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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

SELF-ASSOCIATION AND HYDRATION OF N-METHYLACETAMIDE IN CARBON TETRACHLORIDE

A DISSERTATION

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Norman, Oklahoma

1966

SELF-ASSOCIATION AND HYDRATION OF

N-METHYLACETAMIDE IN CARBON TETRACHLORIDE

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SELF-ASSOCIATION AND HYDRATION OF N-METHYLACETAMIDE IN CARBON TETRACHLORIDE

CHAPTER I

INTRODUCTION

The significance of hydrogen bonding in protein structure has been well established in recent years although much is yet to be learned about the nature of the individual hydrogen bonds. Water undoubtedly plays an indispensable role, but few data are available to explain the interaction between this molecule and polar groups of proteins. Information has been obtained by such methods as protein denaturation; however, the large number and heterogeneity of peptide linkages in proteins makes the interpretation of results very difficult. For this reason a number of workers have attempted to study hydrogen bonding and hydration in proteins by working with smaller, less complicated molecules. N-methylacetamide (NMA) has been the object of much investigation because it is the smallest molecule containing a single peptide group.

At first glance we might consider the investigation of NMA in water to be the logical starting point for hydration studies. It is possible to study the self-association of the amide in aqueous solutions, but very little information on the interaction of the amide with water can be gained if water is to be used as the solvent. Part of the trouble

arises because of the necessarily high activities of water or amide and because of consequent difficulties encountered in measuring such activities. On the other hand, if the investigations could be carried out in an inert solvent, e.g. CCl_4, Henry's law would be applicable and activities could be replaced by concentrations. Both self-association and hydration constants could be calculated for such a system and the strengths of the individual hydrogen bonds could be inferred. If data of this kind were available for amides dissolved in solvents of different dielectric constant, it should be possible to make predictions about the nature of hydrated species present in aqueous media.

Several methods are readily available for studying the self-association of a compound. Infrared absorption measurements, for example, are convenient and provide good results where only monomer-dimer or monomer-trimer equilibria are involved; however,the method is less satisfactory for molecules that associate to form more complicated species. In the present investigation attention was focused on other techniques because N-methylacetamide apparently undergoes self-association to form linear chains containing any number of monomeric units, depending on the stoichiometric concentration of the solution.

Several factors were taken into account in choosing the method of investigation. First,a method was desirable which would allow measurements to be made at several different temperatures to permit the calculation of thermodynamic properties. In addition, because most proteins function normally in a rather narrow temperature range near room temperature, a method was needed which was capable of yielding accurate results

in this range. Second, because it was expected that the self-association results would be needed to correlate the hydration data, measurements were desired under conditions of controlled humidity, preferably under anhydrous conditions. Thus,the experiments needed to be carried out in an enclosed system. Third, because amides ordinarily show appreciable self-association in dilute solutions (less than 0.02 molar) a technique incorporating high sensitivity was desirable.

A method based on colligative-property measurements seemed to be best suited to fill all three requirements. Most of the measurements were carried out with a vapor-pressure lowering apparatus, and average molecular weights were calculated for the species in solution. Such calculations obviously provide self-association information because, for example, if only dimerization is involved, the calculated molecular weight must fall in a range somewhere between the monomer and dimer weight.

A second colligative-property method was also investigated to some extent although the measurements had to be carried out at temperatures above room temperature. A liquid-vapor equilibrium still was utilized to measure NMA partial pressures above solutions of the amide. Deviations of the pressures from Henry's law were then explained by self-association of the compound in the liquid phase.

Two different methods were also used to study the hydration of N-methylacetamide. In the first, solutions of the amide in CCl_4 were suspended over constant humidity solutions (dil. H_2SO_4) in closed containers whereupon water was gained or lost until the activity in the amide solution became identical to that of the acid.[61] Samples were

then withdrawn and analyzed for total water content with a coulometric Karl Fischer titrator similar to that described by Meyer and Boyd.[57] Consequently, increase in water concentration with increasing amide strength was obtained at constant water activity. These data were then analyzed in terms of hydrated species assumed to be present in solution.

The second method involved vapor-pressure measurements on CCl_4 solutions of water and the amide. When water was added to CCl_4 containing no NMA, a linear relationship was found between amount of water added and water activity (determined by measuring the increase in vapor pressure of the solution). This phenomenon was explained by assuming that water does not undergo self-association in CCl_4. On the other hand, when water was added to a solution of NMA in CCl_4, the relationship was still approximately linear, but the slope of the line was increased. By measuring water activity vs. amount of water added for a given concentration of amide, data were obtained which could be evaluated in accordance with the various hydrated species present.

In addition to the experimental studies, considerable emphasis was placed on a literature survey in which an attempt was made to correlate amide self-association with structure. It was possible to conclude that most N-mono-substituted amides, because of the location of the NHR group proton <u>trans</u> to the carbonyl group, undergo self-association to form linear chains. The experimental results of the present investigation also support this conclusion.

CHAPTER II

STRUCTURE AND SELF-ASSOCIATION OF AMIDES

INTRODUCTION

This chapter is devoted to a consideration of the structure of NMA and related compounds in the solid, liquid, dissolved, and gaseous states. Although the evidence is presented primarily to establish the structure of NMA in dilute, non-polar solutions much of it also applies to the structure of the amide group in natural polypeptides and proteins. As would be expected, the structure of amides has a marked effect on their self-association, and though little evidence is yet available, the structure undoubtedly influences the hydration of these compounds.

The structural evidence has been taken from a variety of experimental studies, and where possible, results from two or more independent sources have been compared and analyzed together. When available, self-association data for compounds in solution are included.

The compounds have been classified into unsubstituted and N-monosubstituted amides although the distinction is occasionally overlooked when it seems appropriate to discuss the two classes together. A brief section is also included on the structure of polypeptides and proteins.

UNSUBSTITUTED AMIDES

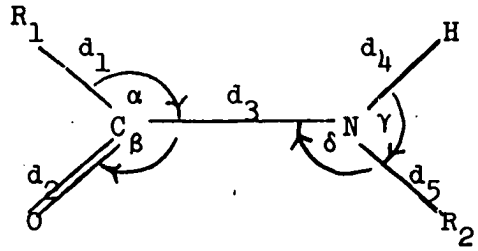
Formamide. The structure of this compound in the crystalline state at -50°C. has been established by the X-ray diffraction study of Ladell and Post.[1] Four molecules are present in a monoclinic unit cell having the dimensions a = 3.69 ± 0.01, b + 9.18 ± 0.025, c = 6.87 ± 0.02 Å, and β = 98 ± 1/4°. The space group is $P2_1/n$. The molecules are arranged to form puckered sheets with a separation between sheets of about 3.1 Å. Within each sheet pairs of formamide molecules associate about centers of symmetry to form almost coplanar "bimolecular" units. The sheets are puckered as a result of tilt in the bimolecular units relative to each other and are situated approximately parallel to the (101) plane. Hydrogen bonds 2.935 Å long join the monomers together in the bimolecular units, which are in turn linked to each other by somewhat shorter bonds, 2.880 Å, to form the sheets.

The C=O and C-N covalent bonds have lengths of 1.255 ± 0.013 and 1.300 ± 0.013 Å, respectively. The latter value deviates significantly from the typical C-N bond length of 1.47 Å[2] due to a resonance structure, which can be represented by $-\overset{O}{\overset{\|}{C}}-N< \leftrightarrow -\overset{O^-}{\overset{|}{C}}=\overset{+}{N}<$. The almost coplanar structure of the group also supports this conclusion. Molecular parameters are summarized in Table 1.

Several investigators have studied the structure of formamide in the gaseous phase by recording and analyzing the microwave spectrum of the compound. In addition to formamide, Kurland and Wilson have studied the isotopic species 1,2 N di-deutero formamide and 1 N mono-deutero formamide.[3,4] From the very small positive value of the inertial defect, these workers concluded that the molecule is planar. Bond

TABLE 1

MOLECULAR PARAMETERS OF SELECTED AMIDES [a]



Molecule	R_1	R_2	Method	State	d_1	d_2	d_3	d_4	d_5	α	β	γ	δ	Structure	Ref.
Formamide	H	H	Microwave spect.	Gaseous	1.094 ± 0.025Å	1.243 ± 0.007 Å	1.343 ± 0.007 Å	0.995 ± 0.007 Å	0.995 ± 0.007 Å	103.9 ± 1.2°	123.58 ± 0.35°	118.98 ± 0.50°	....	Planar	4
			Microwave spect.	Gaseous	1.102 ± 0.010 Å	1.193 ± 0.020 Å	1.376 ± 0.010 Å	1.002 ± 0.005 Å	1.014 ± 0.005 Å	113°14' ± 40'	123°48' ± 40'	118°53' ± 40'	117°9' ± 40'	HCONis planar; CNH_2 forms a shallow pyramid	5
			X-ray diff.	Cryst.	....	1.255 ± 0.013 Å	1.300 ± 0.013 Å	....	....	....	121.5°	....	....	Dimers are formed with the 6 heavy atoms almost coplanar	1

TABLE 1 -- continued

Molecule	R_1	R_2	Method	State	d_1	d_2	d_3	d_4	d_5	α	β	γ	δ	Structure	Ref
Acetamide	CH_3	H	Electron diff.	Gaseous	1.53 ± 0.03 Å	1.21 ± 0.02 Å	1.36 ± 0.02 Å	1.02 Å (assumed)	1.02 Å (assumed)	113 ± 3°	125 ± 3°	....	107° (assumed)	R_1,C,O, and N are assumed to be coplanar	6
			X-ray diff.	Crys	1.51 ± 0.05 Å	1.28 ± 0.05 Å	1.38 ± 0.05 Å	....	....	109 ± 5°	122 ± 5°	....	....	Planar	7
N-Methyl-acetamide	CH_3	CH_3	Electron diff.*	Gaseous	1.53 ± 0.03 Å (assumed)	1.21 ± 0.02 Å (assumed)	1.36 ± 0.02 Å (assumed)	1.02 A (assumed)	1.44 ± 0.04 Å	113 ± 3° (assumed)	125 ± 3° (assumed)	....	117 ± 5°	R_1 C,O, and N are assumed to be coplanar;the H is probably trans to the C=O group	6

TABLE 1 -- continued

Molecule			Method	State	d_1	d_2	d_3	d_4	d_5	α	β	γ	δ	Structure	Ref.
			X-ray diff.	Crys.	1.536 ± 0.016 Å	1.236 ± 0.012 Å	1.290 ± 0.013 Å	....	1.465 ± 0.013 Å	116.5°	123°	....	120.5°	R_1,C,O, N, and R_2 are almost coplanar; the H is *trans* to the C=O group	8
General amide structure according to Pauling and workers			....	....	1.53 Å	1.24 Å	1.32 Å	1.00 Å	1.47 Å	114°	125°	114°	123°	Planar with H *trans* to the C=O group	9, 10

[a] Many additional examples have been cited by Pimentel and McClellan[11], Table 10-I, p. 300. A comparison between normal single and double bond lengths is given on page 304 of the same reference. See also references [12] and [2].

*d_1, d_2, d_3, d_4, α and β were assumed to be the same as in acetamide.

distances and angles were calculated and are included in Table 1. The moments of inertia are: $I_a^\circ = 6.952$, $I_b^\circ = 44.448$, and $I_c^\circ = 51.407$ amu $\mathring{A}^2$; and the inertial defect, $\Delta = I_c^\circ - I_a^\circ - I_b^\circ$, is found to be equal to 0.007 amu $\mathring{A}^2$. For a rigidly planar molecule $I_c^\circ = I_a^\circ + I_b^\circ$. The dipole moment, as determined from Stark-effect measurements, is equal to 3.71 ± 0.06 Debyes and makes an angle of 39.6° with the C-N bond. The quadrupole coupling constants, calculated from the frequency shifts of the hyperfine splittings observed for several rotational transitions are: $\chi_a = 1.9$mc, $\chi_b = 1.7$mc, and $\chi_c = -3.6$mc.

Costain and Dowling[5,13] studied the microwave spectra of 10 isotopic species of formamide and found, in contrast to the work of Kurland and Wilson, quite anomalous results for the inertial defects explainable only by postulating a slightly nonplanar structure in which the CNH_2 group takes the form of a shallow pyramid. The two hydrogen atoms of the NH_2 group apparently move in a direction perpendicular to the principal plane, giving rise to different equilibrium positions for the H atoms on opposite sides of the plane. This effect is well known for many molecules and is best understood for ammonia.[14] The dihedral angle between the H'NC plane (H' <u>trans</u> to the formyl hydrogen) and the NCO plane is $7 \pm 5°$, and the angle between the H"NC plane (H" <u>cis</u> to the formyl hydrogen) and the NCH plane is $12 \pm 5°$. Kurland and Wilson's values for the moments of inertia and the quadrupole coupling constants were confirmed, but several significant differences were found in the bond distances and angles. See Table 1.

The structure of gaseous formamide has also been studied by analysis of its IR spectrum although the interpretation of results is not as

straightforward as in the case of the microwave spectrum. Evans[15,16] recorded the IR spectrum in the range 2.6 to 15.4μ and concluded at first that the molecule has a markedly nonplanar structure in the vapor phase, the main evidence for this conclusion being derived from the shapes of the bands due to the NH_2 stretching modes[15]. Later, the spectrum was reinvestigated in the 2.7 to 3.7μ range with a grating spectrometer having considerably better resolution than the prism instrument used earlier.[16] The high resolution data were not in agreement with the original interpretation, and the slightly nonplanar form proposed by Costain and Dowling was adopted as the most probable structure.

On the basis of his infrared results taken in conjunction with the available Raman data in the literature, Evans[15] made an assignment of the wavelengths observed for gaseous formamide to the normal vibrations of the molecule. Only the more easily identifiable N-H and C=O modes will be discussed here because they are of greatest interest in the comparison of spectra observed for different amides. Also, these bands are sensitive to hydrogen bonding and are oftentimes utilized in quantitative studies of association.

Formamide would be expected to have two infrared peaks in the 3μ region arising from the symmetric and asymmetric stretching vibrations of the NH_2 group. These were recorded by Evans[15] at 2.90 and 2.82μ ,respectively, for the compound in the vapor state. No shifts in the wavelengths were observed when the temperature was varied between 130 and 160°C. and the pressure between 20 and 95 mm, showing that association is nonexistent in the gaseous state under these conditions.

The symmetric stretching vibration of the OCN group was identified with the peak at 7.97μ and the asymmetric stretching mode with the peak

at 5.75μ. The latter vibration is largely confined to the C=O bond. Neither of these wavelengths showed any shift over the temperature and pressure ranges studies[15].

Davies and Evans[17] recorded the N-H stretching vibrations of formamide in carbon tetrachloride solution both for the monomer and the associated species. Absorption peaks due to the monomer were observed at 2.93 and 2.83μ (symmetric and asymmetric stretching modes, respectively) and peaks for the associated species at 2.86, 3.03, 3.10, and 3.15μ.

In chloroform solution the symmetric and asymmetric N-H stretching modes of the formamide monomer were recorded at 2.94 and 2.84μ by Evans[15] and at 2.91 and 2.83μ by Puranik and Ramiah[18]. The latter workers also recorded associated N-H peaks at 2.97, 3.05, and 3.12μ.

The carbonyl stretching mode of the formamide monomer (asymmetric stretching vibration of the OCN group[15]) in chloroform is observed at about 5.84μ[15,18]. Upon association this peak is shifted to ~5.90μ[15,18]. In the 6μ region of the IR spectrum carbonyl stretching vibrations have been recorded at 5.78, 5.83, 5.88, 5.94, and 7.63μ[15,18].

Puranik and Ramiah[18] have attempted to identify the origin of several N-H stretching vibrations observed in the associated formamide species. On the basis of a simple monomer-trimer equilibrium for formamide in chloroform solution, they have shown that the ratio $C\varepsilon^3_{max}(S)/\varepsilon_{max}(T)$ is constant to within 20 per cent, where $\varepsilon_{max}(S)$ is the apparent molecular extinction coefficient for the symmetric N-H stretch (2.91μ), $\varepsilon_{max}(T)$ is the coefficient of the associated peak at 3.05

or 3.12μ, and C is the concentration. Similarly, $C\varepsilon^3_{max}(a)/\varepsilon_{max}(T)$ is constant to within 20 per cent, where $\varepsilon_{max}(a)$ is the extinction coefficient for the asymmetric N-H stretch (2.83μ) and $\varepsilon_{max}(T)$ is that of the 2.97μ associated peak. The authors have therefore concluded that the 3.05 and 3.12μ peaks are due to symmetric N-H stretching modes in the associated molecule and that the peak at 2.97μ is due to the asymmetric N-H stretching vibration of this species.

Acetamide. The crystal structure of rhombohedral acetamide has been determined by the X-ray diffraction study of Senti and Harker[7]. Eighteen molecules are present in a hexagonal unit cell having the dimensions $a = 11.44 \pm 0.03$ and $c = 13.49 \pm 0.03$ Å. The space group is C^6_3v -- R3c. The molecule is planar, with no atom deviating more than 0.01 Å from the plane. Adjacent molecules are held together by N-H--O hydrogen bonds 2.86 ± 0.05 Å long to form rings containing six molecules, which are themselves interconnected by other hydrogen bonds. The molecular parameters are given in Table 1. Ladell and Post[1] have noted that the 1.38 Å C-N bond length is anomalously long and should probably be reinvestigated.

The structure of acetamide in the gaseous state has been determined by an electron diffraction study of Kimura and Aoki[6]. Bond lengths and angles were calculated on the assumption of a planar structure and are included in Table 1. A comparison of the molecular parameters obtained by this method with those of X-ray study of Senti and Harker shows them to be in agreement within experimental error.

The infrared spectrum of acetamide in the rock salt range, 2.50 to 16.0μ, has been studied by Davies and Hallam[19]. Results were obtained

principally in chloroform solution, but where this solvent absorbed too strongly solutions in acetonitrile, carbon tetrachloride, and acetone were utilized. The absorption bands observed in all solvents were then discussed together as a "synthetic" spectrum.

The symmetric and asymmetric N-H stretching modes of the monomer are observed at 2.93 and 2.83μ, respectively. Association leads to the appearance of three new absorption peaks: 2.86, 2.98, and 3.14μ; in addition, a slight shoulder is repeatedly observed near 3.03μ. From the position, intensity, and sharpness of the 2.86μ wavelength Davies and Hallam assigned it to the asymmetric N-H mode of the associated species. This assignment is apparently not in agreement with Puranik and Ramiah's assignment of the 2.97μ peak of formamide to the asymmetric N-H stretch of the corresponding associated species. A comparison of the formamide and acetamide spectra in the 3μ region shows them to be quite similar; thus, it seems unlikely that the asymmetric N-H stretching mode can give rise to the 2.97μ peak in formamide, while producing in acetamide the 2.86μ peak.

The outstanding feature of the 6μ region is the well-known carbonyl absorption band. This can be resolved into two distinct peaks: 5.83 and 5.90μ in CCl_4; 5.85 and 5.94μ in CH_3CN; 5.88 and 5.96μ in $CHCl_3$. The effects of temperature and concentration on the spectrum leads to the conclusion that the peak at 5.83 to 5.88μ arises from the monomer and the one at 5.90 to 5.96μ from the associated molecule[19].

A comparison of the IR spectra in the 3μ region of acetamide and other unsubstituted amides has been made by a number of workers. Badger and Rubalcava[20] noted that acetamide, propionamide, and n-butyramide

exhibit virtually identical spectra in the range from 2.8 to 3.2μ. According to Buswell et al[21] both benzamide and propionamide in carbon tetrachloroide solution have N-H peaks at 2.83, 2.92, 3.03, and 3.15μ. Thus, it is evident that the structure of the amide group in unsubstituted amides is little affected by the nature of the side chain.

Tsuboi[22] has suggested that the 3.15μ peak of unsubstituted amides arises from a cyclic dimer because of its proximity to the 3.11μ wavelength of δ-valerolactam, which forms cyclic dimers as a consequence of the C=O and N-H groups being cis to each other. On the other hand, the 3.03μ peak probably can be attributed to the N-H stretching mode of a linear polymer because it is located near the 2.97 to 3.03μ band of N-methylacetamide (known to form linear chains as will be seen later).

The infrared spectra of primary amides in the NH and CO stretching regions are summarized in Table 2.

Davies and Hallam[19] studied the self-association of acetamide in water, chloroform, acetonitrile, and acetone solutions. Using a freezing point-depression method, they found that acetamide is unassociated in water solutions at 0°C for concentrations up to at least 3 molar. At 6, 25, and 45°C in chloroform (partition method with water) the molecule associates almost exclusively to form a trimer although dimer formation is also detectable at 45°C. The values of the trimerization constants, K_{13}, at the respective temperatures are 51, 34, and 24 $l/mole^2$, and the enthalpy of formation, ΔH_{13}, is equal to -3.3 Kcal/mole of trimer or -1.1 Kcal/mole for each hydrogen bond on the assumption of a cyclic species. Although the enthalpy change appears to be abnormally low, it can be rationalized on the basis of a relatively strong interaction between solvent and solute molecules.

TABLE 2

INFRARED SPECTRA OF PRIMARY AMIDES IN THE NH AND CO STRETCHING REGIONS

Molecule	State	Species					Reference
		Monomer			Polymer		
		ν_s(NH)	ν_a(NH)	ν(CO)	ν(NH)	ν(CO)	
Formamide	Gaseous	2.90μ	2.82μ	5.75μ[a] 7.97μ[b]	----	----	15
	CCl_4 Soln.	2.93μ	2.83μ	----	2.86μ 3.03μ 3.10μ 3.15μ	----	17
	$CHCl_3$ Soln.	2.94μ	2.84μ	~5.84μ[a] 7.70μ[b]	----	5.93μ	15
		2.91μ	2.83μ	~5.84μ	2.97μ 3.05μ 3.12μ	5.88μ	18
	Liquid	----	----	~5.83μ	2.95μ 3.00μ 3.12μ	5.94μ 7.63μ	15
		----	----	----	----	5.78μ 5.88μ	18
Acetamide	CCl_4 Soln.	2.92μ	2.83μ	----	3.15μ	----	21
	$CHCl_3$ Soln.	2.93μ	2.83μ	----	2.86μ 2.98μ 3.14μ	----	17

[a] ν_a(OCN); [b] ν_s(OCN)

TABLE 2 -- continued

Molecule	State	Species					Reference
		Monomer			Polymer		
		ν_s(NH)	ν_a(NH)	ν(CO)	ν(NH)	ν(CO)	
Acetamide	"Synthetic state"	2.93μ	2.83μ	5.87μ	2.86μ 2.98μ 3.03μ 3.14μ	5.96μ	19
Propion-amide	CCl_4 Soln.	2.92μ	2.83μ	----	3.03μ 3.15μ	----	21
		2.92μ	2.83μ	----	3.03μ 3.15μ	----	20
n-Valer-amide	CCl_4 Soln.	2.92μ	2.83μ	----	3.14μ	----	21
Benzamide	CCl_4 Soln.	2.92μ	2.83μ	----	3.03μ 3.15μ	----	21

Boiling point-elevation data in chloroform (61°C) are in agreement with the data obtained from the partition method in that trimers are formed with an increased amount of dimer. In acetonitrile no association occurs up to 0.41 molar at the boiling point (82°C), but in acetone at its boiling point (56°C) a monomer-dimer equilibrium is present with a dimerization constant K_{12} of 0.49 ± 0.04 1/mole.

