PART I: SPECTROSCOPIC STUDIES OF IONIC SOLVATION
PART II: GAS CHROMATOGRAPHIC STUDIES OF CONVULSANT TETRAZOLES

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This is to certify that the

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PART I: SPECTROSCOPIC STUDIES OF IONIC SOLVATION

PART II: GAS CHROMATOGRAPHIC STUDIES
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ABSTRACT

PART I: SPECTROSCOPIC STUDIES OF IONIC SOLVATION

PART II: GAS CHROMATOGRAPHIC STUDIES OF CONVULSANT TETRAZOLES

By

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The solvation of the lithium ion by acetone was studied in acetone-nitromethane solutions by far-infrared, Raman, and ^{7}Li and ^{35}Cl nuclear magnetic resonance spectroscopic techniques. It was confirmed that the 390-cm^{-1} far-infrared acetone band is split by the lithium ion and that a 369-cm^{-1} far-infrared band, which was attributed by other investigators to a Li^{+}-nitromethane vibration, is due to the vibration of acetone molecules in the inner solvation shell of the lithium ion. It was determined that the frequency of the lithium ion vibration, in nitromethane solutions, is strongly dependent on the nature of the counter ion.

Studies of the Li^{+}-acetone-nitromethane system by several different experimental techniques indicate that the primary solvation shell of the Li^{+} ion consists of four acetone molecules. From Raman spectral data of the above system at varying compositions, approximate values of the equilibrium constants for the stepwise solvation reaction were calculated. The values obtained were K_1 = 19.1, K_2 = 2.5, K_3 = 1.3, and K_4 = 0.6. Lithium-7 nuclear magnetic resonance measurements showed that acetone solvates

the lithium ion much more strongly than does nitromethane. Results from chlorine-35 nuclear magnetic resonance studies indicated that in solutions containing less than four acetone molecules per Li^+ ion, the vacant position in the inner solvation shell is occupied by ClO_4^- ion in preference to nitromethane.

The influence of a weak complexing agent, pentamethylenetetrazole, on the $Li^+ClO_4^-$ ion pair formation was investigated. It was determined that the degree of interaction with the lithium ion was acetone > pentamethylenetetrazole > ClO_4^-. Therefore, pentamethylenetetrazole displaces ClO_4^-, but not acetone, from the inner solvation shell of the lithium ion.

Gas chromatography was used as a technique for the analysis of the cyclopolymethylenetetrazoles. The relative retention times were very nearly the same for trimethylenetetrazole, tetramethylenetetrazole, and pentamethylenetetrazole, but varied considerably as the number of methylene groups is increased. Working curves were obtained for the cyclopolymethylenetetrazoles and were found to be linear over the concentration range from millimolar to 0.1 M. At higher concentrations the curves deviated from linearity. These compounds were analyzed routinely at the 50-100 ppm level, and the technique is capable of determining concentrations of less than 10 ppm.

The gas chromatographic technique was also employed to study the distribution of these tetrazoles between

aqueous solutions and carbon tetrachloride. For pentamethylenetetrazole, hexamethylenetetrazole, heptamethylenetetrazole, and 8-tert-butylpentamethylenetetrazole, the distribution ratio was found to be concentration dependent. Cryoscopic measurements were carried out on aqueous solutions of the cyclopolymethylenetetrazoles. The results indicated that in the case of pentamethylenetetrazole some association occurs in the aqueous phase. For hexamethylenetetrazole and heptamethylenetetrazole it was assumed that trimers formed in the organic phase, and on the basis of this assumption, an equation was derived from which the trimerization equilibrium constants and the partition coefficients were calculated.

PART I: SPECTROSCOPIC STUDIES OF IONIC SOLVATION

PART II: GAS CHROMATOGRAPHIC STUDIES OF CONVULSANT TETRAZOLES

By

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Contact Ion Pairs. Pairs of ions, linked electrostatically, but with no covalent bonding between them.

Solvent Shared Ion Pairs. Pairs of ions, linked electrostatically by a single, oriented solvent molecule.

Solvent Separated Ion Pairs. Pairs of ions, linked electrostatically but separated by more than one solvent molecule.

Me_2CO: Acetone

PMT: Pentamethylenetetrazole

C_6CMT: Hexamethylenetetrazole

C_7CMT: Heptamethylenetetrazole

8-tert-butyl PMT: 8-tert-butylpentamethylenetetrazole

8-sec-butyl PMT: 8-sec-butylpentamethylenetetrazole

T: One Tesla = 10 kilogauss

ΔT_f: Freezing point depression in °C.

PART I

SPECTROSCOPIC STUDIES OF IONIC SOLVATION

CHAPTER I

HISTORICAL

INTRODUCTION

Most chemical reactions whether in research laboratories, industry, or biological systems occur in solutions. The association of solvent molecules with metal ions in solutions has been an important field of research for many years, and a wide variety of experimental techniques have been used to study ion-ion, ion-solvent, and solvent-solvent interactions. However, we still have only a rudimentary concept of the structure of electrolyte solutions.

Classical techniques such as electrochemical measurements and the study of colligative properties of solutions have been widely used in studies of electrolyte solutions. These methods, however, measure bulk solution properties and give little information about the chemical nature of the species present in solution. For example, it is very difficult to distinguish between contact and solvent separated ion pairs by the above techniques. Similarly, attempts to determine ionic solvation numbers often give contradictory results. In many cases no clear-cut distinction can be made between the inner and outer solvation spheres and, consequently, for a given ion, vastly different solvation numbers can be obtained with different experimental techniques (1).

Within the last decade spectroscopic techniques such as mid- and far-infrared, Raman, and nuclear magnetic

resonance have been very useful probes for the elucidation of the structure of electrolyte solutions and of the chemical species present in them.

FAR-INFRARED SPECTROSCOPY

In 1965 Evans and Lo (2) studied the far-infrared spectra of tetrabutyl- and tetrapentylammonium halides in benzene solution. They observed a band in the 100-cm^{-1} spectral region which could not be assigned to a vibrational mode of either the solvent or the solute. Since the band position was dependent on the mass of both the cation and anion, the authors assumed that it was due to a cation-anion ion pair vibration. This constituted the first report of an ionic vibration in solution.

