Solvent Interactions in Some Conformation-Regulating Solvents for Polypeptides and Proteins[1,2]

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Abstract: Conductivities, dielectric constants, and infrared spectra have been studied for binary mixtures of anhydrous formic acid with acetonitrile, dioxane, ethylene dichloride, formamide, N-methylformamide, N,N-dimethylformamide, acetamide, N-methylacetamide, N,N-dimethylacetamide, and dimethyl sulfoxide. All of these mixtures can act in regulating polypeptide conformation. There is a very marked conductance maximum at 80% formic acid (by volume) in each of the highly protic diluents. There is no evidence of amide group protonation in any of the infrared spectra. Spectral shifts corresponding to hydrogen bonding interactions predominate. The extent of hydrogen bonding between acid and the amide or dimethyl sulfoxide appears to be directly related to the basicity of the carbonyl or sulfoxyl group. The methyl substitution on the carbonyl has a large effect whereas substitution on the amide N has a lesser effect. Hydrogen bonds form between the amide or sulfoxyl oxygen and the hydroxyl group of the acid. These data imply that anhydrous formic acid is a differentiating solvent for amides, in contrast to the stronger acids, trifluoroacetic and dichloroacetic, which have a leveling effect. The formic acid–amide systems may thus be useful in probing for differences in peptide bond helix forming ability in proteins and polypeptides.

The solvent environment of proteins and polypeptides has a direct effect on the equilibrium constant characterizing the order $\rightleftharpoons$ disorder transconformation reaction. A large number of binary nonaqueous solvent pairs have been used as regulators of polypeptide conformation,[4] the stable equilibrium conformation depending upon the relative amount of each solvent in the mixture. One of the components in these mixtures is usually a proton donor, the other a weak proton acceptor or an inert aprotic diluent. In strongly protic solvent systems it has been assumed that direct protonation at the peptide bond[5,6] converts the polypeptide into a polymer with backbone polyelectrolyte character. This factor is so great that it overwhelms other aspects of structure stabilization and is the predominating factor in randomization of the polypeptide. In the course of the polypeptide disordering reactions the internal peptide hydrogen bonds and the hydrophobic interactions are disrupted and rearranged.

In nonaqueous chemistry the concepts of "leveling" and "differentiating" solvent systems are widely used. In order to "differentiate" between protic acids a poor proton acceptor must be used. Likewise, a weak acid (poor proton donor) must be used to "differentiate" between protic bases. Peptide groups, and other substituted amides, can obviously act as weak proton acceptors.[4–6] In weakly protic solvent systems, solvent–peptide interactions probably depend in large measure upon a competition between solvent and the internal interactions of the polypeptide. Thus, one might expect that by a proper adjustment of the solvent composition, stepwise conformational transitions could be achieved. Furthermore, if one could correlate solvent–solvent interactions with the conformational transition conditions required for particular types of peptide systems, then new insights into the stabilities of different regions of proteins and polypeptides might also be gained. The first requirement for realizing such a detailed mapping of peptide bond character is a thorough understanding of a suitably "differentiating" set of solvent systems.

As the data discussed below indicate, mixtures of anhydrous formic acid with a variety of proton acceptors or with aprotic diluents make up one useful differentiating system. This paper deals exclusively with a description of the nature of the solvent–solvent interaction in such mixtures. The direct demonstrations of the correlation of these interactions with peptide conformation transitions will be treated in another report, since these latter studies involve a quite different set of techniques and materials.

Experimental Section

Materials. Formic acid from Eastman Organic Chemicals was obtained in 97% purity. The anhydrous acid was prepared[7] by distilling the 97% acid from boric acid at 22° (3–8 mm). The specific conductivity, $K_{25°}$, was 5.90–6.35 $\times$ 10^{-5} ohm^{-1} cm^{-1}; dielectric constant, $\epsilon_{25°}$ 57.91.[8,9]

Dimethyl sulfoxide from McKesson and Robbins, Inc. was redistilled at 56–58° (5–7 mm), $n^{22}D$ 1.4785 (lit.[10] $n^{21}D$ 1.4787).

The amides used, with the exception of dimethylformamide, were purchased from Eastman Organic Chemicals. These reagents were redistilled under the following conditions: N-methylformamide, 102–103° (20 mm), $n^{20}D$ 1.4317 [lit.[11] 1.4310 (25°)]; N-methylacetamide, 160–165° (15 mm), $n^{25}D$ 1.4301 [lit.[12] 1.4301 (25°)]; N,N-dimethylacetamide, 83–84° (32 mm), $n^{22}D$ 1.4362 [lit.[13] 1.4358 (25°)]; and N,N-dimethylformamide (from Fischer

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(3) Work done in part during tenure of a postdoctoral fellowship, National Institutes of Health Grant No. 12-14-100-7656.

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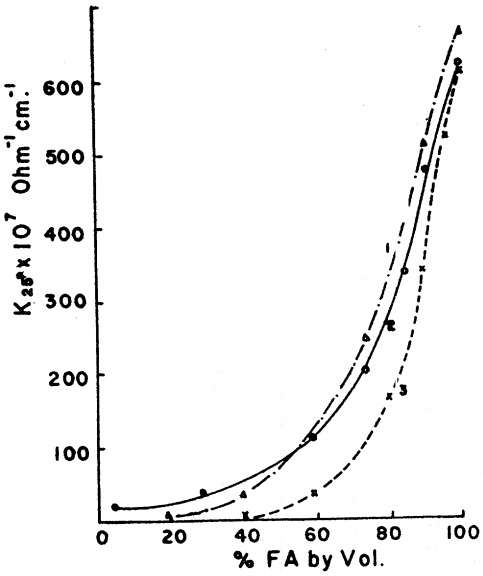

Figure 1. Specific conductances of formic acid–aprotic diluent mixtures as a function of acid composition by volume at 25°: curve 1, formic acid–acetonitrile; curve 2, formic acid–ethylene dichloride; and curve 3, formic acid–dioxane.