Davies and Thomas[23] used an isopiestic method to study the self-association of trichloroacetamide in benzene solution. At 25°C the compound associates to form a series of associated species with successive equilibria all having the same constant, i.e., $K_{12} = K_{23} = \ldots = K_n$. The value of K_n at 25°C is 93 in mole fraction units. At 35 and 45°C, however, the equilibria apparently conform to the case where $K_{12} < K_{23} = K_{34} = \ldots = K_n$. The values of K_{12} and K_n at 35°C are 64 and 77 while those at 45°C are 43.5 and 63. Although the data were treated on the assumption of unlimited equilibria, the results could be accounted for equally well by postulating only dimer and trimer formation.

The heat of dimerization, -7.2 ± 0.3 Kcal/mole, is twice that of the subsequent stage, -3.7 ± 0.1 Kcal/mole. These results suggest the formation of cyclic dimers and trimers, i.e., the dimer is cyclic with two hydrogen bonds and the trimer is also cyclic with one additional bond.

A cyclic dimer was also postulated for another primary amide, propionamide, by Badger and Rubalcava[20], who utilized the N-H absorption peaks in the 3μ region to study the self-association in carbon tetrachloride solution. Results could be explained on the assumption of simple dimerization with a constant K_{12} of 45 ± 6 1/mole at 25°C.

Evidence of the cyclic dimer came from the heat of dimerization, which was calculated to be -7.85 ± 0.22 Kcal/mole.

Table 3 summarizes the self-association data of acetamide, trichloroacetamide and propionamide.

N-MONOSUBSTITUTED AMIDES

N-Methylformamide. Infrared and Raman spectra of N-methylformamide have been recorded by a number of workers and assignments have been made to the majority of observed wavelengths. The IR peak in the vapor spectrum occurring at 2.87μ has been assigned to the N-H stretching mode and that at 5.80μ to the carbonyl stretch[24]. In dilute non-polar solutions the corresponding peaks occur at 2.89[20] and 5.93μ[24], while in the liquid they are observed at 3.03[25] and ≈6.00μ[24,25,26]. See Table 4 for a summary of the infrared spectra in the NH and CO stretching regions of several N-monosubstituted amides.

From an analysis of the band contours in the IR spectrum of NMF recorded between 2.86 and 22.2μ Jones concluded that the isomer having the oxygen and the hydrogen of the amide group cis to each other is present in the vapor phase[24]. However, criticism of this conclusion[27] led to a reexamination of the spectrum between 2.78 and 3.84μ, whereupon it was concluded that neither of the rigid models adopted (cis or trans) provides a satisfactory basis for the interpretation of results.[28]

In a third investigation[29] Jones recorded the IR spectrum of gaseous NMF and several other N-substituted amides in the range between 2.50 and 24.1μ. This time it was concluded that the molecule probably exists in the cis form in the vapor phase although, as noted, the cis configuration

TABLE 3

SELF-ASSOCIATION OF PRIMARY AMIDES

MOLECULE	METHOD AND SOLVENT	TYPE OF ASSOCIATION	TEMP.	ASSOCIATION CONSTANTS	ENTHALPY OF ASSOCIATION	REF.
Acetamide	Freezing point depression; water	Unassociated in water soln. up to at least 3 molar	0°C	----	----	19
	Partition method between chloroform and water; chloroform	Trimer formation in $CHCl_3$	6°C	$K_{13} = 51 \pm 2$ (l/mole)^2	-3.3 Kcal/mole	
			25°C	$K_{13} = 34 \pm 1$		
		Trimer formation with some dimers	45°C	$K_{13} = 24 \pm 2$		
	Boiling point elevation; chloroform	Trimer and dimer formation	61°C	$K_{13} = 3.2 \pm 0.4$ $K_{12} = 1.0 \pm 0.1$ (l/mole)	----	
	Boiling point elevation; acetonitrile	No association up to 0.41 molar	82°C			
	Boiling point elevation; acetone	Dimer formation	56°C	$K_{12} = 0.49 \pm 0.04$	----	

TABLE 3 -- continued

MOLECULE	METHOD AND SOLVENT	TYPE OF ASSOCIATION	TEMP.	ASSOCIATION CONSTANTS	ENTHALPY OF ASSOCIATION	REF.
Trichloro-acetamide	Isopiestic method, benzene	Series of associated species formed with successive equilibria all having the same constant, $K_{12} = K_{23} = K_n$. Results can also be accounted for by dimer and trimer formation	25°C	K_{12} = 93 (mole fraction) K_n = 93	-7.2 ± 0.3 Kcal/mole (dimerization)	23
		Series of associated species formed with successive equilibria having the constants $K_{12} < K_{23} = K_{34} = K_n$. Results can also be accounted for by dimer and trimer formation	35°C	K_{12} = 64 K_n = 77	-3.7 ± 0.1 (higher association)	
			45°C	K_{12} = 43.5 K_n = 63		
Propion-amide	N-H absorption; CCl_4	Dimers formed	25°C*	K_{12} = 45 ± 6 l/mole	-7.85 ± 0.22	20

* Data were taken at two other unspecified temperatures.

alone has been criticized by Miyazawa[27] who suggested that both isomers exist in the vapor phase, with the trans form being predominant.

Miyazawa's conclusion is supported by the IR data of Suzuki[26] recorded in the region between 2.78 and 38.4μ for N-methylformamide and its deuterated derivatives in the liquid and solid states. On the assumption that the molecule is planar except for two hydrogen atoms of the methyl group, normal vibrations were calculated for both configurations and compared with experimental data. It was concluded that the molecule is trans in the liquid state; also, because the skeletal deformation band observed at 13.0μ for the liquid (amide IV band) is only slightly different from the value of 12.7μ observed for the compound in the vapor state, it was concluded that the molecule is predominantly trans in the vapor phase.

DeGraaf and Sutherland[25] also recorded infrared as well as Raman spectra for NMF and N-deutero NMF in the liquid phase. The IR spectra were obtained in the range from 2.78 to 31.3μ and the Raman spectra between 2.78 and 44.4μ. The data obtained made possible the assignment of observed bands to all 21 normal vibrations of the compound. A trans model having a plane of symmetry was adopted as the most probable configuration.

The nuclear magnetic resonance spectra of liquid N-methylformamide and N-methylacetamide at room temperature were recorded along with those of several other compounds by Gutowsky and Holm[30]. Both NMF and NMA show splitting of the N-methyl proton resonance which is not field dependent, indicating spin-spin coupling with another proton. In comparing the spectra of the two compounds no discernible change is

TABLE 4

INFRARED SPECTRA OF N-MONOSUBSTITUTED AMIDES IN THE NH AND CO STRETCHING REGIONS

Molecule	State	Species				Reference
		Monomer		Polymer		
		ν(NH)	ν(CO)	ν(NH)	ν(CO)	
N-Methyl-formamide	Gaseous	2.87μ	5.80μ	----	----	24
	CCl_4 Soln.	2.89μ	----	----	----	20
	Liquid	----	----	3.03μ	6.00μ	25
		----	----	----	6.03μ	24
		----	----	3.03μ	5.97μ	26
	Solid	----	----	3.03μ 3.22μ	----	32
		----	----	----	6.02μ	26
N-Methyl-acetamide	Gaseous	2.86μ	5.78-5.84μ	----	----	33
		2.86μ	----	----	----	32
	CCl_4 Soln.	2.88μ	5.92μ	2.97-3.03μ 3.10μ 3.22μ	----	33
		2.88μ	5.88μ	2.97-3.03μ 3.22μ	----	32

TABLE 4 -- continued

Molecule	State	Species				Reference
		Monomer		Polymer		
		ν(NH)	ν(CO)	ν(NH)	ν(CO)	
N-Methyl-acetamide	CCl_4 Soln.	2.88μ	----	2.97-3.03μ 3.22μ	----	22
		2.88μ	----	2.97-3.03μ 2.92μ 3.23μ		34
	$CHCl_3$ Soln.	2.89μ	----	2.99μ 2.92μ	----	34
	Liquid	----	----	3.03μ 3.23μ	6.03μ	33
		----	----	3.03μ 3.23μ	6.1μ	32
N-Ethyl-acetamide	CCl_4 Soln.	2.89μ	----	2.98-3.03μ	----	21
N-Cyclo-hexylacet-amide	CCl_4 Soln.	2.90μ	----	2.98-3.04μ	----	21
Acetanilide	CCl_4 Soln.	2.90μ	----	2.97μ	----	21
Benzanilide	CCl_4 Soln.	2.86μ	----	----	----	21

noted in the doublet when the HCO group is replaced by CH_3CO; consequently the separation can only be due to interaction with the proton on the nitrogen. The observation of only one doublet instead of two indicates the presence of only one rotational isomer[31,30]. Also, the coincident position of the doublet for both NMF and NMA indicates that the compounds have identical configurations, i.e., both are cis or both are trans; one cannot be cis and the other trans.

However, another interpretation of the present data is plausible[30]. If the energy barrier between cis and trans forms is low enough to allow free rotation about the CO-N bond, the chemical shift between cis and trans N-methyl protons would be averaged out, leaving only the observed spin-spin coupling with the proton on the nitrogen atom.

Although NMR evidence cannot definitely rule out free rotation about the CO-N bond of N-methylamides, the presence of a doublet in the spectra of N,N-dimethylamides leaves no other explanation. The splitting in the N-methyl proton resonance of liquid N,N-dimethylformamide and N,N-dimethylacetamide in contrast to that of the N-methylamides is field dependent, indicating a chemical shift instead of spin-spin interaction[35,31,30,36]. Also, because the doublet occurs in the same position in the spectra of both compounds, any coupling with a formyl or acetyl proton is ruled out. The difference in the hydrogens of the two CH_3 groups can only be due to hindered rotation about the CO-N bond, i.e., one CH_3 is cis and the other trans to the carbonyl. Thus, the two groups reside in different magnetic environments and exhibit resonances at slightly different frequencies.

However, at higher temperatures (above 50°C for DMA) the doublet collapses into a singlet, indicating the onset of free rotation about the CO-N bond[30]. The free energy of activation $\Delta F^{\ddagger}$ for the molecular reorientation is calculated to be 19 Kcal/mole for DMA[30]. This value compares favorably with the resonance energy of amides estimated by Pauling[9] to be 21 Kcal/mole.

Davies and Thomas[37] have studied the self-association of a number of N-substituted amides, including NMF and NMA, in benzene solution. The data were obtained with a thermoelectric osmometer originally developed by Hill[38] and later improved by Brady, Huff, and McBain[39]. The N-methyl derivatives of formamide, acetamide, trichloroacetamide, and benzamide as well as N-propylacetamide were all found to conform to the self-association scheme $K_{12} < K_{23} = K_{34} = \ldots = K_n$. At 25°C the values of K_{12} and K_n for N-methylformamide are 213 $\pm$ 5 and 218 $\pm$ 4, respectively, in mole fraction units, and the corresponding enthalpy changes are -3.49 and -3.87 Kcal/mole, respectively.

It is interesting to note that N-methyltrichloroacetamide, which exists mainly as monomers and dimers in benzene solution, has an enthalpy of dimerization of -7.1 $\pm$ 0.2 Kcal/mole. On the other hand, the enthalpy change for the addition of another monomer to the species to form the trimer is -3.6 $\pm$ 0.4 Kcal/mole. Thus, it appears that N-methyltrichloroacetamide, like trichloroacetamide itself, forms both cyclic dimers and trimers[37]. The formation of cyclic species for the molecule appears to be an unusual phenomenon for an N-substituted amide. As evidenced by an average heat of dimerization of 3 or 4 Kcal/mole, most N-substituted amides dissolved in non-polar solvents form linear associated species.

The self-association of a number of N-monosubstituted amides is summarized in Table 5.

N-Methylacetamide. Using the method of X-ray crystallography, Katz and Post[8,40] established the structure of NMA in the crystalline state. Two orthorhombic crystalline forms were observed, one above and the other below a solid phase transition temperature of 10°C. In both forms the molecule was found to exist in the trans, almost coplanar, configuration. See Table I for bond distances and angles.

The unit cell of the lower temperature form contains four molecules and has the dimensions a = 9.61 ± 0.02, b = 6.52 ± 0.01, and c = 7.24 ± 0.015 Å. The space group is P_{nma}. Molecules are arranged in layers perpendicular to the b axis, with a separation between layers of 3.26 Å. Each layer is made up of chains of molecules oriented approximately parallel to the a axis, and individual molecules are linked together through N-H--O hydrogen bonds 2.825 Å long. The five heavy atoms of the amide residue have been determined to be coplanar within 0.015 Å.

The crystalline modification stable above 10°C. contains only two molecules in a unit cell having the dimensions a = 4.85 ± 0.01, b = 6.59 ± 0.01, and c = 7.30 ± 0.01 Å. The space group is P_{nmm}. Reduction in unit-cell size is brought about by molecular reorientation to a more disordered state.

Kimura and Aoki[6] used the method of electron diffraction to study the structure of NMA in the gaseous state and interpreted the data with the aid of molecular parameters obtained for acetamide. The authors concluded that the molecule probably has the trans configuration but that the N-methyl group oscillates about the CO-N bond; however,

TABLE 5

SELF-ASSOCIATION OF N-MONOSUBSTITUTED AMIDES

MOLECULE	METHOD AND SOLVENT	TYPE OF ASSOCIATION	TEMP.	ASSOCIATION CONSTANTS	ENTHALPY OF ASSOCIATION	REF.
N-Methyl-formamide	Thermoelectric osmometer; benzene	Chain formation with $K_{12} < K_{23}$ $K_{34} = K_n$	24.92°C	$K_n = 218 \pm 4$ (mole fract.) $K_{12} = 213 \pm 5$	$\Delta H_{12} = -3.49$ Kcal/mole $\Delta H_n = -3.87$	37
			35.07°C	$K_{12} = 175 \pm 3$ $K_n = 175 \pm 3$		
N-Methyl-acetamide	N-H absorption; CCl_4	Chain formation with no restrictions placed on values of K	25°C.	$K_{12} = 4.7$ (1/mole)	$\Delta H_{12} = -4.2$[1]	41
	N-H absorption; dioxane	Chain formation with no restrictions placed on values of K	25°C	$K_{12} = 0.52$	$\Delta H_{12} = -0.8$[1]	41
	N-H absorption; water	Chain formation with no restrictions placed on values of K	25°C	$K_{12} = 0.005$	$\Delta H_{12} = 0.0$[1]	41

[1] Enthalpy of association was calculated from association constants obtained at 25 and 60°C. Only 25°C data were given.

TABLE 5 -- continued

MOLECULE	METHOD AND SOLVENT	TYPE OF ASSOCIATION	TEMP.	ASSOCIATION CONSTANTS	ENTHALPY OF ASSOCIATION	REF.
N-Methyl-acetamide	N-H absorption; CCl_4	Chain formation with $K_{12} < K_{23} = K_{34} = K_n$	25°C	$K_{12} = 5.8$ (1/mole)[2]	$\Delta H_{12} = -4.2$ Kcal/mole[1]	41
	N-H absorption; dioxane	Chain formation with $K_{12} < K_{23} = K_{34} = K_n$	25°C	$K_{12} = 0.58$[2]	$\Delta H_{12} = -0.8$[1]	
	N-H absorption; water	Chain formation with $K_{12} < K_{23} = K_{34} = K_n$	25°C	$K_{12} = 0.005$[2]	$\Delta H_{12} = 0.0$[1]	
	Thermoelectric osmometer; benzene	Chain formation with $K_{12} < K_{23}$ $K_{34} = K_n$	24.57	$K_{12} = 69 \pm 3$(mole fract) $K_n = 148 \pm 2$		
			35.07	$K_{12} = 56.5 \pm 2$ $K_n = 118.5 \pm 2$	$\Delta H_{12} = -3.5 \pm 0.2$ $\Delta H_n = -3.8 \pm 0.2$	37
			48.90	$K_{12} = 43.5 \pm 2$ $K_n = 91.7 \pm 2$		

[2]No values of K_n were given.

TABLE 5 -- continued

MOLECULE	METHOD AND SOLVENT	TYPE OF ASSOCIATION	TEMP.	ASSOCIATION CONSTANTS	ENTHALPY OF ASSOCIATION	REF.
N-Propyl-acetamide	Thermoelectric osmometer; benzene	Chain formation with $K_{12} < K_{23} = K_{34} = K_n$	21.80°C	$K_{12} = 50$ (mole fract.) $K_n = 52 \pm 2$	----	37
N-Methyl-trichloro-acetamide	Thermoelectric osmometer; benzene	Chain formation with $K_{12} < K_{23} = K_{34} = K_n$	24.57	$K_{12} = 8.48 \pm 0.2$ $K_n = 28.2 \pm 1.0$	$\Delta H_{12} = -7.1 \pm 0.2$ (Kcal/mole) $\Delta H_n = -3.6 \pm 0.4$	37
			35.07	$K_{12} = 5.56 \pm 0.1$ $K_n = 23.4 \pm 1.0$		
			48.90	$K_{12} = 3.40 \pm 0.05$ $K_n = 17.8 \pm 0.5$		
N-Methyl-benzamide	Thermoelectric osmometer; benzene	Chain formation with $K_{12} < K_{23} = K_{34} = K_n$	24.92	$K_{12} = 5.24 \pm 0.1$ $K_n = 75.0 \pm 1$	$\Delta H_{12} = -3.6 \pm 0.1$ $\Delta H_n = -3.71 \pm 0.04$	37
			35.07	$K_{12} = 4.29 \pm 0.1$ $K_n = 61.3 \pm 1$		
			48.90	$K_{12} = 3.30 \pm 0.05$ $K_n = 47.0 \pm 0.5$		

TABLE 5 -- continued

MOLECULE	METHOD AND SOLVENT	TYPE OF ASSOCIATION	TEMP.	ASSOCIATION CONSTANTS	ENTHALPY OF ASSOCIATION	REF.
N-Methyl-acetamide	Nuclear Magnetic resonance; chloroform-d	Chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$	room	$K_{12} = 13.0$(mole fract.) $K_n = 14.0$	----	42
N-Isopropyl-acetamide	Nuclear Magnetic resonance; cyclohexane	Chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$	room	$K_{12} = 10.5 \pm 2.0$ $K_n = 450 \pm 50$	----	
	Nuclear Magnetic resonance; CCl_4	Chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$	room	$K_{12} = 16.0 \pm 2.0$ $K_n = 150 \pm 10$	----	
	Nuclear Magnetic resonance; chloroform-d	Chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$	room	$K_{12} = 4.5$ $K_n = 8.0$	----	
	Nuclear Magnetic resonance; dioxane	Chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$	room	$K_{12} = 3.5 \pm 0.5$ $K_n = 9.0 \pm 0.5$	----	
	Nuclear Magnetic resonance; diethyl ketone	Chain formation with $K_{12} < K_{23}$	room	$K_{12} = 4.5 \pm 1.5$ $K_n = 6.5 \pm 0.5$	----	

TABLE 5 -- continued

MOLECULE	METHOD AND SOLVENT	TYPE OF ASSOCIATION	TEMP.	ASSOCIATION CONSTANTS	ENTHALPY OF ASSOCIATION	REF.
N-Isopropylacetamide	Nuclear Magnetic resonance; dimethyl sulfoxide	Chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$	room	$K_{12} = 0.65 \pm 0.10$ (mole fraction) $K_n = 0.90 \pm 0.10$	----	42
N-t-Butylacetamide	Nuclear Magnetic resonance; chloroform-d	Chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$	room	$K_{12} = 3.0$ $K_n = 4.0$	----	

free rotation about the bond could not be ruled out. As seen from Table I bond distances and angles are in good agreement with those obtained by Katz and Post.

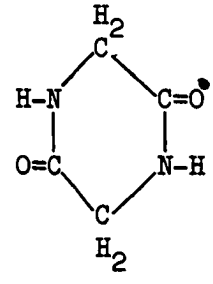
A number of workers have extensively studied the infrared spectrum of NMA in an attempt to correlate the observed wavelengths with the structural features of the molecule. In several investigations frequencies have been calculated by tranference of force constants from simpler molecules. Thus, it has been possible, theoretically at least, to distinguish between proposed molecular configurations by comparison of observed absorption bands with those calculated for a particular configuration.

Bradbury and Elliott[43] recorded the polarized infrared spectrum of crystalline NMA and N-deuterated NMA with the E-vector of the normally incident radiation directed along each of the three crystalline axes. Spectra were recorded at room temperature (where the crystal is not fully ordered) and down to the temperature of liquid nitrogen. The general features of the spectra were found to be in excellent agreement with the crystalline structure proposed by Katz and Post[8].

The most easily identifiable infrared wavelengths of NMA are those arising from N-H and carbonyl stretching, as well as a number of other wavelengths characteristic of N-monosubstituted amides. The free N-H stretch of gaseous NMA occurs at 2.86μ[33], differing only slightly from the value of 2.88μ[33,32,22,34] observed for the compound dissolved in dilute non-polar solvents, e.g., CCl_4 or CS_2. At higher concentrations in the same solvents associated N-H peaks occur at 2.92μ[34], 2.97 to 3.03μ[33,32,22,34], 3.10μ[33], and at about 3.22μ[33,32,22,34]. In liquid

NMA associated wavelengths are present at 3.03 and 3.23μ[33,32]. The free carbonyl stretch of NMA occurs at about 5.90μ in dilute non-polar solutions[33,32] and gives rise to a well-defined doublet at 5.78-5.84μ[33] in the vapor. In liquid NMA the associated carbonyl stretching mode is observed at about 6.1μ[33,32]. See Table 4.

Miyazawa et al[44,45,27] have recorded the IR spectra of a number of compounds including N-methylacetamide and diketopiperazine. To establish the configuration of NMA, the in-plane normal vibrations were calculated for both cis and trans forms and compared with experimental data. The calculated frequencies for the trans form were found to agree well with observed values, while those of the cis form did not. On the other hand, except for the skeletal deformation modes, the cis calculations agreed well with frequencies observed for diketopiperazine, which must take the cis configuration due to its ring structure. It was thus concluded that N-methylacetamide exists in the



diketopiperazine

trans configuration. Although the authors' assignment of frequencies is generally well substantiated, some of the bands are not in agreement with the infrared investigation of Bradbury and Elliott.[43]

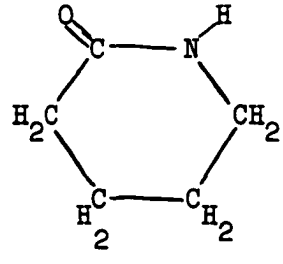
The same conclusion was drawn by Mizushima et al[32,46,47] who recorded both the IR and UV spectra of NMA in addition to the Raman effect. From a comparison of experimental values with skeletal

frequencies calculated for the two possible configurations, the trans form was chosen as the most probable structure.

