqueous solutions. This was interpreted in terms of a simple model and the associated equation,

$$\Delta G^{\circ} = A\,(G^{\circ}_{SL} - G^{\circ}_{MS} - G^{\circ}_{ML})$$

where A is the maximal overlap area and G°_{IJ} is the free energy of interaction per unit area at equilibrium between the "surfaces" I and J (M represents the medium). The empirical slope is equivalent to 64 dynes/cm. Cohen (4) concluded that the term for interaction between S and L, G°_{SL}, is unimportant in comparison with the solvent interaction terms, G°_{MS} and G°_{ML}. This simplified view provided a general first-order description of molecular complex formation in aqueous solution. The deviations from the average line were ascribed to significant second-order effects, perhaps associated with more subtle variations in structure-stability relationships.

The present study started with the suggestive observation that the theoretical and empirical slopes of the area correlation both indicate possible dependence of complex stability on solvent surface tension, or some property related to it. It was found that the stability of the typical molecular complex between methyl trans-cinnamate and theophylline appeared to be

determined only by the surface tension of the solvent for various mixed solvents containing water. Among the solvents used were aqueous binary mixtures with methanol, ethylene glycol, acetonitrile, and dioxane, as well as aqueous solutions of sodium chloride and lithium chloride. Deuterium oxide gave consistent results.

A linear relationship was found between standard free energy changes for the complexation equilibrium and the surface tension of the solvent. This was extrapolated to zero surface tension presumably eliminating the solvent contribution to the overall complex stability. This extrapolation procedure was applied to five complex systems: methyl trans-cinnamate-theophylline; methyl trans-cinnamate-theophylline anion; methyl trans-cinnamate-8-chlorotheophylline anion; naphthalene-theophylline; and methyl 2-naphthoate-8-nitrotheophylline anion. The extrapolated intercepts for these complexes were found to be linearly related to their vertical deviations from Cohen's (4) average line in the maximal overlap area correlation.

If this extrapolation and separation procedure is valid, the extrapolation leads to the quantity ΔG°_{SL}. Comparison of this with the overall free energy change suggests that the S - L interaction may be responsible for a significant (even a major) portion of the complex

stability. Among the assumptions governing this quantitative separation of solvent and intermolecular effects on complex stability are: (1) the overall effect can be partitioned into additive contributions; (2) the extrapolation to zero surface tension eliminates the solvent contribution. At the present time the stability-surface tension correlation appears to be a useful empirical observation, while the solvent effect separation procedure is a potentially valuable approach that must be subjected to future testing before its quantitative results can be accepted.

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APPENDIX