Shortly thereafter, Edgell and co-workers (3,4) observed far-infrared bands due to the motion of the alkali cation in tetrahydrofuran solutions of lithium, sodium, and potassium tetracarbonylcobaltate and pentacarbonylmanganate. Upon extending these studies to other solvents (dimethylsulfoxide, pyridine, and piperidine), the authors observed the band position to be a function of the cation and the solvent.

Popov and co-workers (5-14) extended these far-infrared studies to include several nonaqueous solvents. They found that in highly solvating solvents such as dimethylsulfoxide (5,6), the frequencies of the bands are strongly dependent on the nature of the cation but are completely

independent of the anion. They concluded that these bands were due to the alkali metal ion vibrating in a solvent cage. Thus these bands were named "solvation bands". However, in solvents with very low solvating abilities such as tetrahydrofuran (3,4), some anion dependence is observed. It was postulated that this dependence is due to a change in the nature of the solvent cage around the cation. In these cases a counter-ion replaces a solvent molecule in the inner solvation shell and forms a solvated contact ion pair. Thus the cation is vibrating in a cage composed of solvent molecules and a counter-ion.

While studying the far-infrared spectra of sodium tetrabutylaluminate in cyclohexane solutions, Tsatsas and Risen (15) noted two solvation bands at 195 and 160 cm^{-1}. In tetrahydrofuran solutions, however, only one band, at 195 cm^{-1}, was observed. In addition, a Raman band at 202 cm^{-1} was seen for the cyclohexane solutions. However, Edgell et al. (3,4) had previously shown that the far-infrared solvation bands are Raman inactive, which is indicative of the electrostatic nature of the ion-solvent or ion-ion interaction. Thus in cyclohexane solutions of sodium tetrabutylaluminate, the ion-solvent interaction responsible for the 202-cm^{-1} vibration possesses a significant degree of covalency.

Wong et al. (9) investigated solutions of lithium perchlorate in acetone and acetone-nitromethane binary

solvent mixtures. They observed changes in the far-infrared region of the spectrum where the 390-cm^{-1} acetone band (C-C-C deformation) was split upon the addition of the salt, and a new band appeared at 369 cm^{-1}. This new band was assigned to be due to a vibration of acetone complexed to the lithium ion. Regis and Corset (16), however, recently disagreed with this conclusion and stated that the band at 369 cm^{-1} was due to the lithium ion vibrating in a nitromethane solvent cage.

Erlich et al. (14) studied the variation in the frequency of the sodium solvation band in dimethylsulfoxide-pyridine mixtures. As the solvent composition was changed, the frequency of the solvation band progressed gradually from the frequency characteristic of one solvent to that characteristic of the other. They observed a strong preferential solvation of the sodium ion by dimethylsulfoxide.

Recently, Barker and Yarwood (17) extended the work of Evans and Lo (2). In studying the far-infrared spectra for benzene solutions of tetrabutylammonium chloride, they noticed an asymmetry to the low-frequency side of the 115-cm^{-1} band which suggested the presence of unresolved bands. They showed that a second band was present at $\sim$75 cm^{-1}, and they attributed this band to a perturbed "collisional" or lattice band of the benzene molecule. This implies that the benzene solvent molecules are solvating the aggregate that gives rise to the band at 115 cm^{-1}.

Far-infrared spectroscopy has also been used in studying complexation reactions of alkali metal ions. Risen and co-workers (18) observed far-infrared bands for lithium, sodium, and cesium ions in ethylene-methacrylate copolymers. They also observed far-infrared bands for alkali metal ion complexes of cyclic polyether compounds in dimethylsulfoxide and pyridine solutions (19). More recently, Cahen and Popov (20) observed the far-infrared spectra of sodium and lithium cryptates in several nonaqueous solvents. The spectra were characterized by a broad band whose frequency was independent of the solvent and the anion. The band was assigned to the vibration of the cation in the cryptand cavity.

The frequencies of the alkali metal ion solvation bands in several solvents are presented in Table 1.

MID-INFRARED AND RAMAN SPECTROSCOPY

Day and co-workers (21,22) observed that some infrared bands of tetrahydrofuran are split by the sodium ion, giving rise to new bands characteristic of tetrahydrofuran bound to the sodium ion. From the band intensity measurements, they determined that the sodium ion is solvated by four tetrahydrofuran molecules and calculated stability constants for the stepwise complexation of the sodium ion by tetrahydrofuran. A similar technique was used by Taylor and Kuntz (23) in their study of anion solvation

Table 1. Solvation Band Frequencies of Alkali Metal Ions in Nonaqueous Media.

Solvents	Solvation Band Frequencies (cm^{-1})						Reference
	Li^+	Na^+	NH_4^+	K^+	Rb^+	Cs^+	
Tetrahydrofuran	407	190		150			13,15
Dimethylsulfoxide-d_6	425	200					4,6
Piperidine		183					4
Pyridine		180					4
Dimethylsulfoxide	429	200	214	153	125	110	5,6,8
Dipropysulfoxide	420	220	222	156	123		6
Dibutylsulfoxide	425	224	226	152			6
2-Pyrrolidone	400	207	216	145			7
1-Methyl-2-pyrrolidone	398	204	207	140	106		7,8
1-Vinyl-2-pyrrolidone	419						7
Acetone	425	195		148			9
Acetic Acid	390						10
Pyridine	420	180	200				11
4-Methylpyridine	390	180	200				12
3-Methylpyridine	384						12
2,4-Dimethylpyridine	362						12
3,4-Dimethylpyridine	383						12
2-Chloropyridine	355						12
Propylene Carbonate	400	186	184	144	115	112	13

by phenol.

The solvation of the lithium ion by dimethylformamide (DMF) was investigated by Lassigne and Baine (24) by infrared and nuclear magnetic resonance (NMR) spectroscopy. They monitored the infrared carbonyl band of DMF at 1686 cm^{-1}. As lithium perchlorate was added to the solution, a new band appeared at 1670 cm^{-1}, which was indicative of DMF bound to the lithium ion. The solvation number of lithium by dimethylformamide was found by NMR to be four.