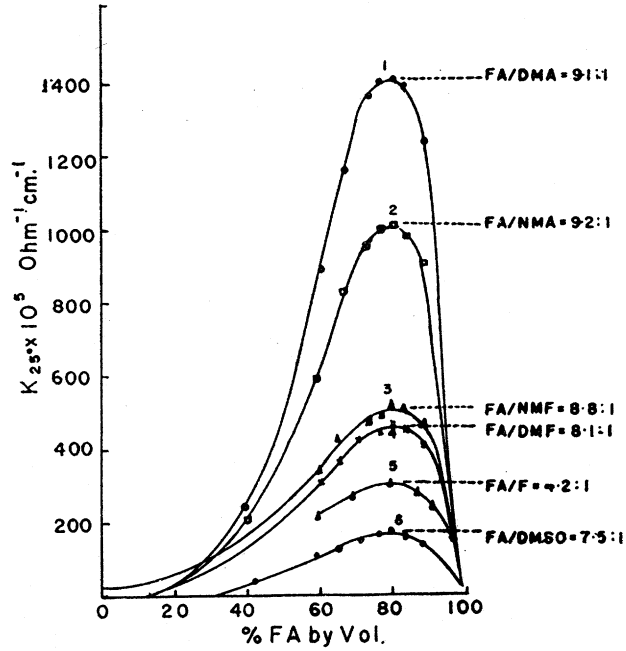

Figure 2. Specific conductances of formic acid–protic diluent mixtures as a function of acid composition by volume at 25°: curve 1, formic acid–dimethylacetamide; curve 2, formic acid–N-methylacetamide; curve 3, formic acid–N-methylformamide; curve 4, formic acid–dimethylformamide; curve 5, formic acid–formamide; and curve 6, formic acid–dimethyl sulfoxide. The notations on the right side of the figure indicate mole ratios of the various formic acid solutions at each conductivity curve maximum.

Scientific Co.), 76° (39 mm), $n^{22}D$ 1.4292 [lit.[14] 1.4290 (22°)]. All other chemicals were reagent grade and used without further treatment.

Conductance. An Industrial Instrument Model RC-16B-1 conductivity bridge was used to measure resistance in the range 0.2–2.5 × 10^6 ohms with an accuracy within ±1% of the reading over all except the extreme ends of the scale. The conductivity cell, with a constant of 0.9425 cm^{-1} at 25°, had unplatinized electrodes and was used to measure specific resistance in the range from 200 to 200,000 ohms.

Dielectric Constant. Measurements were made with a Sargent Model V oscillometer, an instrument which operates on the resonance frequency principle at 10 Mc. The cell was of glass, 10 ml in volume, with concentric external rhodium coated areas and a capacitance range of 135–145 $\mu\mu$f. Since a number of solutions had both high dielectric constants and high conductance, the cell constants were determined using both methanol and water and saturated KCl and water. The cell constants were similar, but not identical with these two calibration procedures. Since the specific resistance of each solution was known from conductance measurements in every case, the instrument scale reading was corrected for the contribution of the conductance to the apparent capacitance of the cell and contents by the equation

$$C = \frac{C_g(R_s^2\omega^2C_0^2\epsilon^2 + 1)}{R_s^2\omega^2C_gC_0\epsilon + R_s^2\omega^2C_0^2\epsilon^2 + 1} - \frac{C_gC_0}{C_g + C_0}$$

In this equation, C is the measured capacitance change, in farads; C_0, the capacitance of the empty cell containing only air; C_g, the capacitance due to the cell walls; R_s, the resistance of the solution in question, in ohms; ω, the angular frequency in radians per second ($2\pi f$, where f is the frequency in cycles per second); and ϵ, the desired dielectric constant corrected for conductance effects. For highly conducting solutions C and C_g have very similar values; hence the precision of the evaluation of ϵ drops off markedly as R_s decreases. The values of ϵ for the conducting solutions must be treated as approximations only. The instrument scale readings were calibrated with the aid of a General Radio 1401-A 100-$\mu\mu$f standard air capacitance ±0.05%.

Infrared Spectra. Spectra were obtained in the 4000–800 cm^{-1} region using either a Beckman IR-12 spectrophotometer or a Perkin-Elmer Model 21 spectrophotometer in cells with NaCl windows. Absorption spectra in the 1.4–1.6-μ region were obtained with a Cary Model 14 recording spectrophotometer. Quartz cells with optical paths of 0.1–1.0 cm were used.

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Volume of Mixing. The densities of the pure substances and of the solution mixtures were measured at 25.00 ± 0.01° in a 25-ml pycnometer similar to that of Mathot and Desmyter.[15] All weighings were reproducible to within ±0.04 mg. The pycnometers were calibrated with conductivity water, the density of which was calculated from the equation of Tilton and Taylor.[16] The conversion factor used to convert the density to units of g/cc was 0.999972 ml/cc. The molar volume change on mixing, ΔV_M, was computed from the equation

$$\Delta V_M = \frac{M_1X_1 + M_2X_2}{\rho} - (V_1^0X_1 + V_2^0X_2)$$

where ρ = the measured density (g/ml), M_1 and M_2 the molecular weights of the solution components, X_1 and X_2 their mole fractions and V_1^0 and V_2^0 the molar volumes of the pure components.

Preparation of Solution. All solutions were made up by volume. Freshly distilled materials were used for mixing. Although all early measurements were made on solutions mixed under nitrogen in a drybox, it was found that comparable results could be obtained if the solutions were mixed quickly at room temperature under normal laboratory conditions. The purity of pure components and solutions were repeatedly checked by conductivity measurements. A given anhydrous formic acid solution was stable for days if stored properly in a desiccator.