Mizushima et al[32] have cited the UV spectrum of NMA as evidence that the molecule has the resonance structure $CH_3-\overset{O}{\overset{\|}{C}}-NHCH_3 \leftrightarrow CH_3-\overset{O^-}{\overset{|}{C}}=\overset{+}{N}HCH_3$, which probably accounts for the planarity of the amide group. If the molecule can be represented simply by the structure $CH-\overset{O}{\overset{\|}{C}}-NHCH_3$, it should have a UV spectrum for the carbonyl group similar to that of acetone. However, the expected peak occurs at a much shorter wavelength (<2100 Å) than in acetone (2650 Å), suggesting the delocalization of carbonyl group electrons. This conclusion is supported by the fact that in strongly acid solution an absorption maximum is observed at nearly the same wavelength (2659 Å) as that for the carbonyl absorption of acetone. This band can be assigned to the N-methylacetamidonium ion $CH_3-\overset{O^+}{\overset{\|}{C}}-\overset{+}{N}H_2CH_3$, which has no resonance form as a consequence of attachment of an additional proton to N.

The evidence for the N-methylacetamidonium ion has, however, been challenged by Bello[48], who was unable to confirm the existence of the reported peak at 2695 Å for NMA in aqueous HCl. He was consequently forced to conclude that the ion does not exist, and thus has case doubt on the UV evidence for the resonance structure of NMA.

Tsuboi[22] and Mizushima et al[32] in comparing the IR spectrum of NMA in CCl_4 with that of δ-valerolactam, have presented evidence to indicate that NMA forms more than one associated species in non-polar solvents.



δ-valerolactam

The spectra of these two compounds in the N-H stretching region are similar except for several distinctions apparently attributable to different configurations of the amide group.

While the wavelength of the free N-H peak (2.88μ) of NMA is constant over a range of concentrations and temperatures, that of the peak due to association first appearing at 2.97μ (low concentration or high temperature) is shifted to 3.03μ as the concentration is raised or the temperature lowered. For δ-valerolactam neither the free N-H stretch (2.92μ) nor the associated peak (3.11μ) is concentration or temperature dependent. The shift in the associated band of NMA indicates that more than one associated species is present. On the other hand, the absence of this shift in the spectrum of δ-valerolactam points to the existence of only one associated species for this molecule. This is to be expected because the cis configuration of the molecule favors the formation of cyclic dimers. Both compounds have an additional associated peak at about 3.23μ.

Klotz and Franzen[41] used the first overtone of the N-H stretching vibration (1.47μ) to study the self-association of NMA in carbon tetrachloride, dioxane, and water. The measurements gave the concentration of the free N-H groups as a function of total concentration, and the resulting data were analyzed on the assumption of a monomer-oligomer equilibrium. Without placing any restrictions on the successive equilibrium constants, K_{12}, K_{23}, ..., the dimerization constant K_{12} was obtained from

$$\lim_{\alpha \to 0} \left(\frac{\alpha}{1 - \alpha} \cdot \frac{1}{C_f} \right) = K_{12}$$

where C_f = concentration of free N-H groups (the measured quantity), $\alpha = C_b/C_t$, C_t = total concentration of NMA residues, and C_b = concentration of bound N-H=$(C_t - C_f)$. The value of K_{12} was compared with that obtained from a second method based on the assumption that $K_{12} < K_{23} = K_{34} \ldots = K_n$. To permit calculation of thermodynamic quantities, the measurements were conducted at both 25 and 60°C. Results at 25°C are summarized below.

Solvent	K_{12}, l/mole	$\Delta F°$, Kcal/mole	$\Delta H°$, Kcal/mole	$\Delta S°$, gibbs/mole
CCl_4	4.7 (5.8)	-0.92	-4.2	-11
Dioxane	0.52 (0.58)	0.39	-0.8	- 4
Water	0.005 (0.005)	3.1	0.0	-10

The values in parentheses were obtained by the second method of data analysis. No values were given for K_n.

It can be seen from the values of K_{12} that NMA associates much more strongly in carbon tetrachloride than in dioxane or water. The fact that solvents of increasing dielectric inhibit the self-association of NMA is also supported by an earlier work of Klotz and Franzen[49] in which they studied the relative extent of association of the amide in water and CCl_4. Although appreciable association was noted at 1.01 molar in CCl_4, the concentration had to be raised to 7 or 8 molar in water before hydrogen-bonded species were observed.

La Planche *et al*[42] used a nuclear magnetic resonance technique to study the self-association of N-methyl-, N-isopropyl-, and N-t-butylacetamide in solvents of varying dielectric constant (see Table 5). The data for all three amideswere explained on the assumption of linear

chain formation with $K_{12} < K_{23} = K_{34} = \ldots = K_n$. The values of the equilibrium constants determined for NMA in chloroform-d solution were $K_{12} = 13.0$ and $K_n = 14.0$ (mole fraction units) at room temperature.

Additional evidence for linear-chain formation in the self-association of NMA has been given by the dipole-moment measurements of Mizushima et al[32]. It is well known that compounds forming cyclic dimers upon self-association, such as carboxylic acids[50] and lactams[32], show a decrease in molecular polarization as the concentration is raised or the temperature lowered. This fact is readily explained by the tendency towards cancellation of the dipoles of two molecules associated in a cyclic dimer, i.e., the two dipoles oppose each other and thus tend to leave the species without net charge separation.

However, the effects of concentration and temperature on the molecular polarization of NMA in CCl_4 or dioxane are in the opposite direction, i.e., the polarization increases as the concentration is raised or the temperature lowered. This behavior can be explained by the formation of linear chains in which the dipoles line up in the same direction, producing a larger moment than in the monomer.

From a statistico-mechanical standpoint Sarolea-Mathot[51] has shown that two association constants, K_{12} and K_n, would be expected for self-association resulting in linear-chain formation. On the basis of the "ideal associated solution" he derived the result that

$$\frac{K_{12}}{K_n} = \frac{1}{p}$$

where p is the number of possible orientations for the monomer. Because p is essentially greater than 1 it follows that $K_{12} < K_n$,

as is actually observed for the great majority of self-association cases involving linear-chain formation. Although the result was derived for the self-association of unhindered alcohols, it should apply equally well to N-monosubstituted amides because of the same type of association, i.e., that in which

$$A + A \rightleftarrows A_2$$

$$A_i + A \rightleftarrows A_i + 1$$

Polypeptides and Proteins. These molecules have been extensively reviewed elsewhere[10,2,11,52,53] and will be discussed only briefly here.

The solution to the problem of polypeptide and protein configuration stems largely from imporvements in X-ray techniques, along with data analysis by high-speed computing equipment. Whereas earlier researchers had to rely on the computation of atomic coordinates from two-dimensional electron density maps, modern methods have made possible the calculation of three-dimensional distributions of electron density, allowing the location of practically all atoms except perhaps the hydrogen atom.

Much of the credit belongs to Pauling and workers, who postulated the fundamental dimensions of the amide group in polypeptides and proteins from a consideration of the structures of a large number of simple amide-containing molecules, such as those discussed previously. These dimensions are given in Table 1.

The collection of data on the amide group stimulated a number of workers to propose configurations for polypeptide chains. The most successful configuration, the α-helix, was proposed by Pauling, Corey, and Branson[54,55]. This structure satisfies all postulated requirements

including the principle of closest packing, planarity of the amide group, and N-H--O hydrogen bonds by all of the N-H and C=O groups. Although other structures have been proposed the α-helix appears to be the most widely occurring polypeptide configuration in proteins.

A diagrammatic representation of the α-helix is shown in Fig.1. If the helical structure were unwound and laid flat on a plane surface it would have an appearance similar to the schematic diagram shown in Fig. 1(a). This projection clearly shows the N-H--O hydrogen bonds joining adjacent turns together in 12-membered rings. Fig. 1(b) shows the structure wound in helical form.

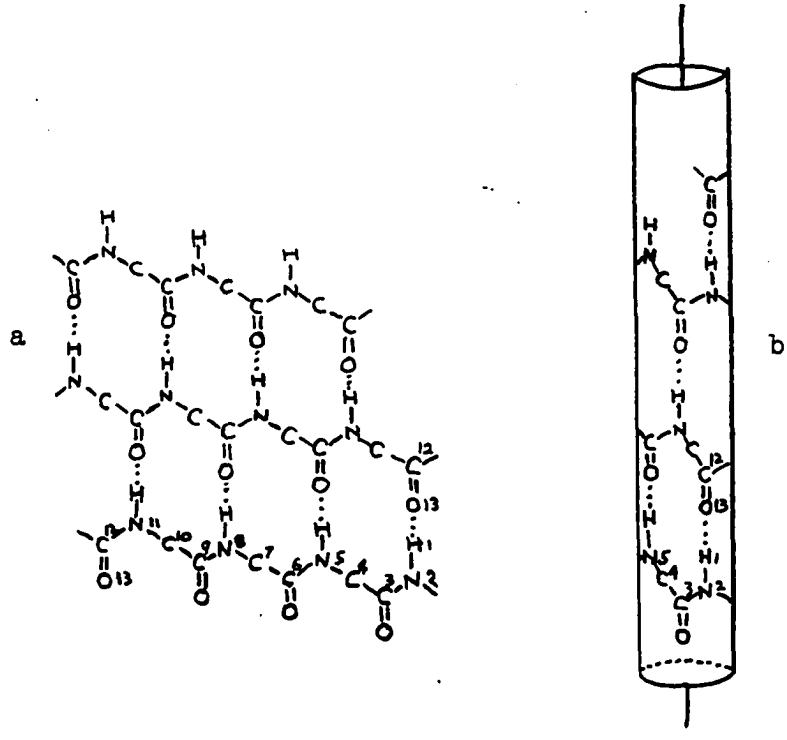


Figure 1. The α-helix. a) Projection onto a plane perpendicular to the helical axis [from Proc. Nat. Acad. Sci. U.S. 37 (1951) 235]; b) Helical configuration.

The α-helix has about 3.6 amide residues per turn and 13 atoms in a ring formed by starting at any given N-H and continuing around the helix until the C=O group situated above the N-H and hydrogen bonded to it is reached. The thirteen atoms are marked in Fig. 1. In the nomenclature of Bragg, Kendrew, and Perutz[56] this configuration is given the symbol 3.6_{13}.

Although the α-helix was largely a hypothetical structure when proposed almost 15 years ago, it now has much support as a fundamental building block of proteins. For example, in the crystalline form of the globular protein myoglobin, it has been determined that 75 per cent of the amino acid residues are present in α-helices[53]. The structure cannot, however, be used to explain the entire configuration of a protein molecule because only the polyamide backbone is actually involved in the helix; side chain interactions must be accounted for by other structures.

SUMMARY AND CONCLUSIONS

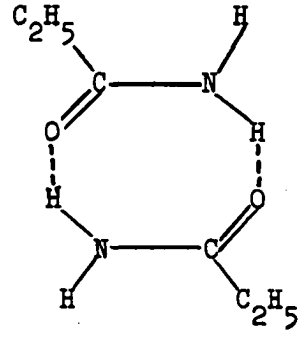
The evidence presented indicated that amides exist in a coplanar or almost coplanar configuration in which the CO-N group has the resonance structure $-\overset{O}{\overset{\|}{C}}-N< \leftrightarrow -\overset{O^-}{\overset{|}{C}}=\overset{+}{N}<$. The main evidence for the latter conclusion is derived from x-ray, microwave, and electron-diffraction studies, which show that the CO-N bond length is significantly shorter than that found in non-resonant molecules. The more recent x-ray and microwave investigations indicate that the amide structure does not exist in a truly coplanar configuration but instead in a slightly pyramidal shape similar to that found in the ammonia molecule.

The almost coplanar configuration of amides appears to be quite stable at room temperatures and is not disrupted by changes in state. Only at higher temperatures, e.g., above 50°C for N,N-dimethylacetamide, does rotation about the CO-N bond occur.

N-monosubstituted amides are found almost exclusively in a trans configuration. The only evidence contrary to this fact is presented by infrared studies in which it has been shown that N-methylformamide may exist in the cis configuration in the vapor phase.

Unsubstituted amides self-associate in nonpolar solutions to form chiefly dimers and trimers. Although the enthalpy of formation is very limited, the available data point to the existence of cyclic species.

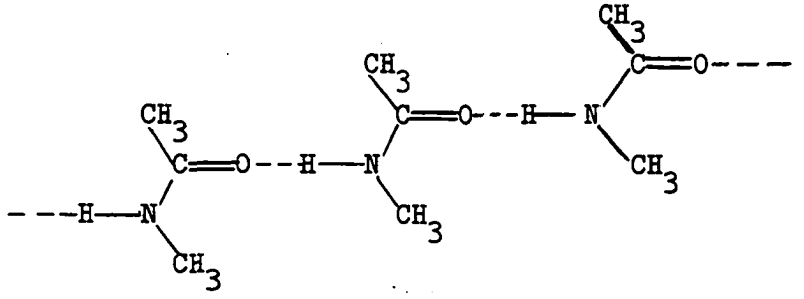
From a consideration of structure, cyclic species would be expected in the self-association of unsubstituted amides because of the existence of a proton of the NH_2 group cis to the oxygen of the carbonyl group. For example, the propionamide dimer probably exists as



N-monosubstituted amides, on the other hand, self-associate in nonpolar solutions primarily to form linear chains with $K_{12} < K_{23} = K_{34} = \ldots = K_n$. The only apparent exception cited is found in the case of N-methyltrichloroacetamide in which cyclic dimers are formed, as evidenced from an exceptionally large enthalpy of dimerization.

From structural considerations linear-chain formation would be expected for N-monosubstituted amides because of the existence of the proton of the NHR group <u>trans</u> to the oxygen of the carbonyl group. It is evident that such a structure would not readily lead to the formation of cyclic species.

The self-association of N-methylacetamide in nonpolar solutions can probably best be represented by



in which only one hydrogen bond exists between adjacent molecules.

CHAPTER III

EXPERIMENTAL

MATERIALS

N-methylacetamide. The compound purchased from K & K Laboratories, Jamaica, New York was first purified by vacuum distillation at 110 mm Hg and 144°C. The distillate was then further purified through fractional crystallization. Starting at 28.0°C the compound was successively recrystallized at increasing temperatures until the melting point was raised to 30.5°C. Ten or more hours were required for each recrystallization. The purified NMA was then stored in a flask equipped with a P_2O_5 drying tube at a temperature of 30.4°C. Before use, any liquid present was removed.

A Kjeldahl analysis performed on the purified NMA indicated the presence of 19.1% nitrogen. By comparison with the theoretical 19.14%, the compound was calculated to be 99% pure N-methylacetamide.

Carbon tetrachloride. Fisher Certified Reagent CCl_4 was first refluxed with mercury from 12 to 24 hours to remove any sulfides present. Next, the compound was distilled in a 30-plate Oldershaw column at a reflux ratio of 10:1. Only the middle third of the distillate was saved, the first and last thirds being discarded. The boiling point of the middle fraction corrected to 1 atm. pressure was 77°C. Before the

purified CCl_4 was used, it was extracted 5 times with distilled water to remove residual, water-soluble impurities.

Naphthalene and benzoic acid. Baker & Adamson and Fisher reagent grades, respectively, were used without further purification.

APPARATUS

Liquid-vapor equilibrium still. The apparatus is shown in Fig. 1. The still is designed to operate at reduced pressures, permitting data to be obtained at temperatures below the normal boiling point of the solvent. The pressure is maintained at reduced values with a capillary-bleeder manostat. The vapor trap is designed to force the distillate down through the center and up along the sides of the trap before its return to the pot. This feature allows rapid equilibration between liquid and vapor phases.

To operate the apparatus a dilute solution of NMA in CCl_4 is placed in the pot and distilled at the desired pressure and temperature for 15 minutes. Then samples are withdrawn from both phases and analyzed for concentrations. The analysis is conducted with a Beckman DK-1 spectrophotometer and a standard absorbance curve plotted in the 3μ region.

Molecular weight-measurement apparatus. The device, shown in Fig. 2, consists of solvent and solution chambers separated by a sulfuric acid manometer. The chambers are equipped with sintered-glass disks sealed with mercury[62]. These seals have a twofold function: (1) they act as one-way valves allowing air and solvent vapors to be removed from the system, and (2) they permit the introduction of solvent and solution

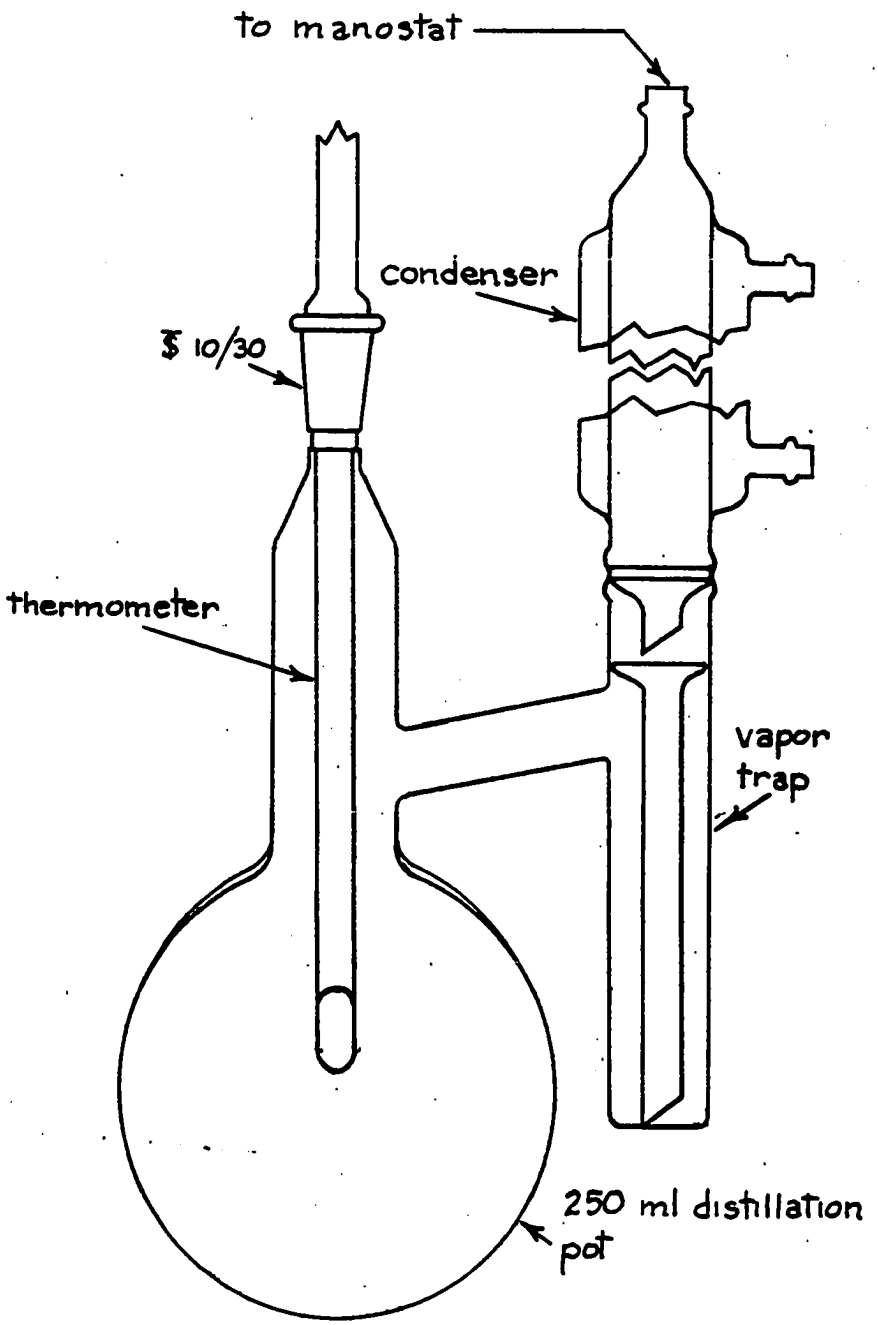


Fig. 1.-- Liquid vapor-equilibrium still

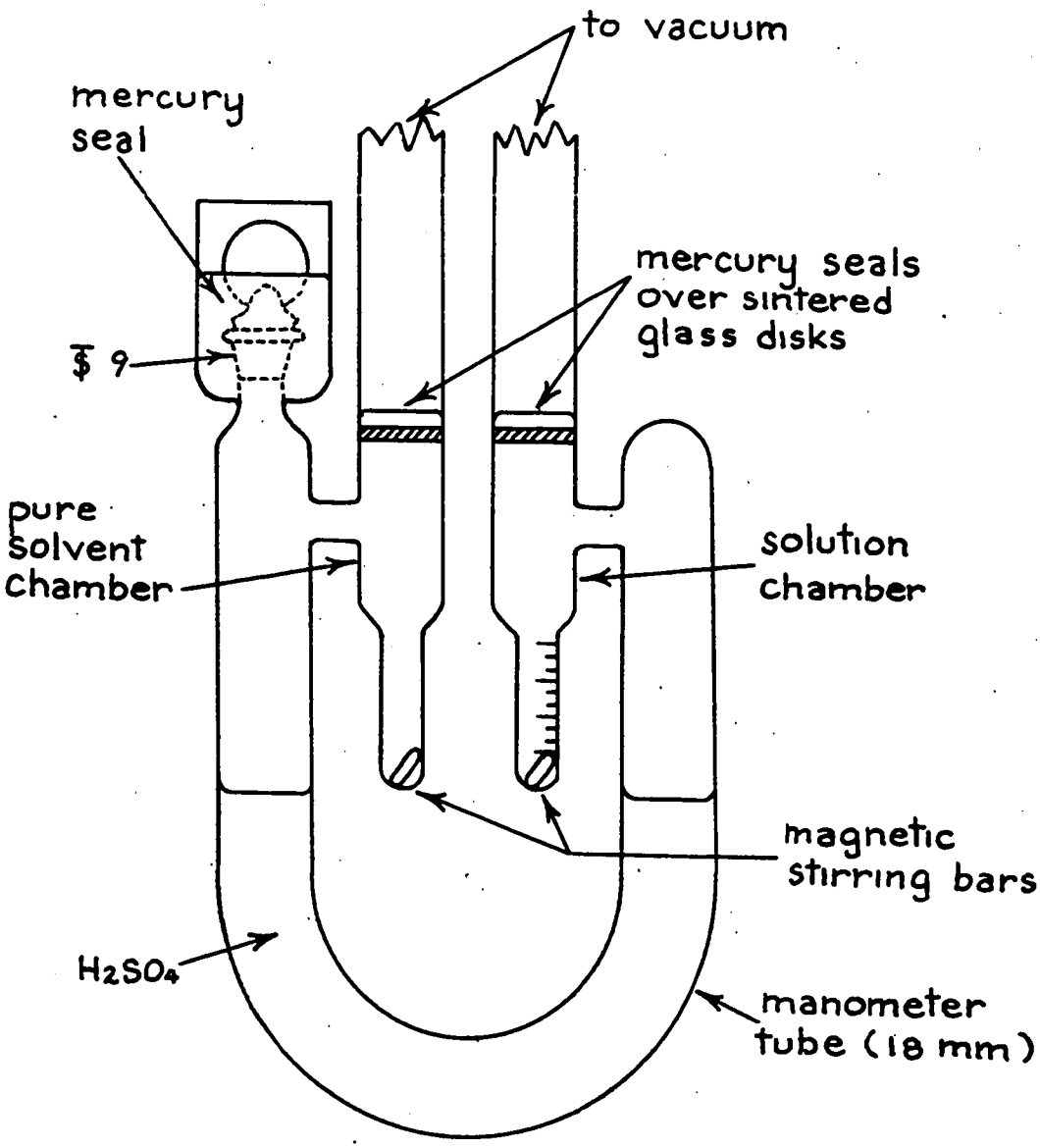


Fig. 2-- Molecular weight-measurement apparatus

into the respective sides of the system. To introduce a liquid through the seal it is only necessary to place the tip of the pipette containing the liquid down through the mercury until the sintered glass disk is touched. Then the liquid will run through the disk and into the chamber. It should be noted that the mercury will not run through the disk even though the system is evacuated. The use of these seals eliminated the need for greased joints and stopcocks, which might cause contamination.