Wong et al. (9) studied the vibrations of the perchlorate ion and of acetone in solutions of lithium perchlorate in acetone-nitromethane mixtures. It was noted that the 935-cm^{-1} Raman band (ν_1 symmetric stretch) of the perchlorate ion remained narrow and at constant frequency in solutions with acetone/lithium mole ratios of ≥ 4, but that it broadened and shifted to higher frequency as the acetone/$LiClO_4$ mole ratio became less than four. It was concluded from these data that the inner solvation shell of Li^+ contained four acetone molecules. Similar results were reported by Handy and Popov (12) in their study of the solvation of the lithium ion by 4-methyl-pyridine.

In a series of studies, Edgell's group (25-27) used the carbonyl stretching frequency of the tetracarbonylcobaltate anion as a probe of the environment of this anion. The 1900-cm^{-1} C-O stretch was monitored for solutions of $NaCo(CO)_4$ in several solvents as a function of

temperature and of salt concentration. In dimethylsulfoxide, dimethylformamide, nitromethane, hexamethylphosphoramide, acetonitrile, and pyridine the band was quite symmetrical which indicates that only solvent molecules are near-neighbors of the $Co(CO)_4^-$ ion in these solutions. However, in piperidine, tetrahydrofuran, and dimethoxyethane solutions, additional bands were observed at the high- and low-frequency side of the main band. The behavior of the two new bands indicates an increasing asymmetrical environment about the anion resulting from contact ion pairing. They observed the spectra of tetrahydrofuran solutions of $NaCo(CO)_4$ at various temperatures. This enabled them to resolve the complex spectra into four band components, which indicated the presence of solvent separated and contact ion pairs. Edgell et al. (28) recently investigated the infrared spectrum of thallium tetracarbonylcobaltate in seven solvents. Only a single ionic environment was found in dimethylformamide, dichloromethane, and dimethylsulfoxide solutions. Several kinds of environments were found in tetrahydrofuran, acetonitrile, and nitromethane solutions which resulted from solvent surrounded ions, contact ion pairs, and triple ions.

Borucka and Kecki (29-31) studied the infrared spectra of several electrolytes in acetone solutions. They noted the splitting of the ν_{C-C-C} and $\nu_{C=O}$ vibrational bands of acetone and correlated the frequencies

of the new components with the charge density of the cations. They also observed the influence of various metal perchlorates and of lithium and zinc halides on the integral intensities of the acetone vibrational bands. In addition, the band frequency changes were related to the electronic structure of acetone molecules complexed with the cations and anions.

In a recent series of papers, Perelygin and Klimchuk (32-36) examined infrared spectra of alkali and alkaline earth metal salts in nonaqueous solutions. For solutions of sodium, lithium, and magnesium perchlorates in acetonitrile (32), they observed perchlorate vibrational bands in the 1100-cm^{-1} spectral region which were due to free ions and contact ion pairs. They also noted changes in the acetonitrile vibration at 2254 cm^{-1} (C≡N stretch). This band is shifted by 10, 21, and 36 cm^{-1} respectively as a consequence of bonding of the acetonitrile molecule with the sodium, lithium, and magnesium cations. From measurements of the band intensities they calculated the association constants of the ions and the coordination numbers of the cations. For Na^+, Li^+, and Mg^{2+} ions the corresponding solvation numbers are four, four, and six. They also investigated the solvation numbers as a function of temperature and found that between -40 and 60°C they did not vary from the above values (33). For acetonitrile solutions of sodium and lithium iodides, they determined the ionic association constants and also found that the

solvent sheath of the iodide ion contained eight acetonitrile molecules (34). Similar studies were carried out on acetone solutions of sodium, lithium, and magnesium perchlorates (35,36). The solvation numbers of the cations were calculated to be four for Na^+ and Li^+ and six for Mg^{2+}, as for the solutions in acetonitrile.

Raman spectroscopy is being used increasingly to investigate ionic association and solvation in solutions. The vibrational modes of polyatomic anions such as nitrate, perchlorate, and sulfate are very sensitive to their environment and can be used as probes in the studies of ionic interactions.

Peleg (37) examined the magnesium nitrate-water system by Raman spectroscopy. The vibrations of the nitrate ion were observed over the range from very dilute solution to the anhydrous molten salt. The results indicated that the interactions in the system varied as the composition varied. In very dilute solutions both ions are completely hydrated, and the nitrate ion is perturbed by the water molecules. As the water content is lowered, the polarization power of the magnesium ion begins to affect the nitrate ion, but no contact ion pairing occurs until the water content is reduced below six moles of water per mole of salt. Upon further decrease of the water content both contact and solvent separated ion pairs exist in solution. From these results it was suggested that the solvation number of the magnesium

cation by water is six.

The magnesium nitrate-water system was also studied by Chang and Irish (38) by infrared and Raman spectroscopy. By very careful computer resolution of the spectral bands, they showed that as the water content decreases, solvent separated ion pairs give way to contact ion pairs in which the nitrate ion is bound to the Mg^{2+} ion in monodentate fashion. On further reduction of the water content, the nitrate becomes bound to the Mg^{2+} ion in a bidentate fashion.

Solutions of silver nitrate in acetonitrile and water were recently investigated by Chang and Irish by infrared and Raman spectroscopy. For acetonitrile solutions (39) they concluded that the nitrate ion exists in three different environments. At salt concentrations of 4 M or less, both free nitrate ions and ion pairs are present in solution. When the salt concentration is greater than 4 M, they noted the formation of multiple ion aggregates. The association constant for the $Ag^{+}NO_3^{-}$ ion pair was calculated, and from a plot of the average number of acetonitrile molecules bound to the silver ion, $\bar{n}$, versus the $AgNO_3$/acetonitrile mole ratio, they determined the solvation number of Ag^{+} by acetonitrile to be four. Aqueous silver nitrate solutions (40) were described in terms of an equilibrium between free ions and ion pairs. The ion pair association constant of 0.1 M^{-1} was obtained from monitoring both the 717-cm^{-1} and 1047-cm^{-1} nitrate vibrations.