Results

All of the systems examined have formic acid (FA) as the strongest proton donor present in mixture with proton acceptors of varying strength. Conductivity data, Figures 1 and 2, clearly indicate that two distinct classes of component interactions can be distinguished. Aprotic diluents, such as acetonitrile, dioxane, and ethylene dichloride on the one hand show more or less ideal mixing behavior. The conductivities of the mixture

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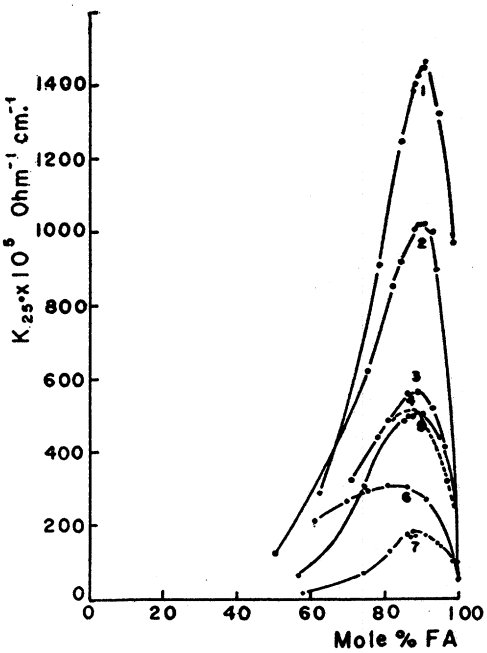

Figure 3. Specific conductances of formic acid–protic diluent mixtures as a function of mole percentage of acid in mixtures at 25°: curve 1, formic acid–dimethylacetamide; curve 2, formic acid–N-methylacetamide; curve 3, formic acid–acetamide; curve 4, formic acid–N-methylformamide; curve 5, formic acid–dimethylformamide; curve 6, formic acid–formamide; and curve 7, formic acid–dimethyl sulfoxide.

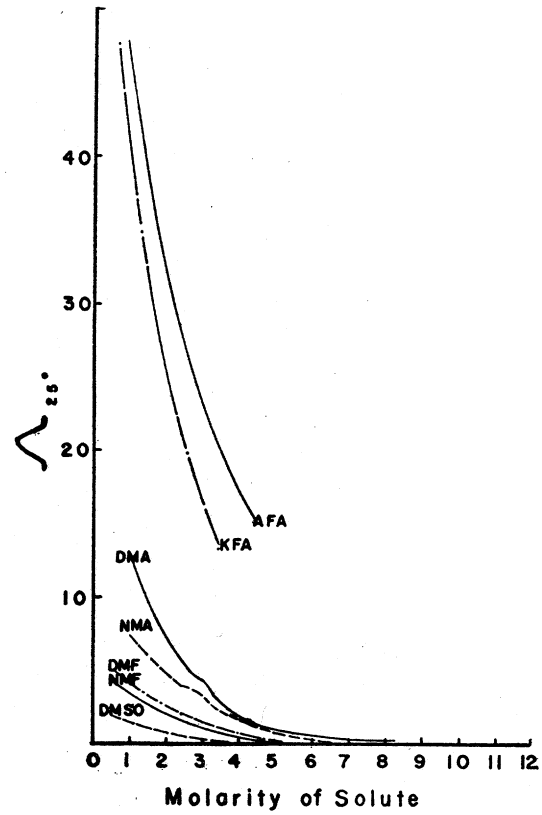

Figure 4. Equivalent conductances of formic acid–protic diluent mixtures as a function of molar concentration of solute at 25°.

drop continuously as the diluent concentration is increased over the entire mixing range (Figure 1). In striking contrast, stronger bases or proton acceptors such as dimethyl sulfoxide (DMSO), formamide (F), N-methylformamide (NMF), N,N-dimethylformamide (DMF), acetamide (A), N-methylacetamide (NMA), and N,N-dimethylacetamide (DMA), all showed a very prominent conductivity maximum at a formic acid content about 80% by volume (Figure 2). The numbers in the figure denote the mole mixing ratio at the conductivity maximum in each case. It is of interest that none of these maxima occur at the anticipated 1:1 mole mixing ratio. This point is emphasized in Figure 3, a plot of the specific conductance of each mixture as a function of the mole % of FA. Acetamide is the only one of the compounds which is normally a solid at room temperature but its behavior is the same as that of the other amides. Among the seven mixtures studied where specific conductivity maxima were found, the relative magnitude of the maxima were in the order: DMSO < F < NMF < DMF < A < NMA < DMA.

Assuming that proton transfer reactions would be most favored at high FA content, the equivalent conductances of the FA–amide and FA–DMSO solutions were determined and compared with the equivalent conductances of the salts, potassium formate and ammonium formate in FA over the same concentration range. The resultant data, Figure 4, show these latter equivalent conductances to be much higher, and indicate that even at the conductance maxima the free ion concentrations must be very low in all the solvent mixtures examined. By expanding the lower five curves in Figure 4 it could be seen that each curve has an inflection point at a solute concentration which corresponds closely to the 80% by volume FA mixtures and the specific conductivity maxima.

One of the distinctive characteristics of interactions in mixed systems is a volume change on mixing.[17,18] In a mixture where the solvent has a permanent dipole moment, the introduction of ions where none existed before usually leads to a larger electrostriction in the solvent and hence a decrease in mixture volume. Hydrogen bond formation may also lead to a volume change but the effect is generally quite small and may be masked by other factors such as differences in molecular sizes. The volume changes on mixing of FA–dioxane, FA–DMSO, and FA–DMF at 25° were determined. The volume change on mixing of FA–DMF at 100° was calculated from the density data of Korolkova and Krichevtsov.[19] The results are summarized in Table I. It appears that ΔV_M at 25° is very small in every case over the entire mixing range. At 100° ΔV_M for FA–DMF does show a slight but significant decrease which is larger at higher acid concentrations. These data can be readily interpreted as due to a small extent of ionization occurring at the elevated temperature. Hanlon, *et al.*,[5] found larger values for volume changes where protonation had occurred.