Because the solvent and solution vapor pressures are very sensitive to slight variations in temperature, the apparatus has to be immersed in a water bath having a temperature fluctuation of no more than about 0.001°C. This control can be maintained through the use of a thermostated double water bath in which the temperature of the inner bath is controlled entirely by heat transfer to the outer bath.

An obviously critical factor in the operation of the apparatus is the removal of air from both sides of the system. No problem is encountered in degassing the vapor phases, but the removal of air from the liquids proves to be more difficult. The process is facilitated by agitation with magnetic stirring bars and a reciprocating magnet, although the method is not very efficient. Probably the results would be improved if chambers large enough to incorporate rotating stirring bars were used.

Sulfuric acid is used as the manometer liquid instead of mercury because its lower density permits a greater change in the heights of the manometer columns for a given pressure differential. Its use appears to be satisfactory at 20°C., but at 40°C. some kind of a reaction

with the NMA occurs resulting in the precipitation of small, white crystals just below the surface of the solution. The nature of these crystals is unknown.

To operate the apparatus the manometer is filled to the proper level with sulfuric acid and sealed with mercury as shown in the figure. Next, a small amount of mercury is poured over each sintered-glass disk and the entire system is evacuated with a vacuum manifold. After the apparatus is evacuated as well as possible, pressure is established on both sides of the system by introducing very small amounts of pure solvent on first one side and then the other until the saturation value is reached. The solvent can be easily introduced with a micropipette made from the capillary of a broken thermometer. After the magnetic stirrers are started, the solvent and solution are introduced into their respective chambers. The system must now be evacuated further to remove air dissolved in the two liquids. After a sufficient number of evacuations all dissolved gases will be removed and the manometer will show a pressure differential which changes only slightly upon further evacuation. This residual change is caused by solvent being removed from the solution, resulting in an increased concentration of NMA and a decrease in vapor pressure of the solution. The volume of the solution is measured by reading the graduated marks on the solution tube.

Coulometric water analyzer. The circuit diagram and cell design are shown in Figs. 3 and 4, respectively. The end point is determined by measuring the resistance of the solution with a simple Wheatstone bridge. A Sargent Model IV Coulometric Current Source, capable of

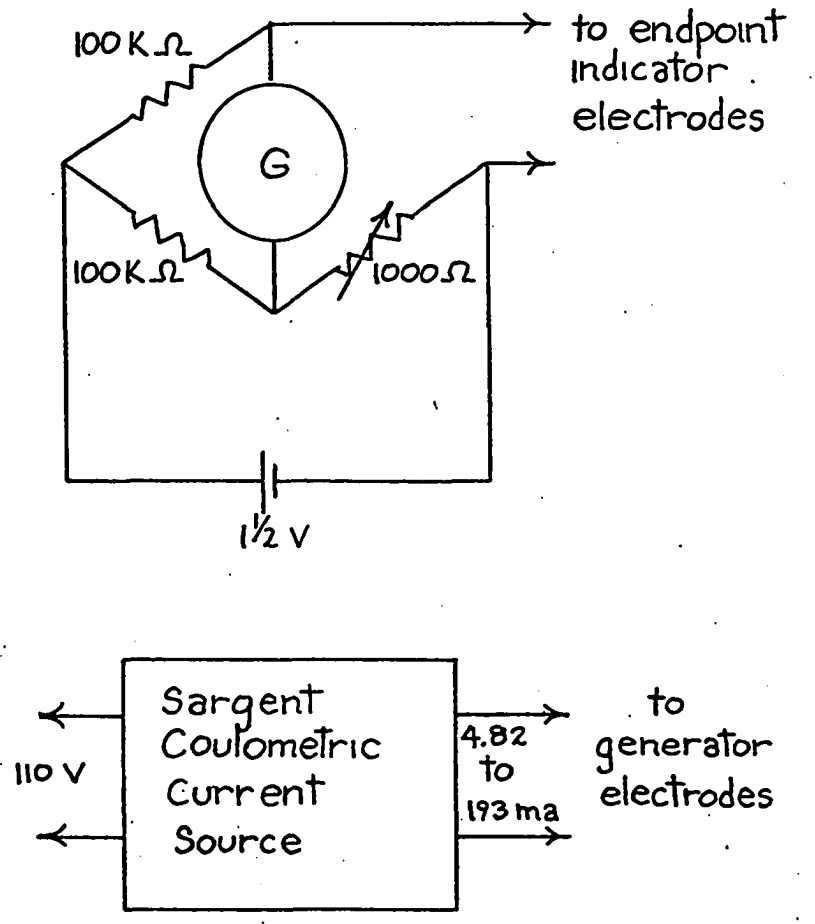


Fig. 3.-- End point-detector circuit and current source for coulometric water analyzer

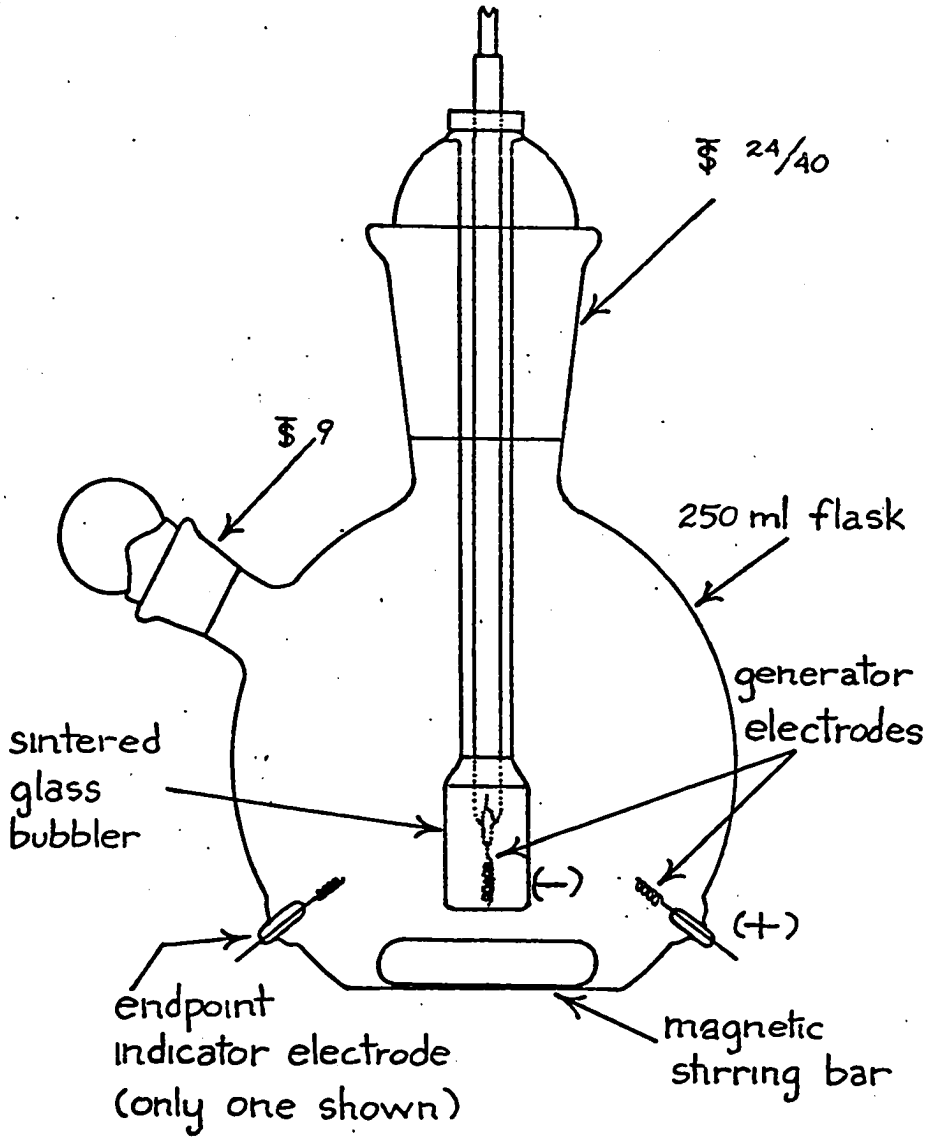


Fig. 4-- Coulometric water analyzer

delivering 4.82 to 193 ma, supplies the current to the generator electrodes. The titration cell consists essentially of a 250 ml flask equipped with platinum indicator and generator electrodes. The latter are separated by a medium porosity sintered-glass bubbler.

The recipe for the Karl Fischer reagent (KFR) is essentially the same as that used by Meyer and Boyd[57]. Forty-five grams of iodine are dissolved in 350 ml of absolute methyl alcohol and then mixed with 213 ml of pyridine. The resulting solution is cooled in an ice bath. While the solution is being cooled, 16 ml of sulfur dioxide are liquified in a dry ice-acetone bath. The liquified SO_2 is now added slowly and with much agitation to the cooled solution. The finished reagent is allowed to stand 2 hours before use.

To put the apparatus into operation the clean, dry cell is first blown out with a stream of dry nitrogen for 1 minute to remove oxygen and residual moisture. The presence of a relatively high oxygen concentration apparently causes the oxidation of iodide to iodine. Next, 100 ml of KFR are added and the magnetic stirrer started. Because the resistance of the solution is sensitive to the rate of stirring, the magnetic stirrer must be able to maintain a constant speed during the titration. The solution is now brought to an end point (color will change from amber to yellow) by the addition of methanol containing 2 to 5 drops of water per 50 ml. If the end point is passed more KFR is added. The final color of the end point should be slightly orange. The liquid level in the cell should now be slightly below the top of the sintered-glass bubbler.

Next, the galvanometer light is plugged in and the battery connected into the end point-detector circuit. The galvanometer should now indicate an end point. If the light beam is not in the center of the scale more methanol or KFR is added dropwise until the end point is reached. The final adjustment may be made by coulometric titration.

The apparatus is next checked for end-point drift. If the end point is drifting to the dry side the cell is blown out with nitrogen for 10 seconds. Drift in the opposite direction is corrected by allowing the solution to stir until the end point becomes steady. Before a sample is introduced, the end point should not be drifting more than 1 scale unit in 3 minutes.

A preliminary run is then made on a sample to obtain the approximate number of microequivalents of water present. The sample size and current setting are chosen to allow the titration to be completed in 5 minutes or less. Usually 1 or 2 ml samples are sufficient. As the sample is introduced the light beam will move rapidly to the wet side. The sample is then titrated coulometrically until the beam returns to the original position. The approximate number of microequivalents may now be calculated.

Next, the end point is readjusted to set the sensitivity (μeq./scale unit) equal to 1% of the approximate number of microequivalents present in a sample. This is accomplished by adding water in methanol solution or KFR (which can be generated coulometrically) and correcting the light beam to the center of the scale by altering the variable resistance in the Wheatstone bridge. For example, suppose the approximate number of microequivalents is 60. The sensitivity should then be

set equal to 0.6μ eq./scale unit. It should be noted that the sensitivity becomes less as the end point is shifted to the dry side.

The end point is again checked for drift and corrected if necessary. Then the sample is introduced and titrated. More precise results are obtained if several samples are run and an average taken. Ordinarily, the reagent does not have to be replaced until the volume of liquid reaches the side arm of the flask.

Total pressure apparatus. The device, shown in Fig. 5, consists essentially of a sintered-galss disk, a flask attached with a ball joint, and a manometer assembly. The sintered-glassed disk permits the introduction of water to the system and acts as a one-way valve for evacuation[62]. The ball joint is sealed with mercury and requires no grease. The seal consists of a glass cup-rubber stopper assembly held in place with springs attached to the flask. The open bottom of the cup is ground to fit the top of the socket. This prevents mercury from running out of the seal. The side pocket attached above the sintered-glass disk serves as a receiver for the mercury above the disk. It is desirable, during long periods of evacuation, to have the mercury removed from the surface of the disk to prevent the formation of scum and consequent clogging. To seal the disk after evacuation it is only necessary to tilt the apparatus to the side causing the mercury to run out of the pocket onto the disk.

In the operation of the apparatus, about a milliliter of mercury is first added above the disk (enough to just fill the side pocket) and the manometer assembly together with the rubber stopper, glass cup, and springs are weighed to the nearest tenth of a gram. The flask is

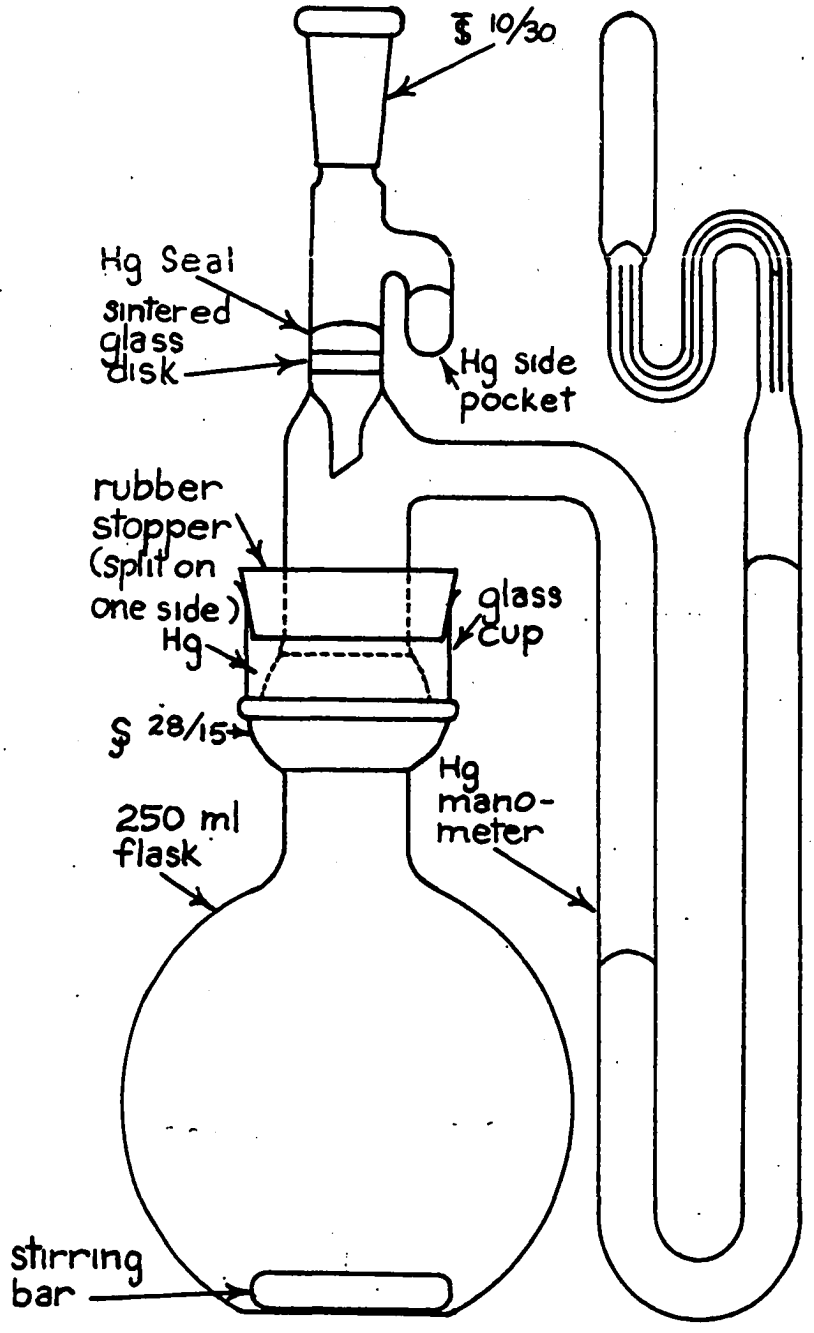


Fig. 5-- Total-pressure apparatus

also weighed to the same accuracy and then filled with the solution of NMA in CCl_4. The apparatus is now assembled with a weighed amount of mercury in the glass cup.

The apparatus is next connected to a water aspirator and placed in a constant temperature bath over a magnetic stirrer. After checking to see if the mercury above the sintered-glass disk is contained in the side pocket, the magnetic stirrer is started and the system evacuated. The evacuation is allowed to continue until the pressure is reduced to approximately the vapor pressure of pure CCl_4, showing that most of the dissolved air and water vapor have been removed. The apparatus is now tilted to the side, causing the mercury to run out of the side pocket onto the sintered-glass disk, and the vacuum line is disconnected. After about 5 minutes the pressure is read with a cathetometer. If the observed value is greater than that for pure CCl_4 (it may be less because of the vapor pressure-lowering effect of the solute) the system is not completely degassed and further evacuation is required.

After all dissolved gases have been removed, as shown by the pressure, the apparatus is removed from the water bath, dried by blotting with paper towels,and weighed to the nearest tenth of a gram. The total weight of the apparatus minus the weight of the manometer assembly, cup, mercury, and flask is equal to the weight of the solution. Thus, the volume of the solution may be calculated and, consequently, the concentration of the NMA.

After the apparatus is again placed in the constant temperature bath, a few milligrams of water are added with a micropipette. The

tip of the pipette is inserted through the mercury until it touches the sintered-glass disk; the delivery is then made and the tip withdrawn. The amount of water added can be accurately measured by weighing the pipette before and after delivery. To facilitate getting the water into the CCl_4 the apparatus is raised until the sintered-glass disk is above the water level, and the region around the disk is heated until the moisture is driven down along the neck.

The apparatus is lowered into the water bath again and allowed to equilibrate. After equilibrium has been attained (ordinarily about two hours are required) the total pressure of the system is measured. The difference between this and the pressure of the dry solution gives the partial pressure of the water. This value divided by the saturation pressure gives the activity of the water in solution. The concentration of the water is obtained by subtracting the amount of water in the vapor phase (calculated from the partial pressure and the previously determined volume in the vapor phase) from the amount of water added to the system. By adding further quantities of water, the total water concentration vs. water activity for a given NMA concentration is obtained.

CHAPTER IV

RESULTS AND DISCUSSION

SELF-ASSOCIATION STUDIES

Theory. If we assume that only two self-association constants -- one for dimerization and one for the formation of species containing 3 or more monomers -- are needed to explain the formation of linear chains, we can write

$$A_1 + A_1 \rightleftarrows A_2 \qquad K_2 = \frac{[A_2]}{[A_1]^2}$$

$$A_2 + A_1 \rightleftarrows A_3 \qquad K_n = \frac{[A_3]}{[A_2][A_1]}$$

$$\vdots$$

$$A_{n-1} + A_1 \rightleftarrows A_n \qquad K_n = \frac{[A_n]}{[A_{n-1}][A_1]}$$

Thus, for the formal or total concentration of the amide

$$[A]t = [A_1] + 2[A_2] + 3[A_3] + \ldots$$

$$= [A_1] + 2K_2[A_1]^2 + 3K_2K_n[A_1]^3 + 4K_2K_n^2[A_1]^4 + \ldots$$

Or $$[A]t = [A_1] + K_2[A_1]^2(2 + 3K_n[A_1] + 4\{K_n[A_1]\}^2 + \ldots)$$

If we let $f(x) = 2 + 3x + 4x^2 + \ldots$

It can be shown that

$$f(x) = \frac{2 - x}{(1 - x)^2}$$

Thus $$\frac{2 - K_n[A_1]}{(1 - K_n[A_1])^2} = 2 + 3\,K_n[A_1] + 4\{K_n[A_1]\}^2 + \ldots$$

Or $$[A]t = [A_1] + \frac{K_2[A_1]^2(2 - K_n[A_1])}{(1 - K_n[A_1])^2} \qquad (4.1)$$

Similarly, if the effective concentration of amide is represented by

$$[A]e = [A_1] + [A_2] + [A_3] + \ldots$$
$$= [A_1] + K_2[A_1]^2 + K_2K_n[A_1]^3 + \ldots$$

by the same mathematical procedure, we can obtain

$$[A]e = [A_1] + \frac{K_2[A_1]^2}{1 - K_n[A_1]} \qquad (4.2)$$

This equation is the same as that derived by Davies and Thomas[23].

Liquid-vapor equilibrium data. For dilute solutions the partial pressure of the amide is given by

$$P_A = P_t \cdot X_A \simeq P_{CCl_4} \cdot X_A$$

where P_t is the total pressure and X_A is the mole fraction of the amide in the vapor phase. With the use of Henry's law, the concentration of the amide monomer in the liquid phase can be expressed by

$$[A_1] = P_A/k \qquad (4.3)$$

where k is the Henry's law constant. After k is evaluated from the initial slope of a plot of P_A vs. [A]t in the liquid phase, the equation can be used to calculate monomer concentrations at any value of the partial pressure. If the monomer concentrations are calculated as a function of total amide concentration, the values can be inserted into eq. (4.1) to obtain the values of the constants K_2 and K_n.

The liquid-vapor equilibrium data for the self-association of NMA in CCl_4 at 60 and 75°C are given in Table 6 and shown in Fig. 6. With the aid of a computer method for least squares analysis developed by Christian and co-workers[58], the best values of the constants K_2 and K_n were found to be 2.7 ± 0.7 and 14.3 ± 1.5 l./mole, respectively, at 60°C. At 75°C, the values were determined to be 1.5 ± 0.4 and 13.8 ± 1.1 l./mole. For both sets of data the Henry's law constants were evaluated by extrapolating the graphs to zero concentration.