The $AgNO_3$ - acetonitrile system was investigated further by Janz and Müller (41). They examined ion pairing by careful and precise Raman measurements and extended these studies to very dilute solutions, so as to overlap the concentration range over which the Fuoss-Onsager conductance theory applies. The value of the ion pair association constant calculated from the Raman data is 84±14; from the Fuoss-Onsager conductance theory, the value obtained is 70.3±1.2, which shows good agreement between the two experimental techniques.

The complexation of the cadmium ion by nitrite ion in aqueous solutions was studied by Irish and Thorpe (42). Upon addition of $Cd(ClO_4)_2$ to aqueous solutions of $NaNO_2$, a new Raman band appears at 861 cm^{-1} and grows in intensity as the Cd^{2+}/NO_2^- mole ratio increases. At the same time, the intensity of the nitrite band at 817 cm^{-1} decreases. The free nitrite concentration was monitored and the average ligand number was evaluated. Four successive formation constants were determined for the species $Cd(NO_2)^+$, $Cd(NO_2)_2$, $Cd(NO_2)_3^-$, and $Cd(NO_2)_4^{2-}$. It was also determined that the chelation occurs through the two oxygen atoms.

Plowman and Lagowski (43) examined Raman spectra of solutions of alkaline earth and alkali metal perchlorates and nitrates in liquid ammonia. They observed low-frequency bands of Li^+, Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} at 241, 194, 328, 266, 243, and 215 cm^{-1}, respectively.

These bands were assigned to the symmetric stretching mode of the solvated cation, which presumably originate from interaction of the first solvation shell.

The results presented in this thesis are, in general, applications of the aforementioned studies. A more extensive historical discussion of solvation studies by vibrational spectroscopy can be found in the doctoral dissertations of B. W. Maxey (44), J. L. Wuepper (45), M. K. Wong (46), and P. R. Handy (47).

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

During the past few years numerous publications have appeared which deal with solvation studies by proton nuclear magnetic resonance (NMR) spectroscopy. In electrolyte solutions the solvent molecules can exist in several environments. These environments may be divided into bulk solvent regions, where solvent molecules are effectively out of range of ionic influence, secondary solvation regions, and primary solvation regions. If the exchange of molecules between all of these environments were very slow, a number of resonance lines would be expected in the NMR spectrum of the solvent nuclei that correspond to the different environments. Generally, however, the exchange is quite rapid. Thus, the separate resonance signals for each environment are population averaged to a single line whose shift from the pure solvent resonance signal reflects the average effect of

the different environments.

Two proton nuclear magnetic resonance methods have been widely used for the determination of ionic solvation numbers. The first involves the cooling of the solution in order that the proton exchange be slowed to an extent that separate signals can be observed for the coordinated and bulk solvent molecules. This method has been extensively employed by Fratiello and co-workers (48-50) in the determination of hydration numbers of several metal ions. In the second method, the chemical shift of the solvent protons is monitored as the solvent/salt mole ratio is varied, and the results are plotted. Often a distinct break in the resulting curve is observed that indicates the solvation number. This technique was used by Schaschel and Day (51) to complement their results obtained by infrared spectroscopy (21,22). In studying the solvation of the sodium ion by tetrahydrofuran (THF), they monitored the chemical shift of the THF protons as a function of the THF/$NaAlBu_4$ mole ratio. From the results the authors concluded that Na^+ was solvated by four THF molecules.

Although proton nuclear magnetic resonance has been extensively used to investigate electrolyte solutions, the protons are usually several atoms removed from the actual site of interaction and, consequently, the chemical shifts are only weakly affected by the solvation. Thus, ambiguous results are often reported. It is obvious that better information can be obtained by observing the

resonance of the solvated species.

Alkali metal and halogen NMR have been used as very sensitive probes in the elucidation of the structure of alkali salt solutions in nonaqueous solvents. Several extensive historical reviews of non-proton NMR have recently been written. A comprehensive discussion of ^{23}Na and ^{7}Li NMR can be found in the doctoral dissertations of M. S. Greenberg (52) and Y. M. Cahen (53), respectively. The use of alkali metal NMR (especially ^{133}Cs) and of halogen NMR in solvation studies was recently reviewed by DeWitte (54). Therefore, the remainder of this discussion will be devoted to a few recent examples.

Greenberg and Popov (55) published results obtained from ^{23}Na NMR studies of preferential solvation in nonaqueous mixed solvents. Generally, these studies reflected the relative donicity of each solvent in a given solvent pair, where the solvent of higher donicity was preferentially contained in the inner solvation shell of the Na^{+} ion.

Cahen et al. (56) examined the solvation and ion pair formation of several lithium salts in nonaqueous solutions by using ^{7}Li and ^{35}Cl NMR. Formation of contact ion pairs in tetrahydrofuran, nitromethane, and tetramethylguanidine was particularly evident as considerable broadening of the ^{35}Cl resonance of the perchlorate ion was observed. Similar results were recently obtained by Berman and Stengle (57) in their studies of metal perchlorate

solutions in nonaqueous solvents.

Very recently the solvation of hexafluorophosphate salts was investigated by DeWitte and Popov (58) by ^{19}F and ^{23}Na NMR measurements. They also reported on the solvation and ionic association of several cesium salts in nonaqueous solutions by ^{133}Cs NMR (59).

The use of alkali metal NMR has yielded considerable information as to the nature of alkali metal ion complexes. Cahen et al. (60,61) determined the formation constants and studied the complexation reaction kinetics for various lithium-cryptand complexes, while Mei et al. (62) investigated complexes of Cs^+ with cyclic polyether compounds ("crowns") by ^{133}Cs NMR.

CONCLUSIONS

It thus appears that spectroscopic techniques can be very useful in the elucidation of the structure of electrolyte solutions. Now, with the advent of laser excitation Raman spectroscopy and Fourier transform infrared and nuclear magnetic resonance spectroscopy, very dilute solutions can be investigated. This makes possible more direct comparisons of results obtained by spectroscopic methods with those obtained by classical techniques.