Infrared spectra have been of considerable use in the study of ion formation and hydrogen bonding in solution.[5,20–27] It has been generally recognized that the

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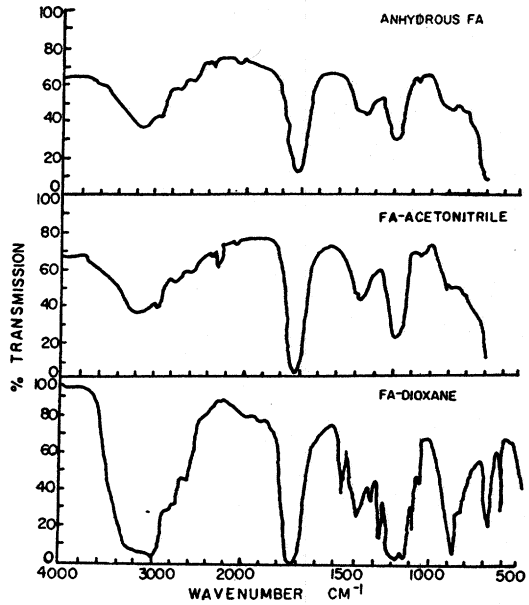

Figure 5. Infrared spectra of formic acid and the aprotic diluent mixtures: upper spectrum, anhydrous 100% formic acid; middle spectrum, formic acid–acetonitrile, 50:50 (v/v); lower spectrum, formic acid–dioxane, 50:50 (v/v). Pertinent spectral features: ~3000 cm^{-1}, bonded OH stretch; 1735 cm^{-1}, C=O stretch of FA.

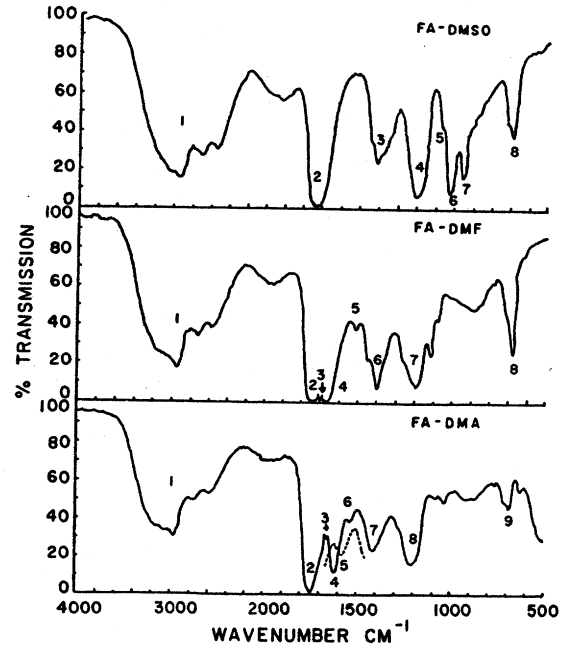

Figure 6. Infrared spectra of formic acid and the protic diluent mixtures: upper spectrum, formic acid–dimethyl sulfoxide, 50:50 (v/v); middle spectrum, formic acid–dimethylformamide, 50:50 (v/v); lower spectrum, formic acid–dimethylacetamide, 50:50 (v/v). Pertinent spectral features: ~3000 cm^{-1}, bonded OH stretch; 1735 cm^{-1}, C=O stretch of FA; 1130 cm^{-1}, S=O stretch; 1015 cm^{-1}, S=O···H stretch; 1680 cm^{-1}, C=O stretch of amide; 1650, C=O···H stretch of amide.

C=O group of an anhydrous saturated carboxylic acid has its stretching frequency between 1700 and 1780 cm^{-1}.[21] The most intense, characteristic C=O frequency of a carboxylate ion, however, is found at between 1550 and 1610 cm^{-1} with an extinction coefficient, $\epsilon \sim 300$ cm^2/mole.[22,23] Klotz and co-workers[5,27] have reported that the ion formed due to protonation of an N-monoalkyl-substituted or unsubstituted amide has an absorption in the near-infrared at about 1.51 μ with an extinction coefficient, $\epsilon \sim 200$ cm^2/mole. Accordingly, the spectra of a large number of FA–diluent mixtures were run in both the medium and near-infrared regions. Representative spectra are shown in Figures 5 and 6. Figure 5 illustrates the spectra of 50:50 (v/v) mixtures of the noninteracting type, FA–acetonitrile and FA–dioxane. Figure 6 shows the interacting system spectra at this same concentration. The dashed line in the lower spectrum of Figure 6 shows the position of the characteristic formate ion absorption, obtained using 2.1 *M* ammonium formate in FA. It is evident that the formate ion spectral peak at 1572 cm^{-1} is absent in all the FA mixtures. Several near-infrared spectra of FA–NMF and FA–NMA mixtures are shown in Figures 7 and 8. It is evident in these spectra that the protonated $-NH_2^+$ peak at 1.51 μ is absent.

Table I. Volume of Mixing of Formic Acid–Organic Solvent Mixtures

Solutions	FA mole fraction	ΔV_M, ml/mole
FA–dioxane (25°)	0.492	0.124
	0.601	0.077
	0.900	0.029
FA–DMSO (25°)	0.447	0.023
	0.815	−0.020
	0.945	0.020
FA–DMF (25°)	0.338	−0.046
	0.754	0.007
	0.891	0.015
FA–DMF[a] (100°)	0.35	−0.137
	0.70	−0.213
	0.90	−0.331

[a] Korolkova and Krichevtsov.[19]

The lowering of certain group stretching frequencies when these are associated in hydrogen bonding is well known. For example, it has been demonstrated that in the medium infrared region the stretching frequencies of both S=O and C=O will be displaced to lower frequencies when these groups are hydrogen bonded.[25,26] Furthermore, the frequency shift of the C=O stretching modes for groups involved in hydrogen bonding is a monotonic and nearly linear function of the O–H···O distance or bond strength. It thus seemed likely that these relationships would be useful in detecting the presence of extensive hydrogen bonding in our FA–amide and DMSO systems. The self-proton donating capacity of the amide–NH groups in unsubstituted or N-monoalkyl-substituted amides precludes the examination of such systems in this fashion. However, Hanlon, *et al.*,[5] recently found that in the near-infrared