Data from molecular weight-measurement apparatus. The measurement of the vapor pressure-lowering effect caused by the presence of a solute in solution gives essentially the effective number of moles of solute, ηe. The relationship between the pressure differential and ηe is given by the equation

$$\eta e = \frac{\Delta P}{P_s^o} \times \frac{V_s}{\nu_m}$$

where P_s^o is the vapor pressure of the pure solvent, V_s is the volume of the solution, ν_m is the molar volume of the solvent, and ΔP is the difference in the vapor pressure of the pure solvent and the solution. For carbon tetrachloride as the solvent at 20°C and with H_2SO_4 as the manometer liquid, the equation becomes

$$\eta e = \Delta P \cdot V_s \cdot 1.540 \times 10^{-5} \frac{\text{moles}}{\text{mm}\cdot\text{ml}}$$

By dividing both sides of the equation with V_s, we obtain the expression for the effective concentration:

$$[A]e = 0.0154\, \Delta P \frac{\text{moles}}{\text{mm}\cdot\text{l}} \qquad (4.4)$$

If the effective concentration is calculated as a function of the total concentration, the average molecular weight of the solute can be

TABLE 6

SELF-ASSOCIATION OF NMA IN CCl_4 AT 60 AND 75°C
LIQUID-VAPOR EQUILIBRIUM DATA.

$[NMA]_t$ in liq. phase (mole/l)	P of NMA (mm Hg)
60°C	
0.0098	0.108
0.015	0.158
0.023	0.26
0.039	0.33
0.048	0.39
0.065	0.43
0.089	0.47
0.114	0.55
75°C	
0.0093	0.15
0.020	0.35
0.033	0.48
0.037	0.53
0.049	0.64
0.069	0.71
0.100	0.86
0.13	0.70

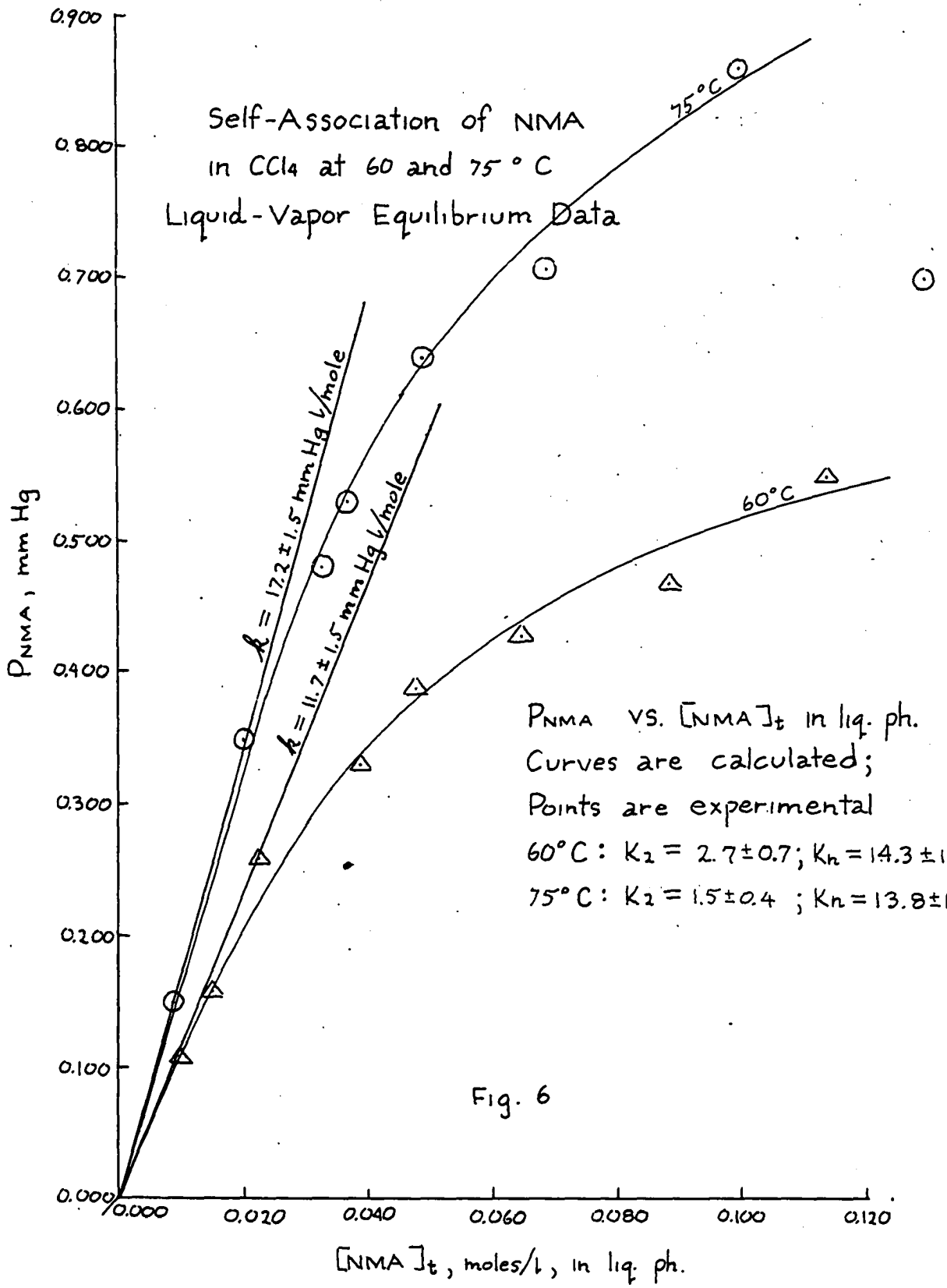

Self-Association of NMA
in CCl4 at 60 and 75 ° C
Liquid-Vapor Equilibrium Data
75°C
60°C
K = 17.2 ± 1.5 mm Hg l/mole
K = 11.7 ± 1.5 mm Hg l/mole
PNMA VS. [NMA]t in liq. ph.
Curves are calculated;
Points are experimental
60° C : K2 = 2.7 ± 0.7; Kn = 14.3 ± 1.5
75° C : K2 = 1.5 ± 0.4 ; Kn = 13.8 ± 1.1
PNMA , mm Hg
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0.000
0.020
0.040
0.060
0.080
0.100
0.120
[NMA]t , moles/l , in liq. ph.

Fig. 6

obtained from

$$\overline{M} = M_m \frac{[A]t}{[A]e} \qquad (4.5)$$

where M_m is the monomer weight of the solute.

Before proceeding with the molecular weight measurements on solutions of NMA the apparatus was tested by measuring the molecular weights of naphthalene and benzoic acid in CCl_4 solutions. The results of the experiments with naphthalene are given in Table 7. The measurements were made at 20°C with concentrations up to 0.0541 mole/l. From the Table it is seen that the average measured value of the molecular weight is 134 $\pm$ 4 as compared to a true molecular weight of 128.16.

The data from the molecular weight-measurements of benzoic acid in CCl_4 at 20°C are given in Table 8. Note that the average measured molecular weight of 245 $\pm$ 3 is twice the value of the true molecular weight (122.12). The doubling of the molecular weight is to be expected because benzoic acid is known to exist predominantly as dimers in non-polar solutions.

The molecular-weight measurement data for the self-association of NMA in CCl_4 at 20°C is given in Table 9 and plotted in Figs. 7 and 8. The observed pressure differentials were not used in the direct calculation of molecular weights and association constants because of an apparent need to correct the values for the partial pressure of NMA above the solution. Without the corrections the pressure differentials would appear to be too small and cause the calculated molecular weights and association constants to be too large.

The need to correct the pressure differentials became apparent from the extrapolation of the Henry's law constant (k) obtained at 60 or 75°C

TABLE 7

MOLECULAR WEIGHT OF NAPHTHALENE IN CCl_4 AT 20°C
MOLECULAR WEIGHT-MEASUREMENT DATA WITH H_2SO_4 AS THE MANOMETER LIQUID
TRUE MOLECULAR WEIGHT = 128

$[C_{10}H_8]_t$ mole/l	ΔP mm H_2SO_4	Mol. Wt.
	Experiment No. 1	
0.0295	1.75	140
0.0299	1.80	137
0.0308	1.83	140
0.0310	1.93	134
0.0387	2.32	137
0.0387	2.46	131
0.0541	3.49	128
	Experiment No. 2	
0.0379	2.41	131
0.0388	2.43	133
0.0399	2.59	128
0.0408	2.58	131
	Experiment No. 3	
0.0444	2.74	135
0.0448	2.75	136
		av. 134
		$\sigma = \pm 4$

TABLE 8

MOLECULAR WEIGHT OF BENZOIC ACID IN CCl_4 AT 20°C
MOLECULAR WEIGHT-MEASUREMENT DATA WITH H_2SO_4 AS THE MANOMETER LIQUID
MOLECULAR WEIGHT OF DIMER = 244.

$[C_6H_5COOH]_t$ (mole/l)	ΔP (mm H_2SO_4)	Mol. Wt.
0.102	3.34	243
0.104	3.40	242
0.107	3.46	245
0.188	6.21	240
0.191	6.21	245
0.199	6.41	246
0.202	6.55	248
0.211	6.67	251
		av. 245
		$\sigma = \pm 3$

TABLE 9

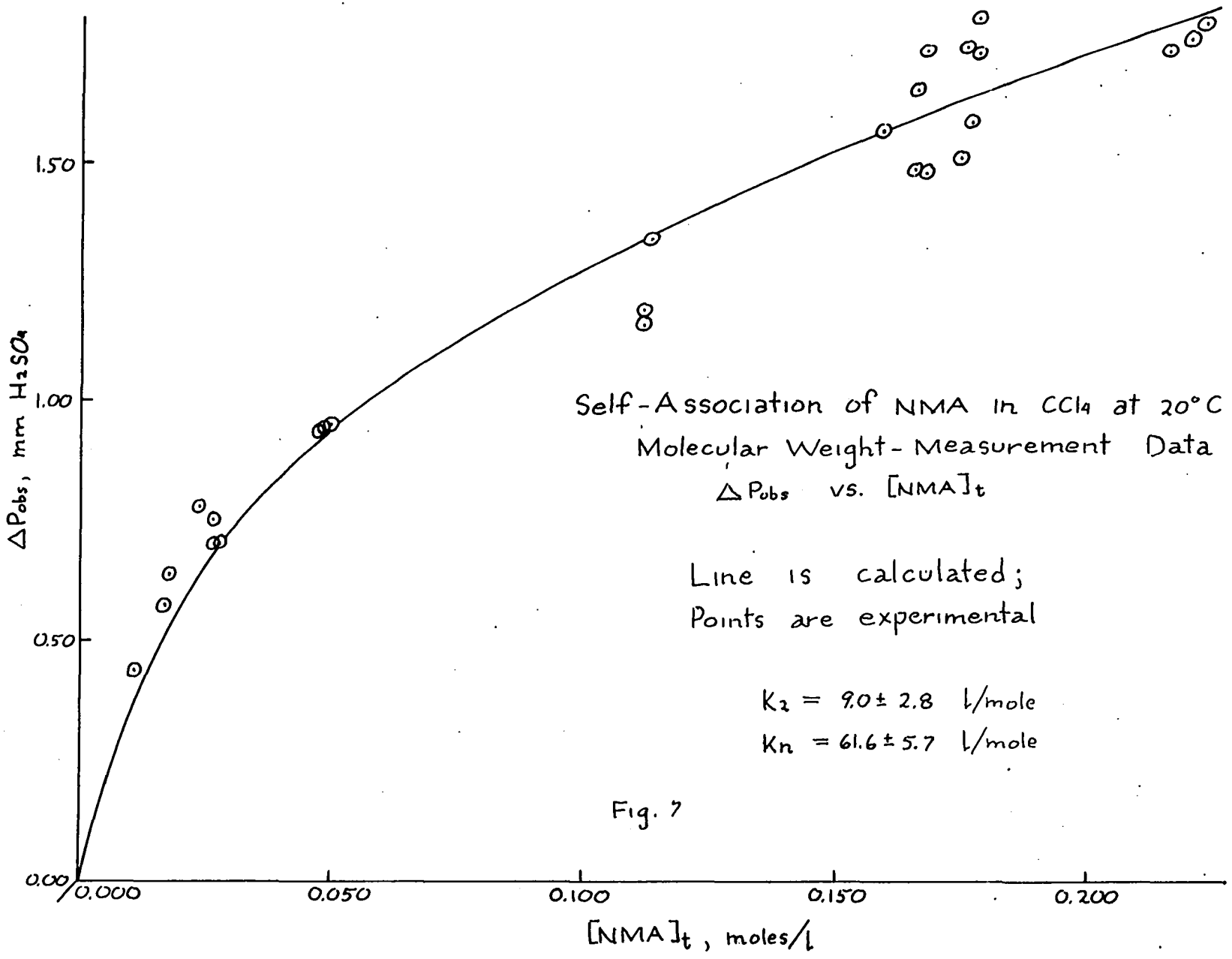
SELF-ASSOCIATION OF NMA IN CCl_4 AT 20°C
MOLECULAR WEIGHT-MEASUREMENT DATA WITH H_2SO_4 AS MANOMETER LIQUID.

$[NMA]_t$ (mole/l)	ΔP_{obs} (mm H_2SO_4)	$\overline{M}^*$
0.0104	0.43	76
0.0170	0.57	97
0.0182	0.64	106
0.0238	0.78	104
0.0263	0.70	126
0.0271	0.70	130
0.0276	0.75	125
0.0475	0.94	178
0.0479	0.94	180
0.0493	0.95	183
0.111	1.19	341
0.111	1.16	348
0.113	1.34	315
0.159	1.56	390
0.165	1.65	387
0.165	1.49	422
0.167	1.48	428
0.167	1.73	377
0.174	1.51	440
0.175	1.74	393

TABLE 9...continued

$[NMA]_t$ (mole/l)	ΔP_{obs} (mm H_2SO_4)	$\bar{M}$*
0.176	1.59	426
0.177	1.73	397
0.177	1.80	385
0.215	1.73	485
0.219	1.76	487
0.222	1.79	486

*$\bar{M}$ was calculated with corrected pressure differentials.

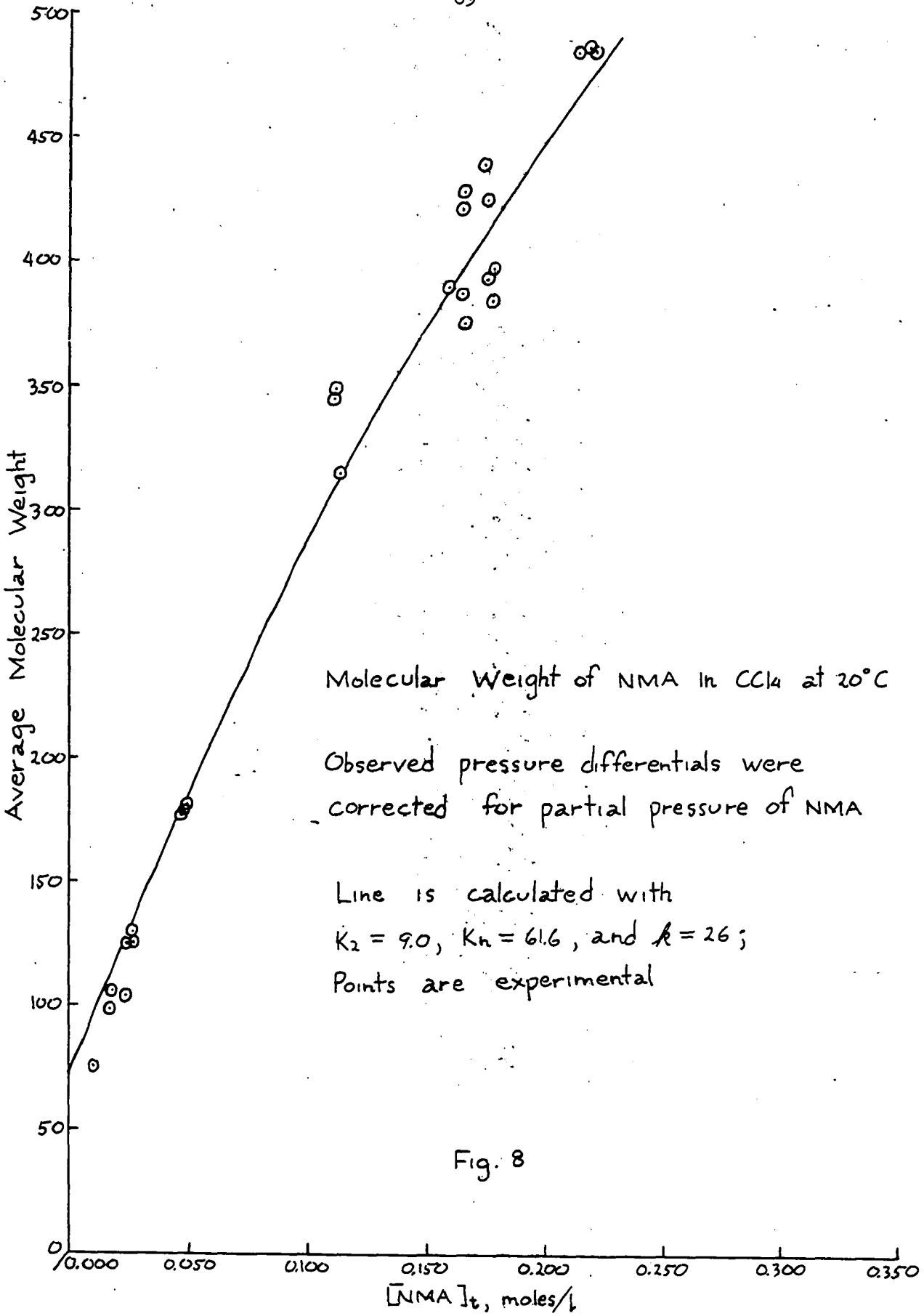


Self-Association of NMA in CCl_4 at 20°C
Molecular Weight-Measurement Data
ΔP_{obs} vs. $[NMA]_t$

Line is calculated;
Points are experimental

$K_2 = 9.0 \pm 2.8$ l/mole
$K_n = 61.6 \pm 5.7$ l/mole

Fig. 7



Molecular Weight of NMA in CCl_4 at 20°C

Observed pressure differentials were corrected for partial pressure of NMA

Line is calculated with $K_2 = 9.0$, $K_n = 61.6$, and $k = 26$;
Points are experimental

Fig. 8

from the liquid-vapor equilibrium data to the temperature at which the molecular-weight measurements were taken (20°C). With k = 11.7 ± 1.5 mm Hg l/mole at 60°C and 17.2 ± 1.5 mm Hg l/mole at 75°C, an enthalpy of vaporization of NMA at infinite dilution was calculated from the Clausium-Clapeyron equation to be 5.9 ± 2.4 Kcal/mole. The value of k at 20°C was then calculated to be 3.5 ± 1.8 mm Hg l/mole or, for sulfuric acid as the manometer liquid, 26 ± 13 mm H_2SO_4 l/mole.

The large error in the Henry's law constant at 20°C naturally introduces large uncertainties in the magnitudes of the corrections to be applied to the pressure differentials. With a value of k equal to 26 mm H_2SO_4 l/mole it can be shown that the magnitudes of the corrections will be approximately 30% of the uncorrected pressure differentials. Thus, with an error in k of ± 50%, the error in the corrected pressure differentials will be ± 50 x 30% = 15%. No attempt was made to apply corrections calculated with values of k other than 26.

Taking the vapor-pressure correction P_A into account, the observed pressure differential can be expressed as

$$\Delta P_{obs} = \Delta P - P_A$$

Substitution for P_A from eq. (4.3) and for ΔP from eq. (4.4) gives

$$\Delta P_{obs} = \frac{[A]_e}{0.0154} - k[A_1]$$

If $[A]_e$ is replaced by the expression in eq. (4.2) we obtain

$$\Delta P_{obs} = \frac{1}{0.0154}\left([A_1] + \frac{K_2[A_1]^2}{1 - K_n[A_1]}\right) - k[A_1]$$

This equation can be solved to give

$$[A_1] = \frac{1 + 0.0154\, K_n \Delta Pobs - 0.0154k}{2(K_n - K_2 - 0.0154kK_n)}$$

$$- \frac{\sqrt{(0.0154k - 0.0154K_n \Delta Pobs - 1)^2 - 4(K_n - K_2 - 0.0154kK_n)0.0154\Delta Pobs}}{2(K_n - K_2 - 0.0154kK_n)}$$

Using the experimental values of the pressure differentials (ΔPobs), the extrapolated value of k (26), plus approximate values of K_2 and K_n (3.5 and 47, respectively), we can solve the equation for the approximate values of $[A_1]$. These values can then be inserted in eq. (4.1) to obtain calculated values of [A]t. By altering the values of K_2 and K_n, a minimum in the sum of the squares of the differences in the experimental and calculated values of [A]t can be found. The values of K_2 and K_n thus obtained are used to obtain new monomer concentrations, whereupon the entire process is repeated until the best values of the constants are found. With the aid of a computer program similar to that developed for the liquid-vapor equilibrium data, the best values of the constants were found to be $K_2 = 9.0 \pm 2.8$ and $K_n = 61.6 \pm 5.7$ l/mole at 20°C. The value of K_2 is in fair agreement with that of 4.7 to 5.8 l/mole at 25°C reported by Klotz and Franzen[41]. No value was given for K_n.

By combining the self-association constants obtained at 20°C from the molecular-weight measurements with those obtained at 60 and 75°C from the liquid-vapor equilibrium data, enthalpies of association for K_2 and K_n were calculated. With K_2 = 2.7 at 60°C and 9.0 at 20°C ΔH (K_2) was calculated to be -5.8 Kcal/mole. At 75°C K_2 was found to be 1.5. This value, when combined with that at 20°C gave a ΔH (K_2) equal to -6.6 Kcal/mole. Thus, the average value of ΔH (K_2) was

calculated to be -6.2 Kcal/mole. According to Klotz and Franzen the value should be -4.2 Kcal/mole.

Similarly, for K_n = 14.3 at 60°C and 61.6 at 20°C, ΔH (K_n) was found to be -7.1 Kcal/mole. At 75°C K_n was calculated to be 13.8. When combined with the K_n obtained at 20°C, this value gave a ΔH (K_n) of -5.5 Kcal/mole. Consequently, the average value of ΔH (K_n) was found to be -6.3 Kcal/mole. No value for the enthalpy change was reported by Klotz and Franzen.

HYDRATION STUDIES

Theory. Because N-methylacetamide self-associates to form chains containing a number of monomeric units, it is reasonable to assume that each chain contains multiple sites for the attachment of water molecules. Based on this assumption, an equation can be derived that adequately explains the relationship between total amide concentration, total water concentration, and water monomer concentration in solution.

Suppose we consider a chain having only one hydration site. The equilibrium in terms of the forward and reverse hydration reactions can be represented as follows:

$$\left.\begin{matrix} \\ \\ \\ \\ X \end{matrix}\right\} + W \underset{r_r^{11}}{\overset{r_f^{11}}{\rightleftharpoons}} \left.\begin{matrix} \\ \\ \\ \\ XW \end{matrix}\right\} \qquad K_{11} = \frac{r_f^{11}}{r_r^{11}}$$

where X is the hydration site on the chain, r_f^{11} is the rate constant for the forward reaction, and r_r^{11} is the constant for the reverse reaction.