CHAPTER II

EXPERIMENTAL

REAGENTS

Lithium perchlorate (Fisher) was dried at 190°C for several days. The water content was found to be 0.2% by weight. Lithium iodide (K & K Laboratories) was prepared as previously described (56). Solutions of lithium triiodide were prepared by the addition of equimolar amounts of iodine to lithium iodide solutions. Reagent grade iodine (Baker) was used without further purification. Pentamethylenetetrazole (Aldrich) was recrystallized from diethyl ether and dried *in vacuo*.

SOLVENTS

Reagent grade acetone (Baker) was refluxed over Drierite and then fractionally distilled. The acetone was further dried over freshly activated 5A Linde molecular sieves and stored in a dry box under nitrogen atmosphere. Acetone-d_6 (Aldrich, 99+%) was dried over molecular sieves and stored in a dry box. Spectroscopic grade nitromethane was fractionally distilled and dried over molecular sieves for 24 hours. Water content was found to be <50 ppm.

The molecular sieves used were activated by heating them at 500°C under dry argon for 12 hours.

WATER ANALYSIS

Analyses for water in salts and solvents, where possible, were carried out with an Aquatest II (Photovolt Corp.)

automatic Karl Fischer titrator.

SAMPLE PREPARATION

Generally, solutions of lithium salts were prepared by weighing out the desired amount of salt into a 5 or 10 ml volumetric flask, transferring the flask to the dry box, and then diluting to the mark with solvent.

The acetone-nitromethane mixed solvent solutions were prepared by taring a small snap-cap vial, adding the desired volume of acetone, weighing, adding the desired amount of nitromethane, and weighing again. From these weights, the solvent composition was determined.

INSTRUMENTAL MEASUREMENTS

Nuclear Magnetic Resonance

Lithium-7 nuclear magnetic resonance measurements were made on a Varian Associates DA-60 spectrometer at a field of 1.4092 T and a frequency of 23.287 MHz. The spectrometer was frequency locked to an appropriate reference solution. The ^{7}Li chemical shifts were measured against an aqueous 4.0 M $LiClO_4$ solution contained in a 1 mm melting point capillary and centered in the Wilmad 506-PP 5 mm OD polished NMR sample tube by Delrin spacers; however when the chemical shifts were so small that the sample was masked by the reference, a secondary reference of 5.0 M $LiClO_4$ in methanol was used. In the latter case, the shifts were corrected

to the 4.0 M aqueous $LiClO_4$ reference solution. A positive shift from the reference is assumed to be upfield.

The chemical shifts reported are corrected for differences in bulk diamagnetic susceptibility between the sample and the reference according to the equation

$$\delta_{corr} = \delta_{obs} + \frac{2\pi}{3} (X_v^{ref} - X_v^{sample}) \qquad (1)$$

in which X_v^{ref} and X_v^{sample} are the volume susceptibilities of the reference and sample solutions, respectively. δ_{obs} and δ_{corr} are the observed and the corrected chemical shifts. Published values of magnetic susceptibilities for the solvents (63) were used to calculate δ_{corr}.

Chlorine-35 NMR measurements were also performed on the DA-60 spectrometer at a field of 1.0378 T and a frequency of 4.33 MHz. The spectra were obtained by using the modulation technique previously described (56). All measurements were made at room temperature (25°C) and cylindrical nonspinning sample tubes of about 15 mm diameter were used. The spectra were recorded in the dispersion mode and linewidths were determined with an estimated accuracy of ±10% as an average of two to four measurements.

Far-infrared Spectra

The far-infrared spectra were obtained with a Digilab FTS-16 spectrometer. The FTS-16 is essentially a rapid-scan Michelson interferometer operated under computer

control. The theory and operation of this instrument have been previously described (47). Most of the spectra were obtained by using the 3- or 6-μm mylar beam splitters which cover the ranges of 600-150 and 425-100 cm^{-1}, respectively. Most of the spectra were obtained at nominal resolutions of either 2 or 4 cm^{-1}, which give a data point every 1 or 2 cm^{-1}, respectively. The instrument was operated in the single beam mode. The reference spectrum was stored in the computer memory and subtracted from the solution spectra. Standard demountable cells (Barnes Engineering Co.) were used with 2-mm polyethylene discs, and the path length was maintained at 0.1 or 0.2 mm. All spectra were smoothed by using the 9-point smoothing routine developed by P. R. Handy (47).

Laser Raman Spectra

Raman spectra were obtained on a Spex Ramalog 4 Laser-Raman spectrometer equipped with a model 1401 double monochromator. The 5145 Å line of a Spectra Physics model 164 argon ion laser was used as the excitation source, and the data were obtained in the pulse counting mode. Samples were injected into 1.6-1.8 X 90 mm melting point capillary tubes and sealed. In most cases the instrument was interfaced with a Digital Equipment Corp. PDP-8/E lab minicomputer to obtain digitized spectra. (This program is listed and its application described in Appendix I.) The digitized spectra were punched onto paper tape and then transfered

to cards for computer analysis.

DATA PROCESSING

Extensive use of the CDC-6500 computer was made to evaluate the Raman spectral data. The Fortran IV program KINFIT (64) was employed to fit and resolve the spectral bands. The application of this program was described by M. S. Greenberg (52).

CHAPTER III

SPECTROSCOPIC STUDIES OF LITHIUM ION SOLVATION IN ACETONE AND ACETONE-NITROMETHANE MIXTURES

INTRODUCTION

Previous studies in our laboratories (9,56,65) and elsewhere (21,23,27,43) have shown that nuclear magnetic resonance as well as infrared and Raman vibrational spectroscopy are very useful probes for the elucidation of the structure of electrolyte solutions and of the species present in them. These techniques are primarily sensitive to the nearest-neighbor interactions and have been used by several investigators for the determination of primary solvation numbers of ions.