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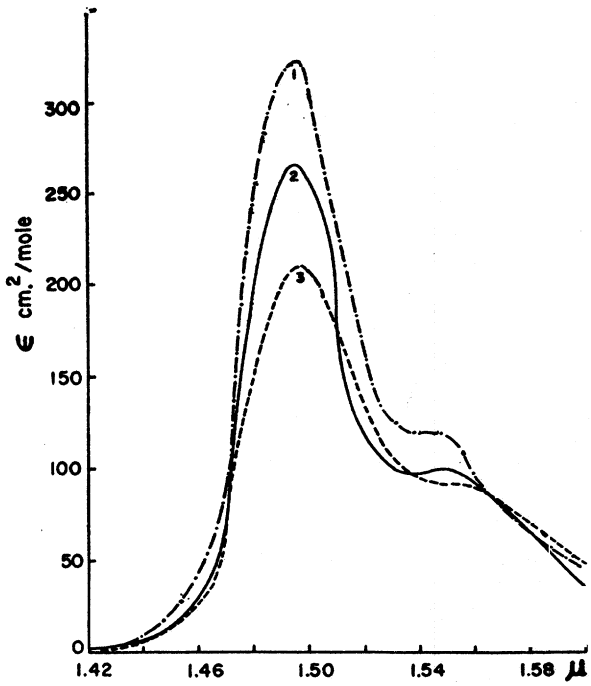


Figure 7. Absorption spectra of N-methylformamide in formic acid: curve 1, 85% formic acid by volume; curve 2, 60% formic acid by volume; and curve 3, 30% formic acid by volume.

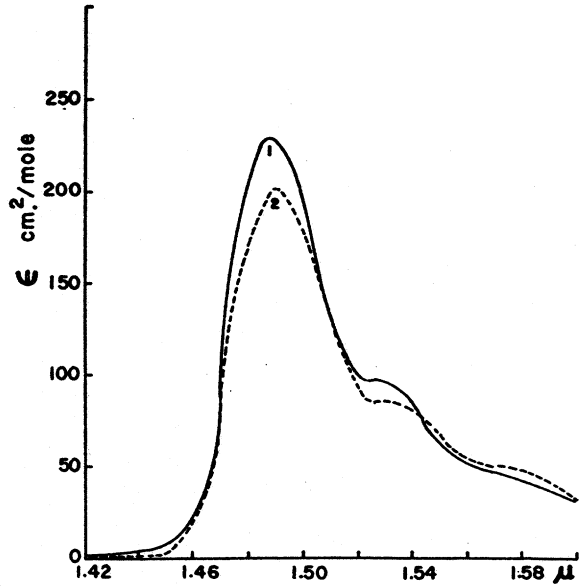


Figure 8. Absorption spectra of N-methylacetamide in formic acid: curve 1, 86% formic acid by volume; and curve 2, 50% formic acid by volume.

region the hydrogen bonding between NH and C=O of either self-associated amides (homogenic association, absorption at 1.53–1.54 μ and 1.57–1.58 μ) or between an amide and another type of proton acceptor (heterogenic association, absorption at 1.48–1.49 μ) can be studied. The relative strengths of these interactions are reflected in changes in the molar extinction coefficients at different mixture compositions. With these comments in mind, we can now examine the spectra of Figures 5 and 6 in more detail.

The aprotic diluents are generally unaffected by the formic acid. The spectral bands of the ether groups in dioxane (1130, 1187, 1052, and 1018 cm^{-1}) and the C≡N group of acetonitrile (2250 cm^{-1}) were not shifted upon the addition of formic acid (Figure 5). However, alone of all diluents examined, acetonitrile led to a marked diminution of the hydrogen-bonded OH stretch at ~3000 cm^{-1}, seen in pure anhydrous formic acid as the result of dimerization. The free OH absorption, which is weaker than the bonded OH stretch, was not observed. It is evident that acetonitrile has a considerable effect in breaking down FA dimers.

With the more highly protic diluents the most prominent spectral shift in the infrared is the appearance of an absorption corresponding to hydrogen-bonded sulfoxyl or carbonyl groups and the disappearance of the nonbonded C=O or S=O absorption. The stretching frequency of the sulfoxyl group in liquid DMSO is 1057 cm^{-1}. As shown in Figure 9a, the addition of only 5% FA to DMSO causes a spectral perturbation and the appearance of the 1021-cm^{-1} band. With increasing FA concentration, the hydrogen-bonded sulfoxyl peak increases in intensity and is shifted progressively to still lower frequency. At 60% FA (Figure 9b) the free sulfoxyl peak is very small. This peak disappears completely at 80% FA.

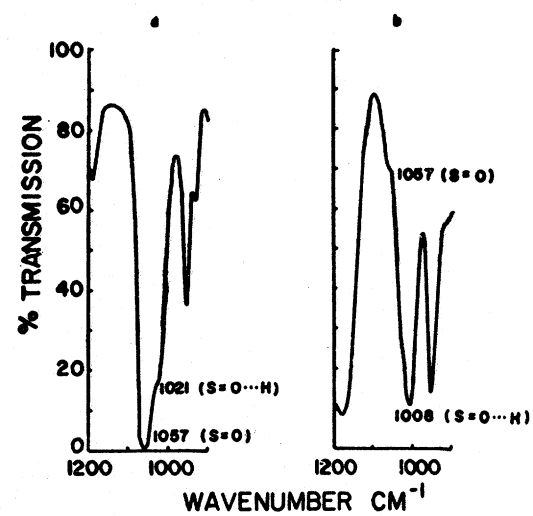


Figure 9. Infrared spectra of formic acid–dimethyl sulfoxide mixtures showing the influence of the hydrogen bonding on the S=O stretching frequency: curve a, 5% formic acid by volume; and curve b, 60% formic acid by volume.

A similar spectra displacement was found for the carbonyl group of DMF. The stretching frequency of the carbonyl group in pure DMF is ~1680 cm^{-1}. At 5% FA concentration in the mixture a small amide carbonyl–hydrogen bond peak appears at ~1620 cm^{-1} (Figure 10). The main carbonyl peak is also perturbed and shifted to ~1665 cm^{-1}. Again at 60% FA the free carbonyl peak appears to be very small whereas the hydrogen bonded carbonyl peak is quite large. The same situation also prevails in the FA–DMA system although the resolution of bonded and nonbonded amide carbonyl absorptions is not as satisfactory due to broadening of the peaks (Figure 11).