For the attachment of one water molecule to a chain having two independent sites, the equilibrium would be

$$\left.\begin{matrix} \\ \\ \\ \\ \\ \\ \\ X \\ X \end{matrix}\right\} + W \underset{r_r^{21}}{\overset{r_f^{21}}{\rightleftharpoons}} \left.\begin{matrix} \\ \\ \\ \\ \\ \\ \\ X \\ XW \end{matrix}\right\} \qquad K_{21} = \frac{r_f^{21}}{r_r^{21}} = \frac{2r_f^{11}}{r_r^{11}} = 2K_{11}$$

and, in general, for the attachment of one water molecule to a chain having n independent sites, we have

$$\left.\begin{matrix} . \\ . \\ . \\ . \\ . \\ X \\ X \\ X \end{matrix}\right\} + W \underset{r_r^{n1}}{\overset{r_f^{n1}}{\rightleftharpoons}} \left.\begin{matrix} . \\ . \\ . \\ . \\ . \\ X \\ XW \\ XW \end{matrix}\right\} \qquad K_{n1} = \frac{r_f^{n1}}{r_r^{n1}} = \frac{nr_f^{11}}{r_r^{11}} = nK_{11}$$

To attach a second water molecule to a chain having n sites

$$\left.\begin{matrix} . \\ . \\ . \\ . \\ . \\ X \\ X \\ XW \end{matrix}\right\} + W \underset{r_r^{n2}}{\overset{r_f^{n2}}{\rightleftharpoons}} \left.\begin{matrix} . \\ . \\ . \\ . \\ . \\ X \\ XW \\ XW \end{matrix}\right\} \qquad K_{n2} = \frac{r_f^{n2}}{r_r^{n2}} = \frac{(n-1)r_f^{11}}{2r_r^{11}} = \frac{(n-1)}{2} K_{11}$$

and to bind a third water

$$\left.\begin{matrix}\cdot\\ \cdot\\ \cdot\\ \cdot\\ X\\ XW\\ XW\end{matrix}\right\} \quad + W \underset{r_r^{n3}}{\overset{r_f^{n3}}{\rightleftharpoons}} \quad \left.\begin{matrix}\cdot\\ \cdot\\ \cdot\\ \cdot\\ XW\\ XW\\ XW\end{matrix}\right\}$$

$$K_{n3} = \frac{r_f^{n3}}{r_r^{n3}}$$

$$= \frac{(n-2)r_f^{11}}{3r_r^{11}}$$

$$= \frac{(n-2)}{3} K_{11}$$

Thus, by generalizing we can say that if n is the number of independent hydration sites and υ is the number of attached water molecules in the product of the reaction,

$$K_{n\upsilon} = \frac{h - \upsilon + 1}{\upsilon} K_{11}$$

By using this equation we can derive a general equation for the concentration of the species A_nW_υ in terms of the concentration of the water monomer and the concentration of A_n. For the reaction

$$A_nW_{\upsilon-1} + W \rightleftharpoons A_nW_\upsilon$$

the equilibrium constant can be expressed by

$$K_{n\upsilon} = \frac{[A_nW_\upsilon]}{[A_nW_{\upsilon-1}][W]}$$

$$= \frac{n - \upsilon + 1}{\upsilon} K_{11}$$

Therefore,

$$[A_nW_\upsilon] = \frac{n - \upsilon + 1}{\upsilon} K_{11}[W][A_nW_{\upsilon-1}]$$

Similarly, for the reaction

$$A_nW_{\upsilon-2} + W \rightleftharpoons A_nW_{\upsilon-1}$$

we can write

$$K_{n(\upsilon-1)} = \frac{[A_n W_{\upsilon-1}]}{[A_n W_{\upsilon-2}][W]}$$

$$= \frac{n-(\upsilon-1)+1}{\upsilon-1} K_{11}$$

Thus,

$$[A_n W_{\upsilon-1}] = \frac{n-(\upsilon-1)+1}{(\upsilon-1)} K_{11} [W][A_n W_{\upsilon-2}]$$

Consequently,

$$[A_n W_\upsilon] = \frac{n-\upsilon+1}{\upsilon} \cdot \frac{n-(\upsilon-1)+1}{\upsilon-1} (K_{11}[W])^2 [A_n W_{\upsilon-2}]$$

By repeating the procedure for $[A_n W_{\upsilon-2}]$, etc. it can be shown that

$$[A_n W_\upsilon] = \frac{(n-\upsilon+1)(n-\upsilon+2)\cdots n}{\upsilon!}(K_{11}[W])^2 [A_n]$$

Or $$[A_n W_\upsilon] = \frac{n!}{\upsilon!(n-\upsilon)!} (K_{11}[W])^\upsilon [A_n]$$

Thus for any n and υ, the concentration of the species $[A_n W_\upsilon]$ can be written as a function of K_{11}, $[W]$, and $[A_n]$.

Using the above result we can derive a general equation for the total concentration of water in solution. In terms of the water monomer concentration and all possible hydrated species in solution the total water concentration can be expressed as

$$\begin{aligned}[W]_t = {} & [W] + [AW] + [A_2W] + [A_3W] + \ldots \\ & + 2[A_2W_2] + 2[A_3W_2] + 2[A_4W_2] + \ldots \\ & + 3[A_3W_3] + 3[A_4W_3] + 3[A_5W_3] + \ldots \\ & + \ldots\end{aligned}$$

By substitution for the concentrations from the equation for $[A_n W_\upsilon]$, we obtain

$$[W]_t = [W] + K_{11}[W][A_1] + \frac{2}{1\cdot1} K_{11}[W][A_2] + \ldots$$
$$+ 2\frac{2}{2\cdot1} (K_{11}[W])^2[A_2] + 2\frac{2\cdot3}{2\cdot1} (K_{11}[W])^2[A_3] + \ldots$$
$$+ 3\frac{2\cdot3}{2\cdot3\cdot1} (K_{11}[W])^3[A_3] + 3\frac{2\cdot3\cdot4}{2\cdot3\cdot1} (K_{11}[W])^3[A_4] + \ldots$$
$$+ \ldots$$

Or

$$[W]_t = [W] + K_{11}[W]([A_1] + 2[A_2] + \ldots)$$
$$+ (K_{11}[W])^2(1\cdot2[A_2] + 2\cdot3[A_3] + \ldots)$$
$$+ \frac{(K_{11}[W])^3}{1\cdot2} (1\cdot2\cdot3[A_3] + 2\cdot3\cdot4[A_4] + \ldots)$$
$$+ \ldots$$

Through the same procedure a similar equation for $[A]_t$ can be derived. Let the total concentration of the amide be represented as

$$[A]_t = [A_1] + 2[A_2] + 3[A_3] + \ldots$$
$$+ [AW] + 2[A_2W] + 3[A_3W] + \ldots$$
$$+ 2[A_2W_2] + 3[A_3W_2] + 4[A_4W_2] + \ldots$$
$$+ \ldots$$

Using the equation for $[A_nW_v]$,

$$[A]_t = [A_1] + 2[A_2] + 3[A_3] + \ldots$$
$$+ K_{11}[W][A_1] + 2\frac{2}{1\cdot1} K_{11}[W][A_2] + 3\frac{2\cdot3}{1\cdot2} K_{11}[W][A_3] + \ldots$$
$$+ 2\frac{2}{2\cdot1} (K_{11}[W])^2[A_2] + 3\frac{2\cdot3}{2\cdot1} (K_{11}[W])^2[A_3] + \ldots$$
$$+ \ldots$$

Or

$$[A]_t = [A_1] + 2[A_2] + 3[A_3] + \ldots$$

$$+ K_{11}[W]\left(\frac{1!}{0!}[A_1] + \frac{2!}{1!} \cdot 2[A_2] + \frac{3!}{2!} \cdot 3[A_3] + \ldots\right)$$

$$+ \frac{(K_{11}[W])^2}{2!}\left(\frac{2!}{0!} \cdot 2[A_2] + \frac{3!}{1!} \cdot 3[A_3] + \frac{4!}{2!} \cdot 4[A_4] + \ldots\right)$$

$$+ \ldots$$

The equation expressing the relationship between $[W]_t$, $[A]_t$, and $[W]$ can now be derived through a combination of the equations for $[A]_t$ and $[W]_t$. Separate the series for $[A]_t$ into the two series:

$$[A]_t = [A_1] + 2[A_2] + 3[A_3] + \ldots$$

$$+ \frac{K_{11}[W]}{1!}\left(1\cdot2\,[A_2] + 2\cdot3\,[A_3] + 3\cdot4\,[A_4] + \ldots\right)$$

$$+ \frac{(K_{11}[W])^2}{2!}\left(1\cdot2\cdot3\,[A_3] + 2\cdot3\cdot4\,[A_4] + 3\cdot4\cdot5\,[A_5] + \ldots\right)$$

$$+ \ldots$$

$$+ \frac{K_{11}[W]}{0!}\left([A_1] + 2[A_2] + 3[A_3] + \ldots\right)$$

$$+ \frac{(K_{11}[W])^2}{1!}\left(1\cdot2\,[A_2] + 2\cdot3\,[A_3] + 3\cdot4\,[A_4] + \ldots\right)$$

$$+ \ldots$$

From the equation for $[W]_t$ it is seen that the first series is simply

$$\frac{[W]_t - [W]}{K_{11}[W]}$$

and that the second series is

$$[W]_t - [W]$$

Therefore,

$$[A]_t = \frac{[W]_t - [W]}{K_{11}[W]} + [W]_t - [W]$$

Or, by rearranging terms,

$$\frac{[W]_t}{[W]} = 1 + K_{11}([A]_t - [W]_t + [W]) \qquad (4.6)$$

Note that the equation does not contain terms depending on the self-association of the amide. Also note that the original equations for $[W]_t$ and $[A]_t$ limit the number of attached water molecules to the number of amide monomers in the chain. However, the possibility of having more than one water per amide monomer cannot be ruled out without deriving and testing the appropriate equations.

Data from constant water-activity equilibrators. Before the coulometric Karl Fischer titrator was used to analyze samples from the equilibrators, it was checked out by three different methods. First, the water in 1 ml samples taken from a flask of CCl_4 was titrated repeatedly with the current ranging from 4.82 to 193 ma. Any dependence of the results on the current setting would have appeared as a variation in the number of microequivalents of water per sample. In fact, as seen from Table 10, no significant difference in the amount of water was found from one sample to another.

As a second test of the apparatus the concentration of water in samples taken from a flask of benzene was determined by the coulometric Karl Fischer titrator, by a Beckman KF-3 Aquameter, and by a P_2O_5 moisture balance. From Table 11, it is seen that the concentration of water found by the coulometric method is somewhat higher than that found by the other two methods.

A third check of the apparatus was obtained by comparing the coulometrically determined concentration of water in CCl_4 vs. water activity

TABLE 10

DETERMINATION OF WATER IN A SAMPLE OF CCl_4 BY COULOMETRIC KARL FISCHER TITRATION. TEST FOR DEPENDENCE OF RESULTS ON CURRENT MAGNITUDE.

Timer		Current Multiplier		Microequivalents of H_2O
571	x	0.02	=	11.42
560	x	0.02	=	11.20
590	x	0.02	=	11.80
570	x	0.02	=	11.40
1200	x	0.01	=	12.00
1100	x	0.01	=	11.00
113	x	0.10	=	11.30
116	x	0.10	=	11.60
59	x	0.20	=	11.80
2325	x	0.005	=	11.63
				ave. 11.52
1 ml samples were used.				$\sigma = \pm 0.31$

TABLE 11

DETERMINATION OF WATER IN A SAMPLE OF BENZENE
A COMPARISON OF RESULTS OBTAINED FROM COULOMETRIC KARL FISCHER TITRATOR WITH THOSE FROM BECKMAN KF-3 AQUAMETER AND P_2O_5 MOISTURE BALANCE.

METHOD	$[H_2O]$
Coulometric Karl Fischer titrator	0.0180 mole/l (2.00 ml samples)
Aquameter standardized against sodium tartrate dihydrate	0.0161 mole/l (25.00 ml samples)
P_2O_5 moisture balance	0.0175 mole/l (10.00 ml samples)

(Table 12) with that determined by the total-pressure apparatus (Table 13). As seen from Figs. 9 and 10, the Henry's law constant at 25°C was found to be 0.0098 mole/l by the coulometric method and 0.0088 mole/l by the total pressure-measurement technique. The latter value is in excellent agreement with the value of 0.0087 mole/l obtained by Johnson[59] (from solute-isopiestic technique) and by Clifford[60] (transpiration technique). Thus, it appears that the coulometric titrator gave values for the water concentrations about 10 to 12% too high. However, the errors apparently did not have much affect on the value of the hydration constant obtained by this method, as will be seen below.

The data obtained from the constant water-activity equilibrators consist of total water concentrations vs. total amide concentrations at constant water activities. The experimental points can be used to test the hydration theory if eq. (4.6) is put into the form

$$[W]_t = [W] + \frac{K_{11}[W]}{1 + [W]} [A]_t \qquad (4.7)$$

Thus, a plot of total water concentration vs. total amide concentration at constant water monomer concentration or water activity should produce a straight line. The data obtained at 25°C for activities of 0.69, 0.56, 0.47, and 0.31 are given in Table 14 and plotted in Figs. 11 and 12. The constant K_{11} was found to be equal to 10.7 $\pm$ 0.5 from a least-squares analysis of the data plotted for eq. (4.6), as shown in Fig. 11. The water monomer concentrations required in the calculations were obtained from the equation

$$[W] = 0.0098\ P/P^{\circ}$$

TABLE 12

CONCENTRATION OF WATER IN CCl_4 VS. WATER ACTIVITY AT 25°C
DATA FROM CONSTANT WATER-ACTIVITY EQUILIBRATORS.

P/p°	$[H_2O]$ mole/l
0.31	0.00352
0.47	0.00455
0.47	0.00449
0.49	0.00463
0.56	0.00552
0.69	0.00657
0.69	0.00669
0.69	0.00677
0.88	0.00874
0.89	0.00867
0.95	0.00989
1.00	0.0101

TABLE 13

CONCENTRATION OF WATER IN CCl_4 VS. WATER ACTIVITY AT 25°C
DATA FROM TOTAL-PRESSURE APPARATUS

P/p°	$[H_2O]$ mole/l
0.112	0.00095
0.153	0.00130
0.242	0.00214
0.267	0.00224
0.272	0.00233
0.372	0.00321
0.397	0.00351
0.503	0.00443
0.532	0.00453
0.534	0.00448
0.625	0.00541
0.659	0.00585
0.757	0.00681
0.767	0.00673
0.777	0.00679
0.862	0.00767
0.904	0.00825

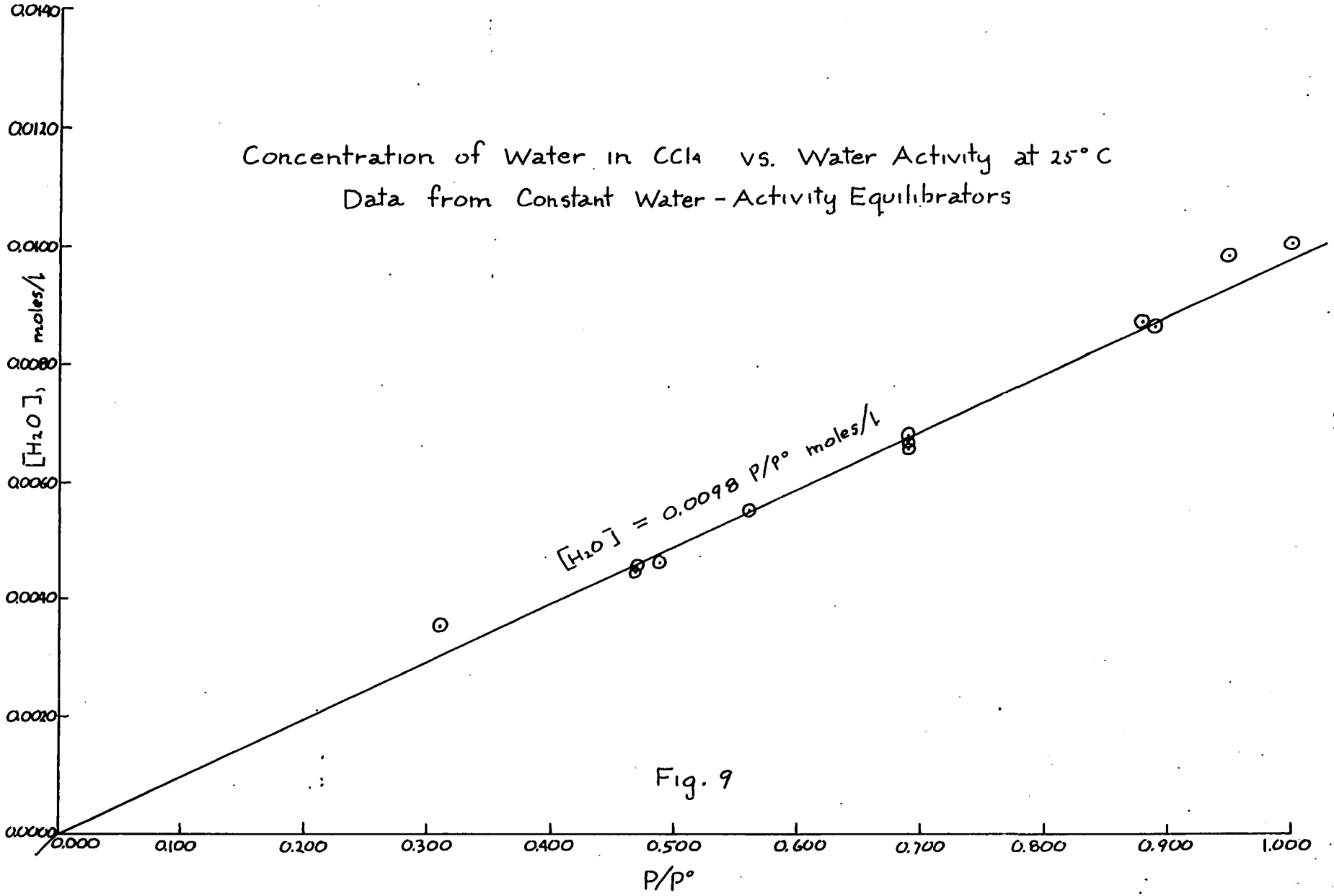

Concentration of Water in CCl4 vs. Water Activity at 25° C
Data from Constant Water - Activity Equilibrators
[H2O] = 0.0098 P/P° moles/l
[H2O], moles/l
0.0140
0.0120
0.0100
0.0080
0.0060
0.0040
0.0020
0.0000
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
0.800
0.900
1.000
P/P°

Fig. 9

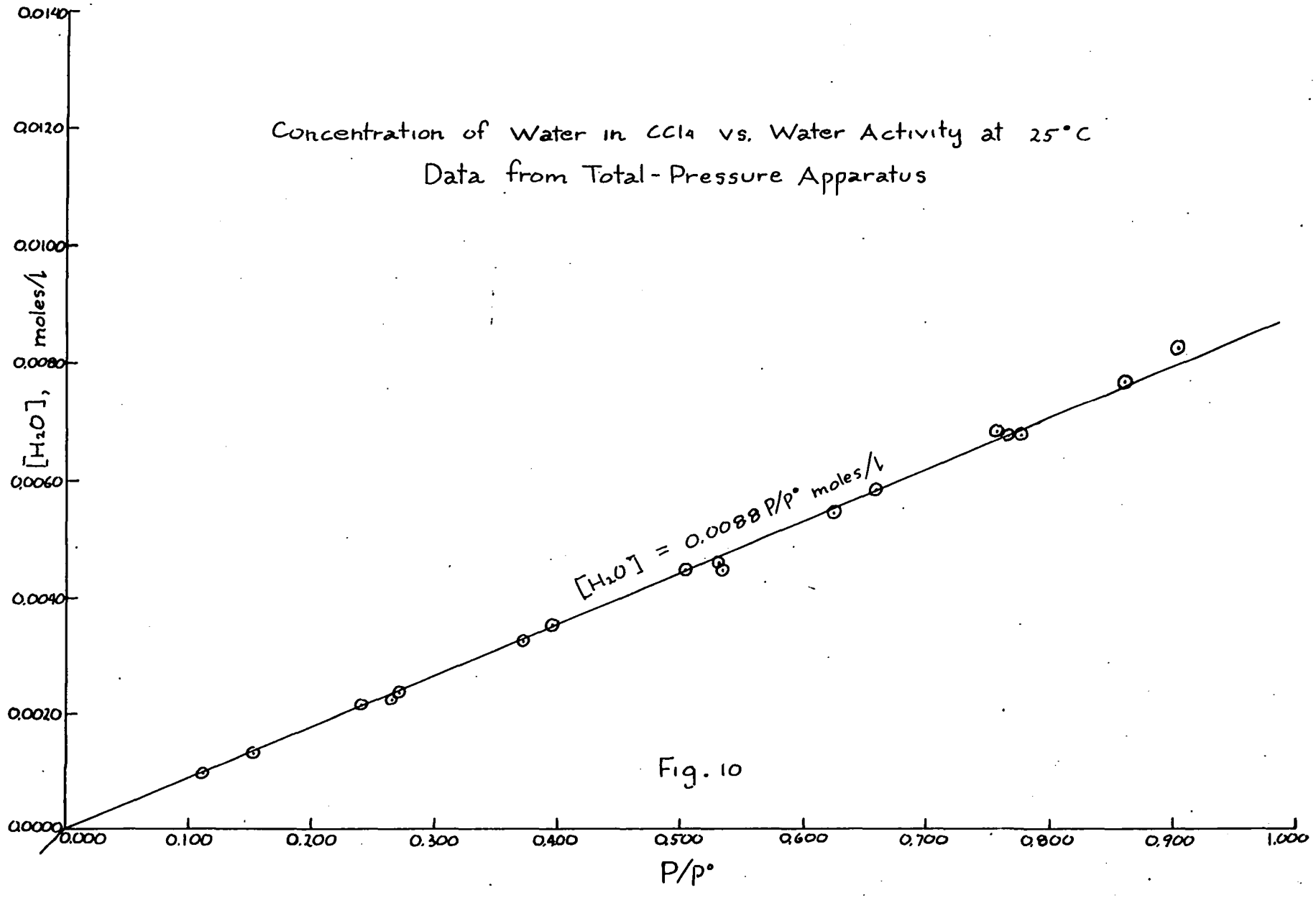

Concentration of Water in CCl4 vs. Water Activity at 25°C
Data from Total-Pressure Apparatus
[H2O], moles/l
0.0140
0.0120
0.0100
0.0080
0.0060
0.0040
0.0020
0.0000
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
0.800
0.900
1.000
P/p°
[H2O] = 0.0088 P/p° moles/l

Fig. 10

TABLE 14

HYDRATION OF NMA IN CCl_4 AT 25°C
DATA FROM CONSTANT WATER-ACTIVITY EQUILIBRATORS
CONCENTRATION OF WATER VS. CONCENTRATION OF NMA AT CONSTANT WATER ACTIVITY.