In this study far-infrared, Raman, and nuclear magnetic resonance spectroscopic techniques were employed in the investigation of lithium perchlorate solutions in acetone and acetone-nitromethane mixtures. This work was undertaken to study quantitatively the solvation of the lithium ion by acetone and to investigate the solvating ability of nitromethane as compared to that of acetone to determine if the results agree with those reported by Regis and Corset (16). In addition, the influence of a weak complexing agent, pentamethylenetetrazole, on the $Li^{+}ClO_4^{-}$ ion pair formation was investigated.

RESULTS AND DISCUSSION

Far-infrared Spectra

In performing a quantitative solvation study, it is very desirable to control the concentration of the

solvating agent. This procedure, however, requires the use of an inert solvent as diluent. In previous work (9), nitromethane was used as the inert solvent. If, however, the interpretation of Regis and Corset (16) is correct (i.e., the 369-cm^{-1} infrared band, observed for solutions of $LiClO_4$ in acetone-nitromethane mixtures, is due to Li^+ solvated by nitromethane), then nitromethane competes with acetone for sites in the lithium ion solvation sphere and thus would be a very poor choice for the diluting solvent. To determine whether the 369-cm^{-1} far-infrared band is due to a complexed acetone vibration or to a lithium-nitromethane vibration, the nitromethane-acetone-lithium ion system has been studied in some detail.

Previously, Wong et al. (9) monitored the intensity of the 425-cm^{-1} Li^+-acetone solvation band as a function of the lithium perchlorate concentration in nitromethane solutions which were 1.5 M in acetone. A linear Beer's law plot of intensity versus concentration was obtained for solutions which were $\leq$0.4 M $LiClO_4$. At higher concentrations, however, although the plot remained linear, the slope was considerably different. It was concluded from these data that below 4:1 acetone/Li^+ mole ratio a new absorbing species was formed, which indicated either a possible change in the solvation number of Li^+ or a replacement of an acetone molecule in the solvation shell by the perchlorate ion.

In this investigation the intensity of the 369-cm^{-1}

band was monitored as a function of the acetone/Li^+ mole ratio for solutions of 0.2 M $LiClO_4$ in nitromethane in which the acetone concentration was varied. A near linear relationship was observed (Figure 1) for solutions in which the acetone/Li^+ mole ratio was ≤ 4. Beyond this point, however, the curve began to deviate from linearity. If it is assumed that the 369-cm^{-1} band is due to complexed acetone, the data indicate that as the concentration of acetone increases, the concentration of complexed acetone increases proportionally; but when the acetone/Li^+ mole ratio reaches four, the intensity of the 369-cm^{-1} band begins to level off since the excess acetone does not solvate the lithium ion any further. If the 369-cm^{-1} band were due to a lithium-nitromethane vibration as suggested by Regis and Corset (16), its intensity should be fairly high when no acetone is present, but should decrease as acetone is added to the system. Thus, these data seem to indicate that the 369-cm^{-1} band is due to complexed acetone.

To confirm this assignment further, far-infrared spectra were obtained for solutions of $LiClO_4$ in nitromethane which were 1.5 M in acetone-d_6. In no case was there evidence of a band at 369 cm^{-1}. In addition, the far-infrared spectra of lithium perclorate solutions were obtained in pure acetone (Figure 2). It is seen that as the concentration of $LiClO_4$ increases, the intensity of the 390-cm^{-1} band (C-C-C deformation) decreases, while at the same time the intensity of the 369-cm^{-1} band increases. Thus,

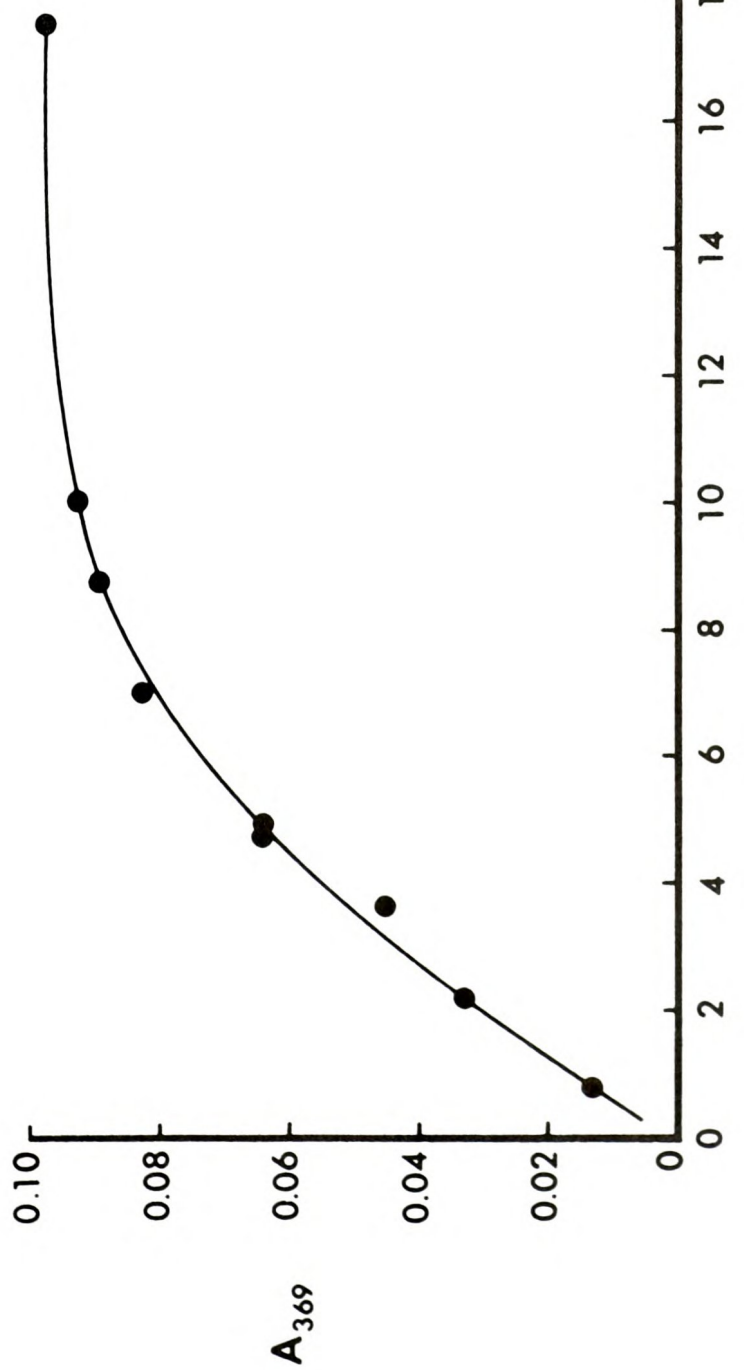


Figure 1. Absorbance of 369-cm^{-1} band vs acetone/$LiClO_4$ mole ratio. Concentration of acetone = 0.2 M; solvent: nitromethane.