Aside from the appearance of the intense hydrogen bonded S=O or C=O absorptions at high formic acid concentrations noted above, it was also found that these hydrogen bonded spectral peaks show small but definite

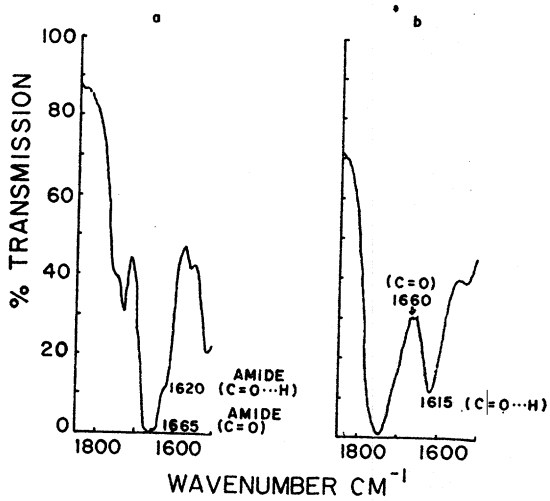


Figure 10. Infrared spectra of formic acid–dimethylformamide mixtures showing the influence of the hydrogen bonding on the C=O amide stretching frequency: curve a, 5% formic acid by volume; and curve b, 60% formic acid by volume.

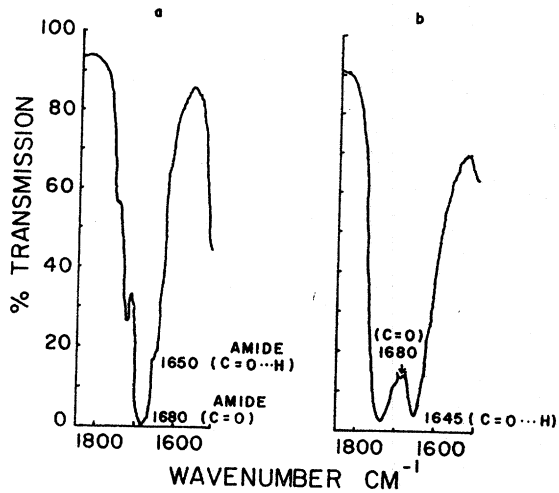


Figure 11. Infrared spectra of formic acid–dimethylacetamide mixtures showing the influence of the hydrogen bonding on the C=O amide stretching frequency: curve a, 5% formic acid by volume; and curve b, 60% formic acid by volume.

changes in spectral displacement with increasing formic acid content. These displacements are summarized for DMSO, DMF, and DMA in Table II. It can be seen that in all cases the frequencies of the hydrogen bonded spectral peaks decrease with increase in the acid concentration in the 0–80% FA range. Above 80% FA the frequencies increase again. The fact that all these peak frequencies have their minimum values at about 80% FA where the absorption is maximized indicates that these hydrogen bonding distances are the shortest and their strengths the strongest.

The near-infrared spectra, in the range of 1.45–1.60 μ, are shown in Figures 7 and 8 for NMF– and NMA–FA mixtures, respectively, at several different mixture ratios. These curves were obtained using mixtures of FA and DMF or DMA as the blanks. Thus, the OH absorption of FA in this region has been canceled out. The absorption at 1.48–1.49 μ represents the hydrogen bonding between the hydrogen of the amide N and the carbonyl of the acid.[5] The absorption at 1.54–1.56 μ

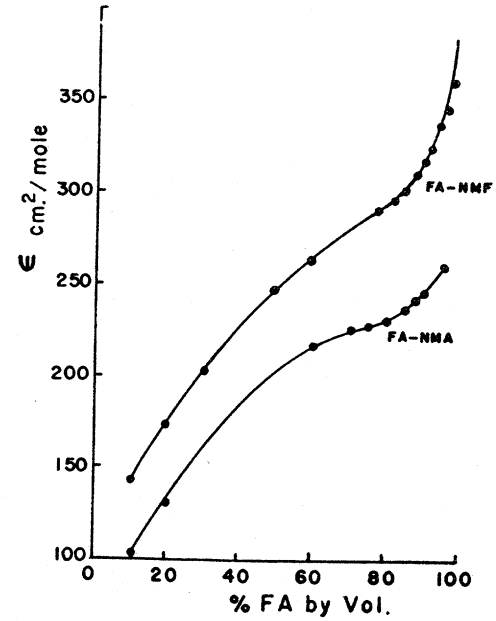


Figure 12. Variation in extinction coefficients, ϵ, of amide NH in formic acid–N-methylformamide, at 1.48 μ; and formic acid–N-methylacetamide, at 1.49 μ.

which appears at FA concentrations below 60% represents the amide self-association hydrogen bonding. It is evident in both sets of spectra that the 1.51-μ peak of a protonated amide nitrogen (NH_2^+) is absent. A plot of the molar extinction coefficient of the 1.48-μ peak is shown in Figure 12 for the entire mixing range for

Table II. Stretching Vibrations of C=O · · · H and S=O · · · H in the Infrared Spectra of Formic Acid–Dimethyl Sulfoxide, Formic Acid–Dimethylformamide, and Formic Acid–Dimethylacetamide Mixtures

FA, vol %	ν_{max}, cm^{-1} S=O···H (FA–DMSO)	C=O···H (FA–DMF)	C=O···H (FA–DMA)
0 (Free of H bonding)	1057	1680	1665
5	1021	1650	1620
15	1018	...	...
20	1015	1650	1620
40	1015	1650	1618
60	1008	1645	1615
70	1004	...	1603
80	1000	1642	1599
90	1002	1657	1604

both NMF and NMA. The values of ϵ are comparable in magnitude to those reported for the protonated amide peak at 1.51 μ and even exceed this in the NMF case at FA concentration exceeding 80%. If amide protonation had occurred to any significant extent it should have been possible to detect it in these systems. Another view of the interactions in these systems is afforded by dielectric constant measurements. Figure 13 shows the expected near-ideal behavior for the noninteracting aprotic diluents, with the dielectric constant, $\epsilon_{25°}$, increasing regularly as the concentration of FA is increased. The protic diluents exhibit marked deviations from this ideal behavior. Upon making