Water monomer concentrations were taken from graph of concentration of water in CCl_4 vs. water-activity (constant water-activity equilibrators)

$[W] = 0.0098\ P/p^\circ$ mole/l

$[W]_t$ (mole/l)	$[NMA]_t$ (mole/l)	$[W]_t/[W]$	$[NMA]_t-[W]_t+[W]$ (mole/l)
	$P/p^\circ = 0.31$; $[W] = 0.00304$ mole/l		
0.00352	0.0000	1.16	------
0.00312	0.0108	1.025	0.0107
0.00420	0.0323	1.38	0.0312
0.00432	0.0538	1.42	0.0529
	$P/p^\circ = 0.47$; $[W] = 0.00460$ mole/l		
0.00455	0.0000	0.99	------
0.00522	0.0160	1.14	0.0154
0.00592	0.0285	1.29	0.0272
0.00664	0.0406	1.44	0.0386
0.00722	0.0534	1.57	0.0508
0.00449	0.0000	0.98	------
0.00473	0.00466	1.03	0.00453
0.00515	0.0136	1.12	0.0130
0.00579	0.0304	1.26	0.0292
0.00687	0.0497	1.49	0.0474
0.00570	0.0227	1.24	0.0216
0.00634	0.0367	1.38	0.0350

TABLE 14...continued

$[W]_t$ (mole/l)	$[NMA]_t$ (mole/l)	$[W]_t/[W]$	$[NMA]_t-[W]_t+[W]$ (mole/l)
	$P/p^\circ = 0.56$; $[W] = 0.00548$ mole/l		
0.00552	0.0000	1.01	------
0.00619	0.0108	1.13	0.0101
0.00742	0.0323	1.35	0.0304
0.00877	0.0537	1.60	0.0504
	$P/p^\circ = 0.69$; $[W] = 0.00676$ mole/l		
0.00669	0.0000	0.99	0.00007
0.00699	0.00698	1.03	0.00675
0.00808	0.0172	1.20	0.0159
0.00810	0.0412	phase separation	
0.00677	0.0000	1.00	------
0.00722	0.00509	1.07	0.00463
0.00716	0.0100	1.06	0.0096
0.00761	0.0164	1.13	0.0156
0.00813	0.0204	1.20	0.0191

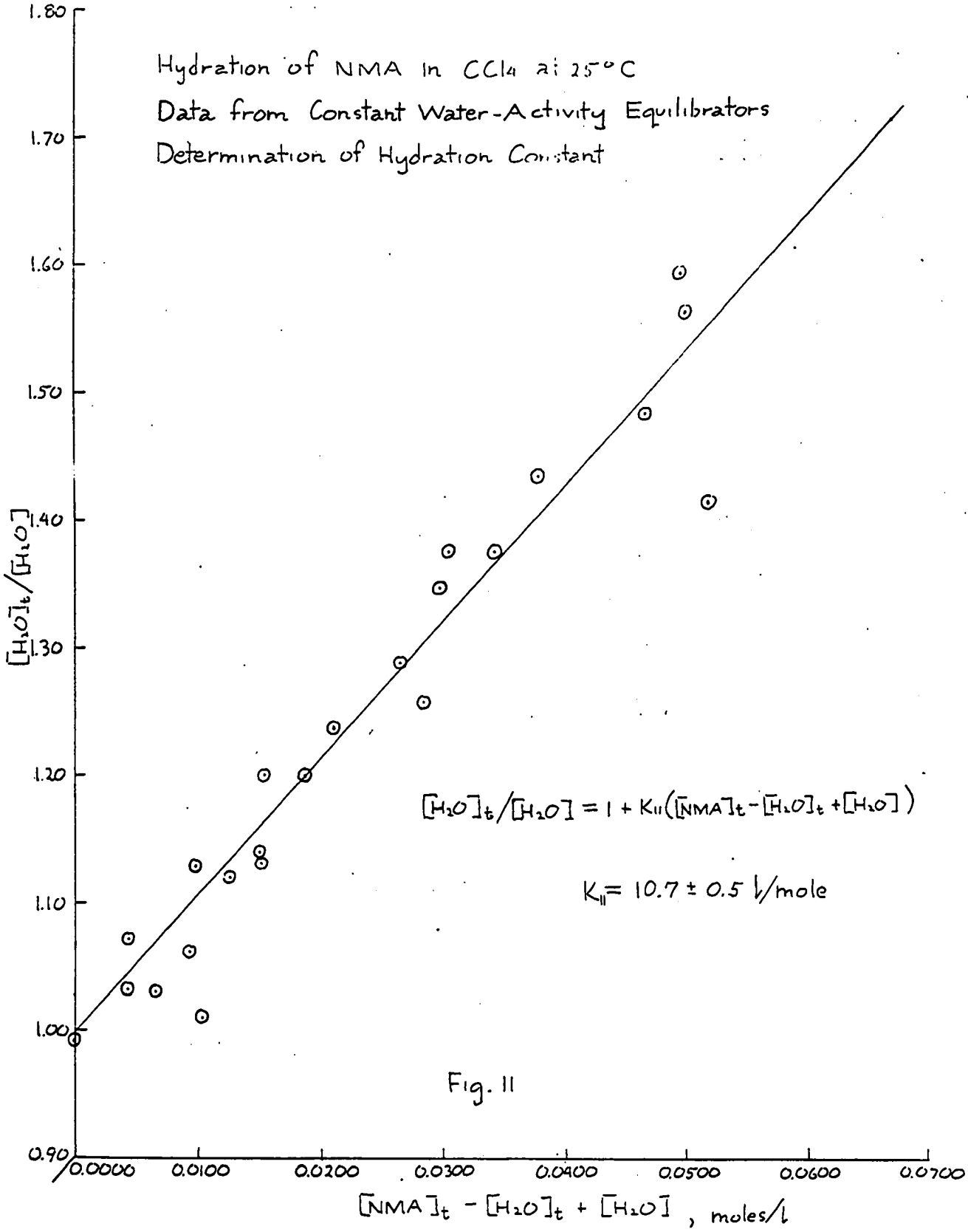

Hydration of NMA in CCl4 at 25°C
Data from Constant Water-Activity Equilibrators
Determination of Hydration Constant
$[H_2O]_t/[H_2O]$
$[H_2O]_t/[H_2O] = 1 + K_{II}([NMA]_t - [H_2O]_t + [H_2O])$
$K_{II} = 10.7 \pm 0.5$ l/mole
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.0000
0.0100
0.0200
0.0300
0.0400
0.0500
0.0600
0.0700
$[NMA]_t - [H_2O]_t + [H_2O]$, moles/l

Fig. 11

The Henry's law constant (0.0098) was evaluated from the data plotted in Fig. 9.

Data from total-pressure apparatus. The experimental points from the total-pressure apparatus consist of total water concentrations vs. water activities at constant amide concentrations. See Table 15. To obtain the value of the hydration constant, a plot of the data was made for eq. (4.6) as shown in Fig. 13. From the least-squares computer program K_{11} was found to be 12.9 ± 0.6 . The water monomer concentrations used in the calculations were obtained from

$$[W] = 0.0088\ P/P^{\circ}$$

in which the Henry's law constant was evaluated from Fig. 10.

The original experimental data can be reproduced by plotting a modified form of eq. (4.7), which is obtained as follows:

$$[W]_t = \left(1 + \frac{K_{11}[A]_t}{1+K_{11}[W]}\right)[W]$$

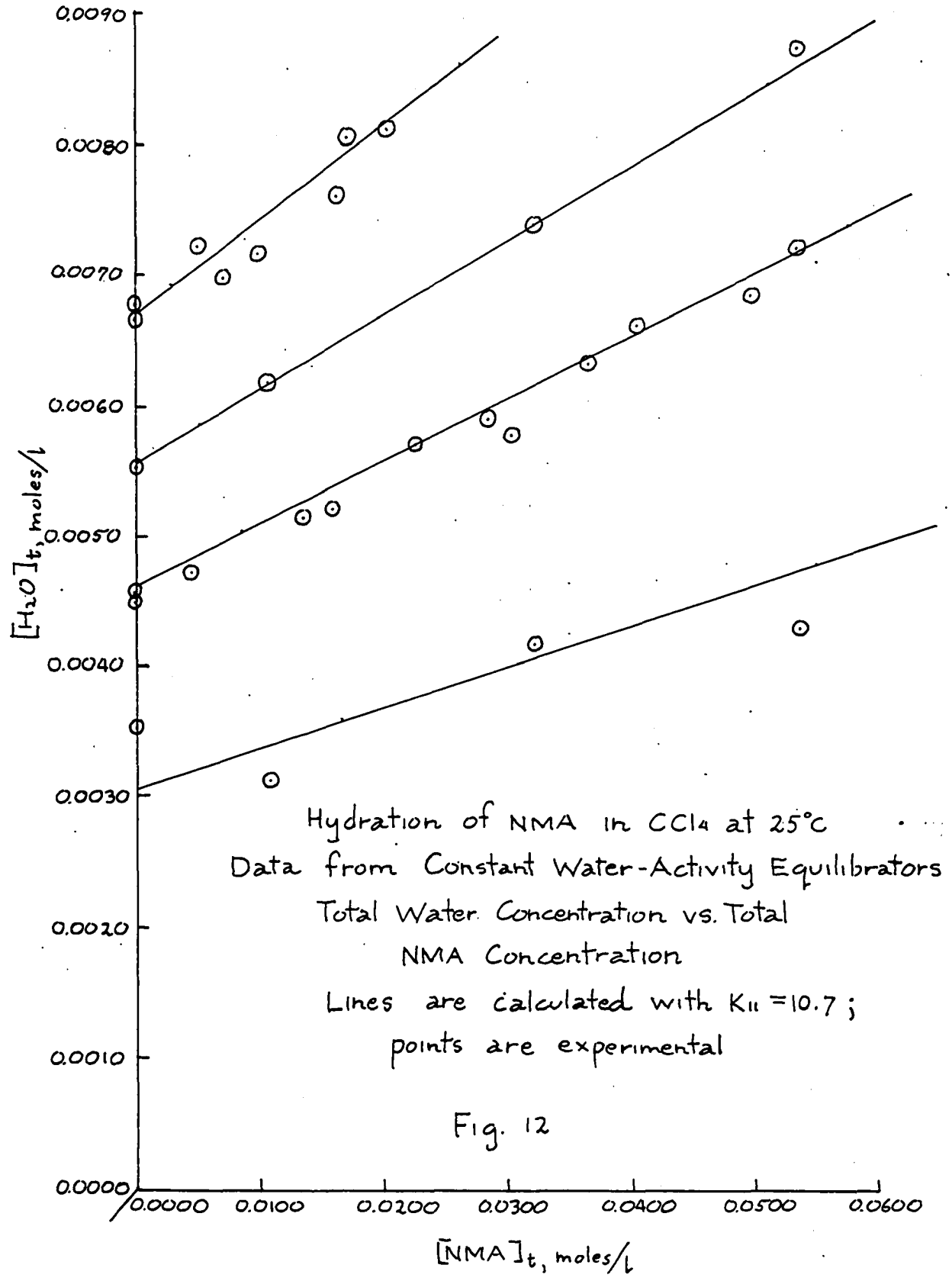
If $K_{11}[W] \ll 1$,

$$[W]_t \simeq (1 + K_{11}[A]_t)\,[W]$$

Replacing [W] by $0.0088\ P/P^{\circ}$,

$$[W]_t \simeq 0.0088\ (1 + K_{11}[A]_t)\ P/P^{\circ}$$

Thus, a plot of $[W]_t$ vs. P/P° for constant $[A]_t$ should produce a straight line. The graphs plotted with $K_{11} = 12.9$ are shown along with the original data points in Figs. 14 through 18.



Hydration of NMA in CCl_4 at 25°C
Data from Constant Water-Activity Equilibrators
Total Water Concentration vs. Total
NMA Concentration
Lines are calculated with $K_{11} = 10.7$;
points are experimental

Fig. 12

TABLE 15

HYDRATION OF NMA IN CCl_4 AT 25°C
DATA FROM TOTAL-PRESSURE APPARATUS
CONCENTRATION OF WATER VS. WATER ACTIVITY AT CONSTANT NMA CONCENTRATION

Water monomer concentrations were taken from graph of concentration of water in CCl_4 vs. water-activity (total pressure apparatus)

$$[W] = 0.0088\ P/p^\circ \text{ mole/l}$$

P/p°	$[W]_t$ mole/l	[W] mole/l	$[W]_t/[W]$	$[NMA]_t-[W]_t+[W]$ mole/l
		$[NMA]_t = 0.00943$ mole/l		
0.227	0.00186	0.00200	0.93	0.00957
0.324	0.00281	0.00280	1.00	0.00942
0.466	0.00421	0.00405	1.04	0.00927
0.559	0.00513	0.00493	1.04	0.00923
0.685	0.00655	0.00610	1.07	0.00898
0.760	0.00750	0.00680	1.10	0.00873
0.806	0.00894	0.00725	1.23ph.sep.	0.00774
		$[NMA]_t = 0.00929$ mole/l		
0.156	0.00138	0.00135	1.02	0.00926
0.391	0.00371	0.00342	1.09	0.00900
0.626	0.00603	0.00555	1.09	0.00881
0.781	0.00789	0.00700	1.13	0.00840
		$[NMA]_t = 0.00957$ mole/l		
0.277	0.00237	0.00240	0.99	0.00960
0.52	0.00474	0.00457	1.04	0.00940

TABLE 15 -- continued

P/p°	$[W]_t$ mole/l	[W] mole/l	$[W]_t/[W]$	$[NMA]_t-[W]_t+[W]$ mole/l
0.752	0.00712	0.00673	1.09	0.00918
0.807	0.00957	0.00725	1.32ph.sep.	0.00725
		$[NMA]_t$ = 0.00989 mole/l		
0.126	0.00122	0.00110	1.11	0.00977
0.261	0.00244	0.00227	1.09	0.00972
0.374	0.00367	0.00325	1.13	0.00947
0.487	0.00489	0.00425	1.15	0.00925
0.592	0.00612	0.00522	1.17	0.00899
0.714	0.00734	0.00635	1.16	0.00890
0.790	0.00859	0.00710	1.21	0.00840
0.790	0.00987	0.00710	1.39ph.sep.	0.00712
0.824	0.01240	0.00740	1.68ph.sep.	0.00489
		$[NMA]_t$ = 0.01875 mole/l		
0.148	0.00161	0.00130	1.24	0.01844
0.233	0.00260	0.00205	1.27	0.01820
0.355	0.00393	0.00310	1.27	0.01792
0.450	0.00502	0.00390	1.29	0.01763
0.572	0.00650	0.00505	1.29	0.01730
0.648	0.00764	0.00575	1.33	0.01686
0.684	0.00856	0.00610	1.40ph.sep.	0.01629
0.693	0.01036	0.00615	1.68ph.sep.	0.01454

TABLE 15 -- continued

P/p°	$[W]_t$ mole/l	[W] mole/l	$[W]_t/[W]$	$[NMA]_t-[W]_t+[W]$ mole/l
		$[NMA]_t$ = 0.01965 mole/l		
0.0859	0.000915	0.00075	1.22	0.01949
0.176	0.00185	0.00155	1.19	0.01935
0.313	0.00374	0.00270	1.39	0.01861
0.406	0.00469	0.00355	1.32	0.01851
0.531	0.00620	0.00470	1.32	0.01815
0.612	0.00722	0.00540	1.34	0.01783
0.676	0.00861	0.00600	1.44ph.sep.	0.01704
		$[NMA]_t$ = 0.0282 mole/l		
0.139	0.00149	0.00120	1.24	0.02791
0.230	0.00252	0.00200	1.26	0.02768
0.352	0.00399	0.00310	1.29	0.02731
0.430	0.00484	0.00375	1.29	0.02711
0.554	0.00628	0.00490	1.28	0.02682
0.615	0.00724	0.00545	1.33	0.02641
0.643	0.00882	0.00570	1.55ph.sep.	0.02508
		$[NMA]_t$ = 0.0311 mole/l		
0.0729	0.00085	0.00065	1.31	0.03090
0.161	0.00181	0.00140	1.29	0.03069
0.303	0.00342	0.00265	1.29	0.03033
0.385	0.00458	0.00335	1.37	0.02987
0.503	0.00615	0.00440	1.40	0.02935

TABLE 15 -- continued

P/p°	$[W]_t$ mole/l	[W] mole/l	$[W]_t/[W]$	$[NMA]_t-[W]_t+[W]$ mole/l
0.572	0.00716	0.00505	1.42	0.02899
0.609	0.00861	0.00540	1.59ph.sep.	0.02789
		$[NMA]_t$ = 0.0442 mole/l		
0.111	0.00143	0.00095	1.51	0.04372
0.186	0.00248	0.00160	1.55	0.04332
0.284	0.00384	0.00245	1.57	0.04281
0.373	0.00495	0.00325	1.52	0.04250
0.467	0.00629	0.00410	1.54	0.04201
0.539	0.00731	0.00475	1.54	0.04164
0.564	0.00883	0.00500	1.79ph.sep.	0.04037
		$[NMA]_t$ = 0.0612 mole/l		
0.117	0.00159	0.00100	1.59	0.06061
0.173	0.00244	0.00150	1.63	0.06026
0.268	0.00393	0.00235	1.67	0.05962
0.330	0.00498	0.00285	1.75	0.05897
0.417	0.00642	0.00365	1.76	0.05843
0.482	0.00729	0.00420	1.74	0.05811
0.527	0.00896	0.00465	1.93ph.sep.	0.05689

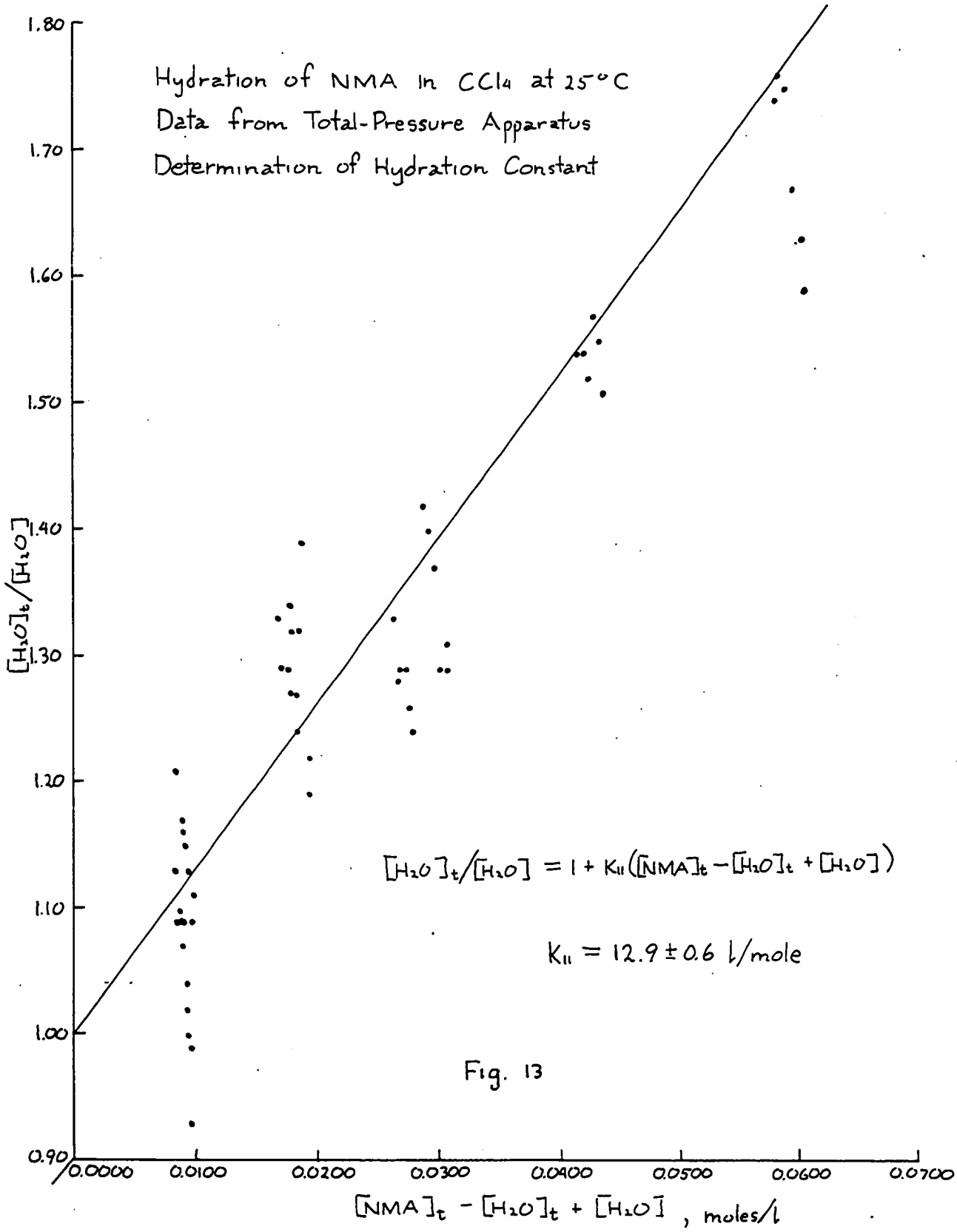

Hydration of NMA in CCl4 at 25°C
Data from Total-Pressure Apparatus
Determination of Hydration Constant
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
[H2O]t/[H2O]
0.0000
0.0100
0.0200
0.0300
0.0400
0.0500
0.0600
0.0700
[NMA]t - [H2O]t + [H2O] , moles/l
[H2O]t/[H2O] = 1 + K11([NMA]t - [H2O]t + [H2O])
K11 = 12.9 ± 0.6 l/mole