Figure 2. Far-infrared spectra of solutions of $LiClO_4$ in acetone.

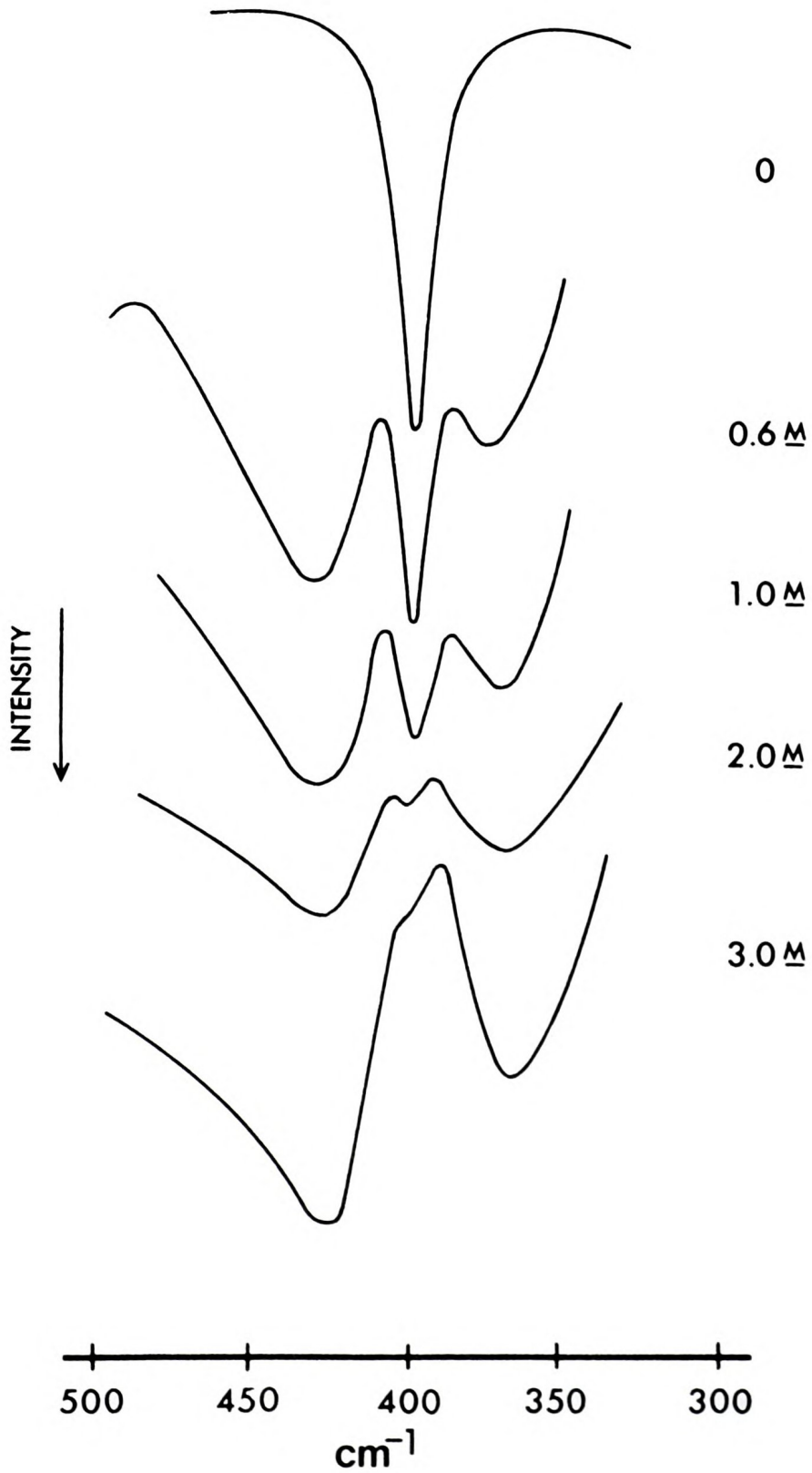


Figure 2

these data give additional strong indications that lithium perchlorate causes the splitting of the 390-cm^{-1} band and that Regis and Corset (16) were in error when they assigned the 369-cm^{-1} band observed in acetone-nitromethane mixtures to lithium-nitromethane vibrations.

Far-infrared spectra were also obtained for solutions of lithium perchlorate and lithium triiodide in nitromethane. In the case of the $LiClO_4$ solution, a broad band was observed at 368 cm^{-1}, which confirmed the results of Regis and Corset (16). This band must be due to the Li^+ ion vibration in a nitromethane solvent cage. Consequently, it appears that while the band at 368 cm^{-1} in $LiClO_4$ solutions in pure nitromethane is indeed due to the Li^+-nitromethane vibration, the 369-cm^{-1} band in acetone-nitromethane mixtures is a displaced acetone band.

For solutions of LiI_3 in nitromethane, however, the solvation band occurs at 340 cm^{-1}. Thus, the frequency of the Li^+ solvation band in nitromethane depends on the anion. It seems reasonable to assume that in these cases some ion pair formation may take place. Therefore, the solvation shell must incorporate anions as well as solvent molecules, thus making the frequency of the Li^+ solvation bands anion dependent.