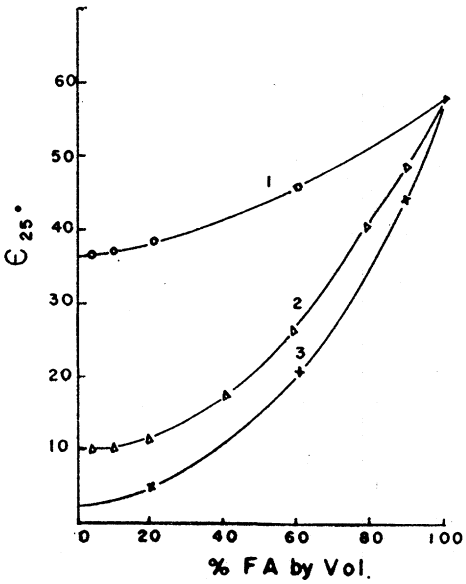


Figure 13. Dielectric constants of formic acid–aprotic mixtures as a function of acid composition by volume at 25°: curve 1, formic acid–acetonitrile; curve 2, formic acid–ethylene dichloride; and curve 3, formic acid–dioxane.

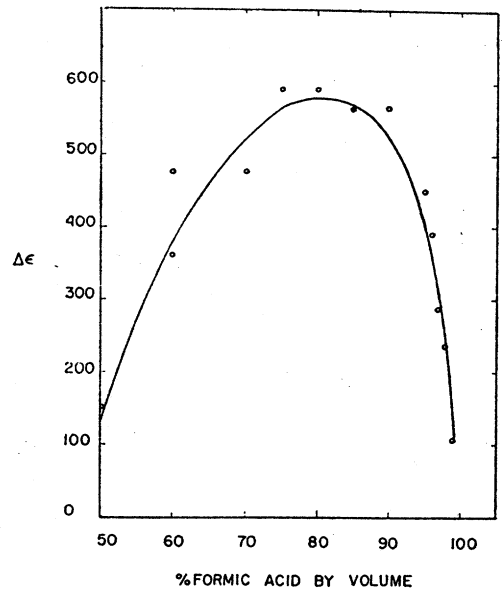


Figure 14. Dielectric increment (Δε) between formic acid–dimethyl sulfoxide solutions and aqueous KCl solutions (of the same specific conductance) *vs.* mole % formic acid in the FA–DMSO solutions. All temperatures about 24°.

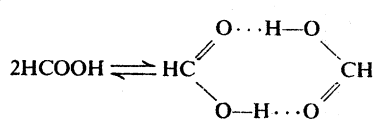
suitable corrections for the conductivity of the solutions, as outlined in the Experimental Section, it was found that ε went through a maximum in the region of the conductance maximum. The dielectric constant appears to the second power in the correction equation; hence, two values were obtained. Both sets of solutions gave real, positive values for ε, with a maximum at about 80% FA concentration. The validity of the conductance correction procedure was checked by determining the dielectric constants of standard aqueous KCl solutions up to 0.01 *M* and covering specific conductances from 10^{-5} to 10^{-2} ohm^{-1} cm^{-1}. The aqueous solutions had computed values of within 10% of the literature value and showed no regular deviations of the type seen in the FA–DMSO mixtures in the same conductance range. The FA–DMSO mixture data are shown in Figure 14. Every one of the amides behaves in a similar fashion.

Discussion

Two competing intermolecular interactions occur in pure anhydrous FA: autoionization[28]

$$2HCOOH \rightleftharpoons HCOOH_2^+ + HCOO^-$$

and hydrogen bond dimerization[29,30]



Using *o*-nitroaniline as an indicator, Hammett and Deyrup[28] found the acidity function, H_0, to be about −2 and that the molar concentration of the protonating species, $HCOOH_2^+$, was on the order of 10^{-3}. Stewart and Mathews[31] confirmed these results (H_0 = −2.19 at 30°) and demonstrated as well that the $[HCOOH_2^+]$ concentration drops sharply upon the addition of a weak base such as water. This concentration decreases to less than 0.1 of its original value on dilution of FA to 95–96%. Schaefgen and Trivisonno[32] determined, also by the *o*-nitroaniline indicator titration method, that ε-caprolactam and a number of poly-ε-caproamides are substantially (22–23%) protonated when in low concentration in anhydrous formic acid. Again, the fraction ionized decreased to very low values as the polyamide concentration was increased. Thus, in all cases examined, there is no evidence of any particular maximum in ionization at a FA concentration of 80% by volume, and protonation seems to be an unacceptable explanation for the specific conductance maxima noted with DMSO and the amides. This is confirmed most particularly in the present study by the infrared spectra of Figures 5–11, none of which show any evidence for substantial formate ion concentration in any of the mixtures or of the corresponding protonated amides or DMSO. Hanlon, *et al.*,[5] were able to pick up the protonated amide absorption in trifluoroacetic acid, a much stronger acid.