Fig. 13

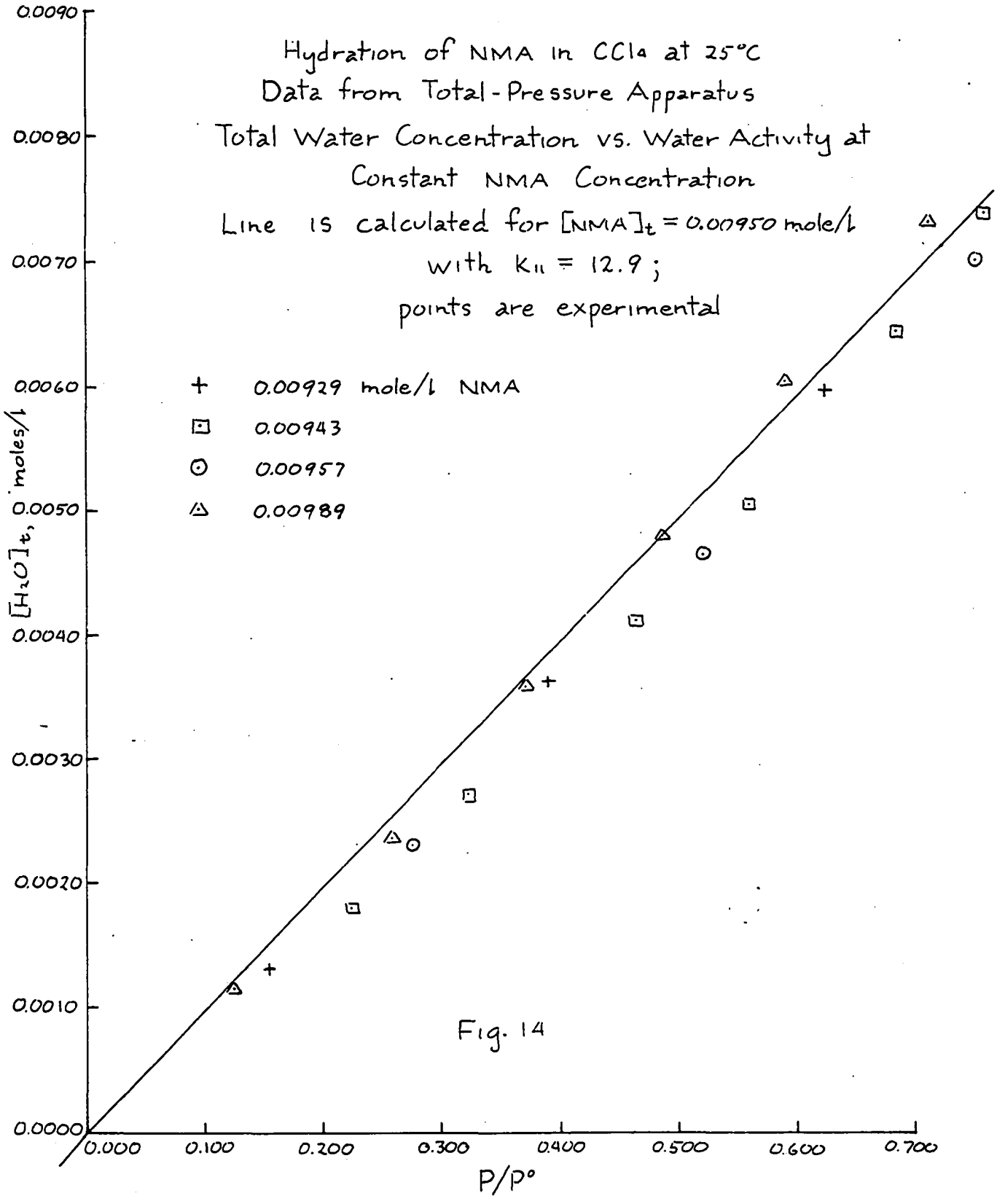

Hydration of NMA in CCl4 at 25°C
Data from Total-Pressure Apparatus
Total Water Concentration vs. Water Activity at
Constant NMA Concentration
Line is calculated for [NMA]t = 0.00950 mole/l
with K11 = 12.9;
points are experimental
+ 0.00929 mole/l NMA
⊡ 0.00943
⊙ 0.00957
△ 0.00989
[H2O]t, moles/l
0.0090
0.0080
0.0070
0.0060
0.0050
0.0040
0.0030
0.0020
0.0010
0.0000
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
P/P°

Fig. 14

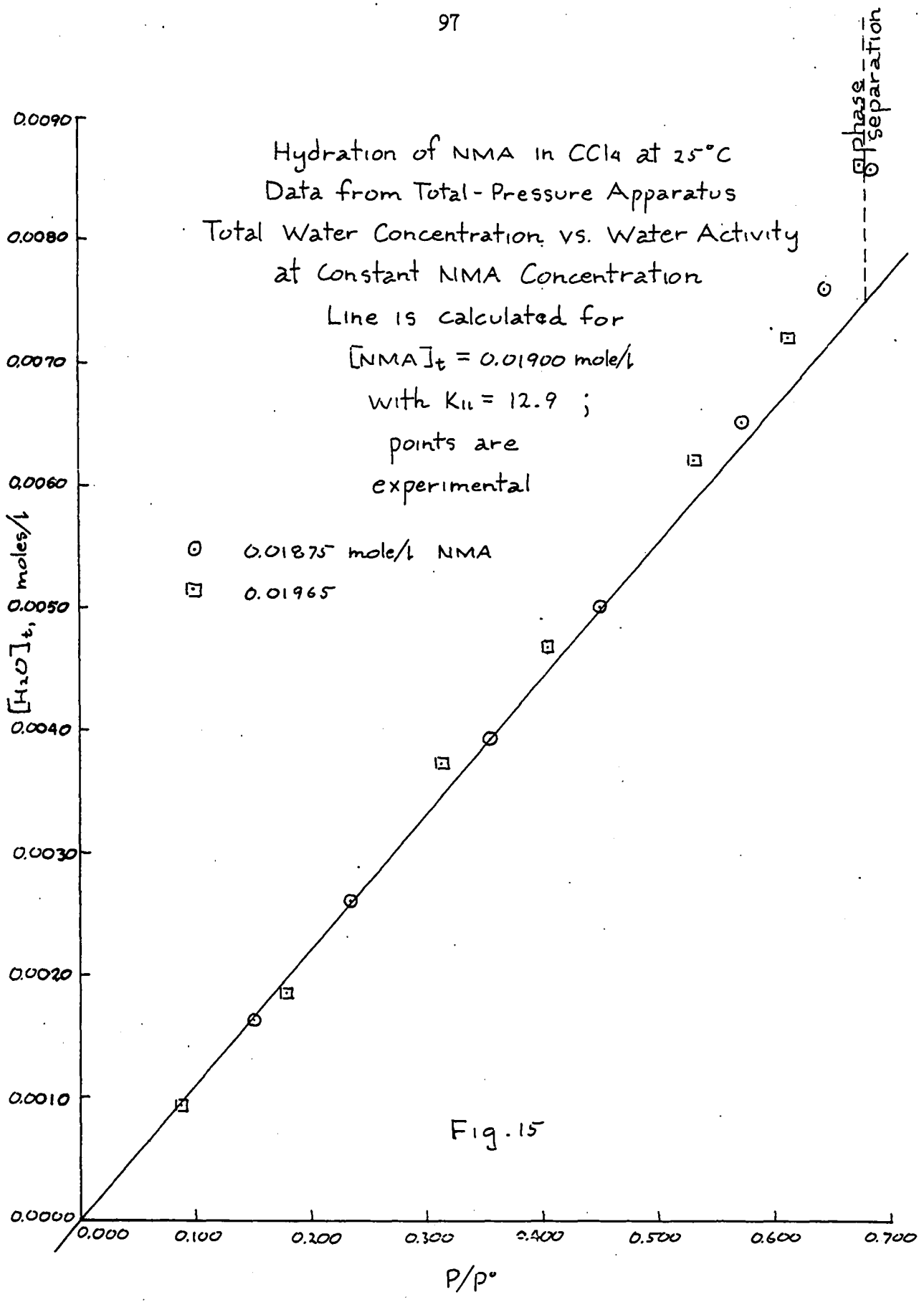

Hydration of NMA in CCl4 at 25°C
Data from Total-Pressure Apparatus
Total Water Concentration vs. Water Activity
at Constant NMA Concentration
Line is calculated for
[NMA]t = 0.01900 mole/l
with K11 = 12.9 ;
points are
experimental
0.01875 mole/l NMA
0.01965
phase separation
[H2O]t, moles/l
0.0090
0.0080
0.0070
0.0060
0.0050
0.0040
0.0030
0.0020
0.0010
0.0000
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
P/P°

Fig. 15

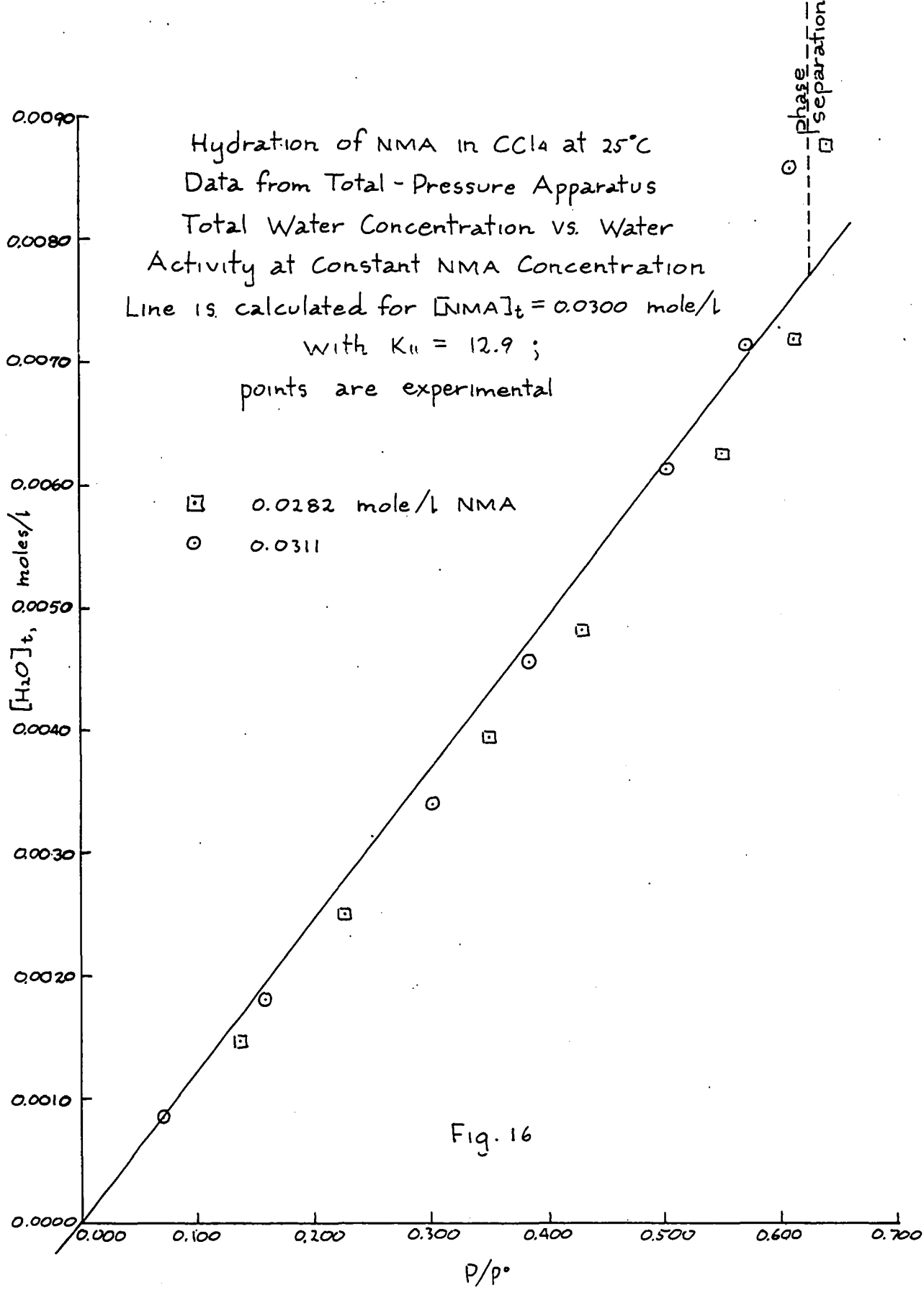

Hydration of NMA in CCl4 at 25°C
Data from Total-Pressure Apparatus
Total Water Concentration vs. Water Activity at Constant NMA Concentration
Line is calculated for [NMA]t = 0.0300 mole/l with K11 = 12.9 ;
points are experimental
0.0282 mole/l NMA
0.0311
phase separation
[H2O]t, moles/l
0.0090
0.0080
0.0070
0.0060
0.0050
0.0040
0.0030
0.0020
0.0010
0.0000
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
P/P°

Fig. 16

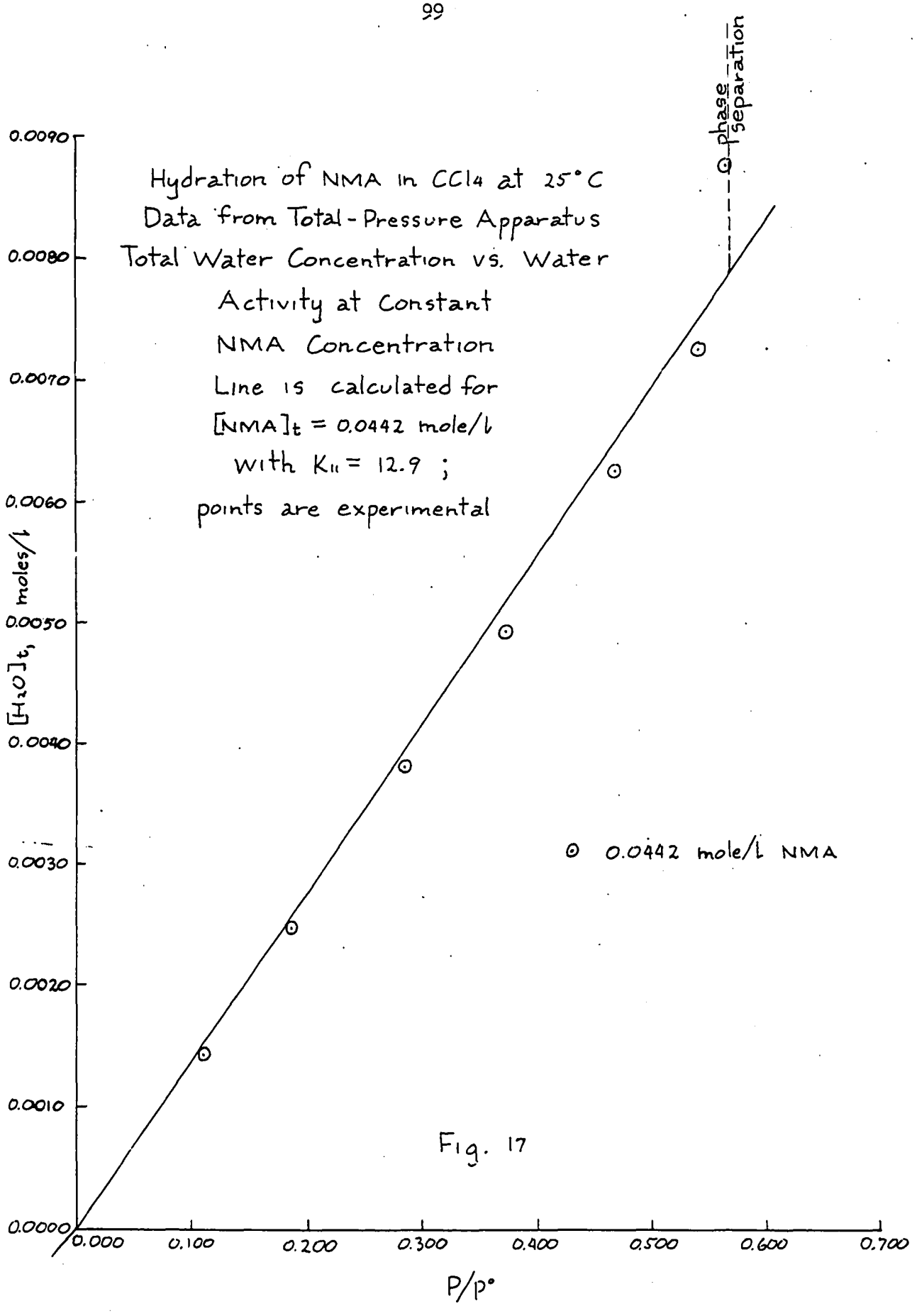

Hydration of NMA in CCl4 at 25°C
Data from Total-Pressure Apparatus
Total Water Concentration vs. Water Activity at Constant NMA Concentration
Line is calculated for
[NMA]t = 0.0442 mole/l
with K11 = 12.9 ;
points are experimental
phase separation
0.0442 mole/l NMA
[H2O]t, moles/l
P/p°
0.0000
0.0010
0.0020
0.0030
0.0040
0.0050
0.0060
0.0070
0.0080
0.0090
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700

Fig. 17

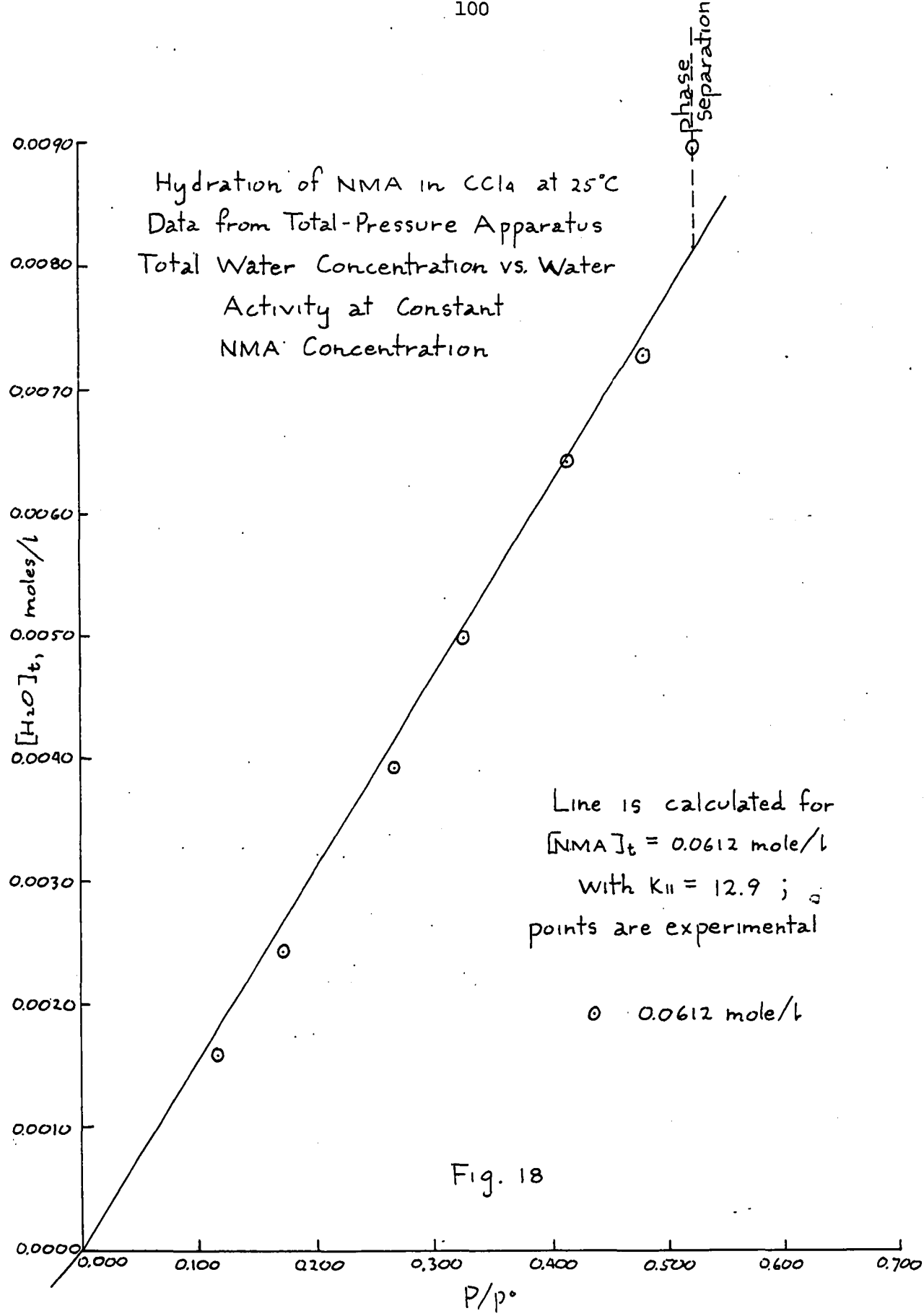

Hydration of NMA in CCl4 at 25°C
Data from Total-Pressure Apparatus
Total Water Concentration vs. Water Activity at Constant NMA Concentration
Phase separation
[H2O]t, moles/l
0.0090
0.0080
0.0070
0.0060
0.0050
0.0040
0.0030
0.0020
0.0010
0.0000
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
P/p°
Line is calculated for
[NMA]t = 0.0612 mole/l
with K11 = 12.9 ;
points are experimental
⊙ 0.0612 mole/l

Fig. 18

CHAPTER V

CONCLUSIONS

Both the data from the liquid-vapor equilibrium still and the molecular weight-measurement apparatus support the theory of chain formation in the self-association of NMA. However, the values obtained for the self-association constants at 20°C are somewhat in doubt because of the relatively large uncertainties in the corrections applied to the pressure differentials. Also, the enthalpies of association for K_2 and K_n of approximately -6 Kcal/mole appear to be rather high. As seen from Tables 3 and 5, the enthalpy change for the formation of one hydrogen bond between molecules is closer to -4 Kcal/mole in solvents of low dielectric constant such as CCl_4 or benzene.

The self-association of NMA at 20 or 25°C should probably be reinvestigated. If a vapor pressure-lowering technique is used, the partial pressure of NMA above the solution must be accurately known. The partial pressure could probably be obtained by collecting and analyzing the vapor in equilibrium with the liquid phase.

The molecular weight-measurement apparatus could be improved by enlarging the solution chambers and by utilizing a better device for measuring the pressure differentials. The sulfuric acid manometer could probably be replaced by a sensitive Bourdon gage or perhaps a capacitance manometer. Electronic Space Products, Inc., Los

Angeles, has available a glass Bourdon gage claimed to be capable of detecting a pressure difference of less than 0.01 mm Hg.

The self-association of NMA should be studied in solvents of varying dielectric constant to allow inference of the hydrogen-bond strengths between polar groups of proteins in aqueous media. As noted in Chapter II, some investigations of this nature have been carried out by Klotz and co-workers.

The hydration data from both the constant water-activity equilibrators and the total-pressure apparatus support the theory that each NMA polymer has n hydration sites capable of binding up to n water molecules. In the derivation of the equations it was assumed that n is the number of NMA monomers in the chain. The hydration equation predicts a slight decrease in the slope of the graph of total water concentration vs. water activity at constant NMA concentration (data from the total-pressure apparatus). Actually the data points appear to give a slight increase in slope although the change is not significant if the error in the points is taken into consideration.

The value of the hydration constant, K_{11}, obtained from the data of the total-pressure apparatus (12.9 $\pm$ 0.6 l/mole) is in good agreement with that obtained from the constant water-activity equilibrators (10.7 $\pm$ 0.5 l/mole). This is true even though the water concentrations determined for the equilibrators appear to be 10 to 12% too high.

A hydration constant of about 12 is in close agreement with the value obtained by Mueller[63] (also about 12) for the formation of a monomer monohydrate of N,N-dimethylacetamide in CCl_4 at 25°C. In addition to the monomer monohydrate, Mueller also noted the presence

of a dimer monohydrate and a monomer dihydrate in solution. The detection of the latter two species is not in disagreement with the results of the present investigation because N,N-dimethylacetamide undergoes a completely different type of self-association. The only self-associated species formed in dilute solution are dimers, which are held together by dipole-dipole interaction instead of hydrogen bonding.

Because the self-association constants for NMA do not appear in the hydration equation, it appears that the equation should apply to hydrated polymers in which the monomers are linked together by covalent bonds instead of by hydrogen bonds. Thus, it would be interesting to test the theory with data obtained for the hydration of small polyamides. If these molecules were found to hydrate in the same manner as N-methylacetamide, then with data from solutions of increasing dielectric constant it should be possible to formulate some kind of a model for a hydrated protein in aqueous media.

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