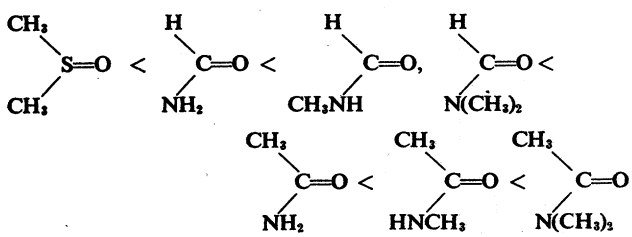
A number of investigators have demonstrated the formation of 1:1 addition compounds between FA and formamide,[33,34] NMF, DMF, and acetamide.[35] Becker and Davidson,[35] in particular, made detailed studies of the temperature–mixing composition phase diagrams, and isolated the 1:1 compounds exhibiting a maximum melting point at 50 mole % FA. Korolkova and Krichevtsov[19] and Maltese and Valentini[36] also found evidence for 1:1 addition compounds. From studies of the dissociation constants of carboxylic acids in formamide Mandel and Decroly[34] proposed the existence of hydrogen-bonded complexes between

(28) L. P. Hammett and A. J. Deyrup, *J. Am. Chem. Soc.*, **54**, 4239 (1932).
(29) R. C. Lord and R. E. Merrifield, *J. Chem. Phys.*, **21**, 166 (1953).
(30) B. W. Allen and L. E. Sutton, *Acta Cryst.*, **3**, 46 (1950).
(31) R. Stewart and T. Mathews, *Can. J. Chem.*, **38**, 602 (1960).
(32) J. R. Schaefgen and C. F. Trivisonno, *J. Am. Chem. Soc.*, **73**, 4580 (1951).
(33) S. English and W. E. S. Turner, *J. Chem. Soc.*, **107**, 774 (1915).
(34) M. Mandel and P. Decroly, *Nature*, **201**, 290 (1964).
(35) B. Becker and A. V. Davidson, *J. Am. Chem. Soc.*, **85**, 159 (1963).
(36) P. Maltese and G. Valentini, *Chim. Ind.* (Milan), **40**, 548 (1958).

acid and amide over a wide mixing range. The present infrared data are direct in confirming the presence of extensive hydrogen bonding in the FA–amide and FA–DMSO systems.

The stoichiometry of the acid–amide interaction is not clear. Although the 1:1 addition compounds form in the solid state, the infrared data suggest that the interaction is strongest at about 80% FA (volume, 85–90 mole % FA). The specific conductances of the mixtures are not maximized at the 1:1 mixing ratio, but are very prominent at 80% FA. In addition to reporting a conductivity maximum in the FA–DMF system at 80% FA, Korolkova and Krichevtsov[19] found a distinct maximum in solution viscosity at this mixing composition. As reported in Table I, neither DMF nor DMSO show any peculiarities in mixture densities over the entire mixing range.

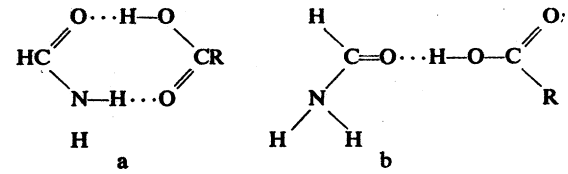
The infrared data indicate that both the carbonyl groups of the amide and sulfoxyl group of DMSO (Figures 6 and 9–11) and the amide NH (Figures 7, 8, and 12) participate in hydrogen bonding. However, the strength of the interaction at 80% FA, as indicated by the height of the conductivity maximum, seems to be governed by the substitution on the carbonyl group rather than on the nitrogen. The electronegativity of the oxygen in the carbonyl group is in the order



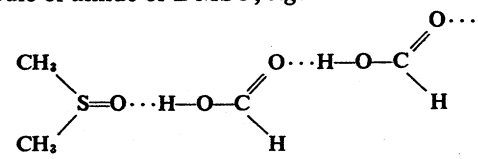
which is exactly the relative order of increasing magnitude of the conductivity maxima, with amide nitrogen methylation having a relatively rather small effect (Figure 2). A similar small effect of amide nitrogen substitution was noted by Higuchi, *et al.*[37] The fact that DMSO, DMF, and DMA fit into the same series as F, NMF, and NMA is further strong evidence of the C=O and S=O dominance in the interaction since none of the first set can act as proton donors.

Mandel and Decroly[34] proposed that carboxylic acid–amide hydrogen bonding could be depicted as a or b. At the freezing point 1:1 complexes of type a might well crystallize from the mixtures of FA and F, NMF, A or NMA, but such complexes cannot be invoked for DMF, DMA, or DMSO. Hence, the more open complexes of type b would seem to be favored.

(37) J. Higuchi, C. H. Barnstein, H. Ghassemi, and W. E. Perez, *Anal. Chem.*, 34, 400 (1962).



At FA:amide > 1 even larger transient aggregates can be easily imagined based on the equilibrium between FA–FA dimers and FA–amide complexes. Several molecules of FA could be complexed with a single molecule of amide or DMSO, *e.g.*



These proposed transient hydrogen bonded aggregates could then account for the enhanced viscosity, and conductance of the mixtures at FA:amide > 1, in the same way that hydrogen bonded transient aggregates of water molecules account for the enhanced transference number of hydronium ions. The anomalous dielectric constants found in the FA–DMSO mixtures near the conductivity maximum, after correction for conductance effects, may reflect the fact that the molecular dipoles in the aggregates are aligned so that the net aggregate dipole moments and the mixture dielectric constants are increased. If dimers were formed, as in the case of anhydrous acetic acid where $\epsilon \approx 6$ due to the dimer formation, then ϵ should not have indicated a maximum at the 80% FA concentration.

The peptide bonds in polypeptides and proteins can be considered as analogs of the simple substituted amides discussed above, and their interaction with formic acid should be similar in nature. Veis, Kaufman, and Chao[38] have demonstrated that a conformational transition in gelatin in FA–diluent mixtures follows the FA composition in each mixture in exactly the order which would be predicted from the FA–diluent interaction intensity described here. Although much remains to be done, we would like to suggest that hydrogen bond formation between formic acid and the peptide carbonyl group might be the interaction competing with the amide–formic acid interaction which ultimately regulates the conformational transition. In contrast to the stronger acid solvents, anhydrous formic acid is a differentiating solvent for amides and peptide groups. Thus, it appears possible to selectively probe differences in peptide bond basicities and polypeptide helix stabilities with these amide or DMSO systems.

(38) A. Veis, E. D. Kaufman, and C. C. Chao in "Proceedings of the International Symposium on the Conformation of Biopolymers," G. N. Ramachandran, Ed., Academic Press Inc., New York, N. Y., in press.