To compare the solvating ability of acetone with that of nitromethane, far-infrared spectra were obtained for nitromethane solutions of lithium triiodide to which varying amounts of acetone were added. As the concentration

of acetone increased, the 340-cm^{-1} band was replaced by two new bands at 425 cm^{-1} and 369 cm^{-1} which were attributed to the Li^+-acetone solvation band and the complexed acetone band, respectively. These data again indicate that acetone solvates lithium ion much more strongly than does nitromethane.

Nuclear Magnetic Resonance Spectra

To establish further the relative inertness of nitromethane in cation solvation, ^7Li NMR studies were carried out on solutions of lithium perchlorate in acetone-nitromethane mixtures. Two studies were performed. In the first study the ^7Li chemical shift was monitored as a function of mole fraction of acetone for solutions which were 0.4 M $LiClO_4$ in various acetone-nitromethane binary mixtures. The data are presented in Table 2. From the results, it is seen (Figure 3) that the chemical shift progresses smoothly and quite rapidly from that in neat nitromethane (+0.76 ppm) to that in neat acetone (-1.05 ppm). The fact that a curve is obtained and not a straight line is indicative of preferential solvation by one of the solvents. In this case, the data indicate that acetone solvates Li^+ much more strongly than does nitromethane, since the limiting shift of $LiClO_4$ in acetone is reached at a low mole fraction of acetone. Since the most dramatic change in the chemical shift occurs between acetone mole fractions of 0 to 0.3, it was decided to extend this study,

Table 2. Variation of the Lithium-7 Resonance as a Function of Mole Fraction of Acetone for Solutions of $LiClO_4$ in Acetone-Nitromethane Mixtures.

Mole Fraction Acetone	Δppm
0.0000	0.76
0.0365	0.00
0.1032	-0.59
0.1486	-0.76
0.1955	-0.87
0.2514	-0.95
0.3286	-1.01
0.4019	-1.02
0.6007	-1.07
0.7967	-1.05
1.0000	-1.05

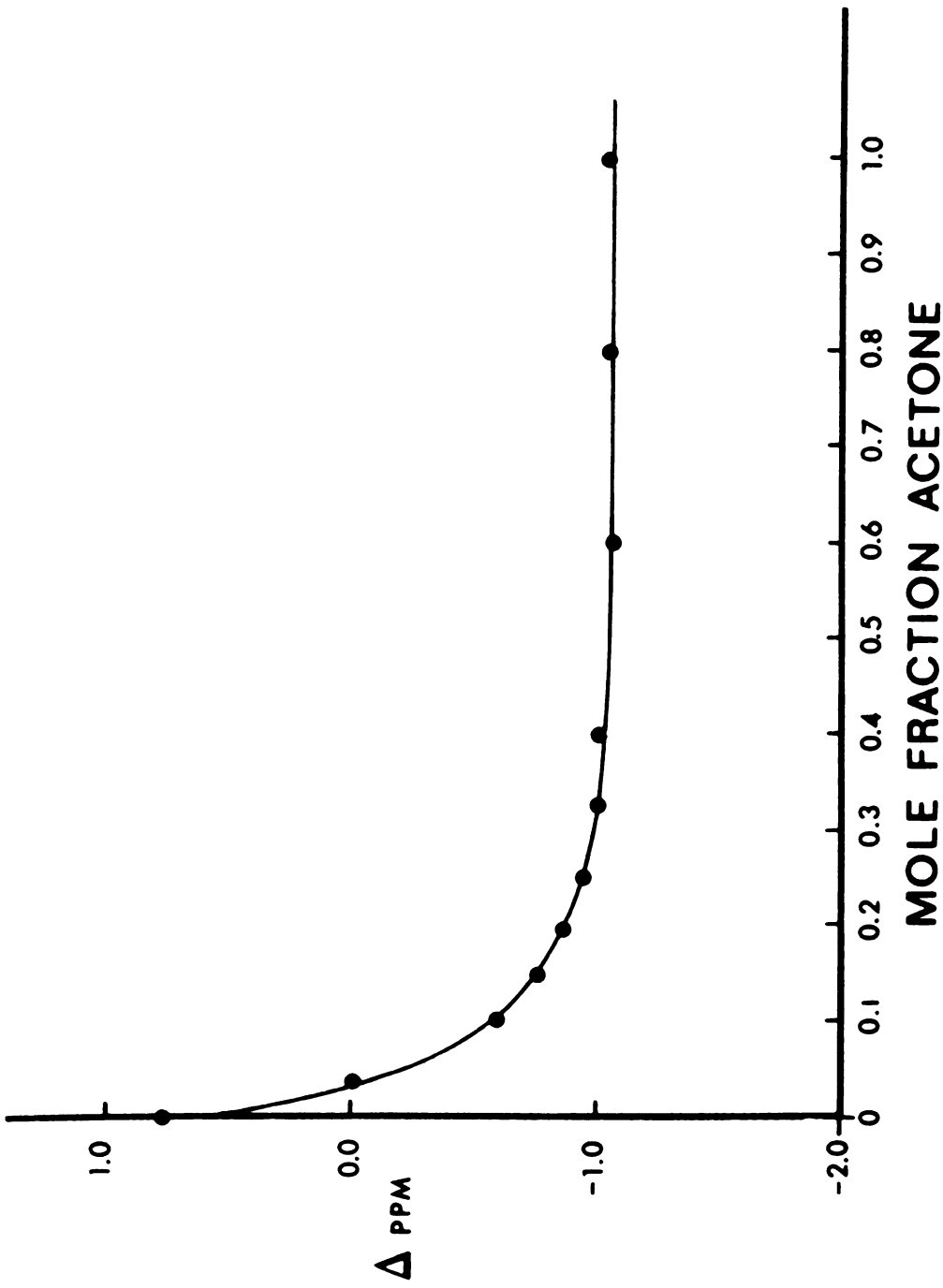


Figure 3. ^{7}Li chemical shifts vs mole fraction of acetone for solutions of $LiClO_4$ in acetone-nitromethane mixtures. Concentration of $LiClO_4$ = 0.4 M.

by focusing on the region where the most sudden changes were occurring. In this investigation, the ^{7}Li chemical shift was again monitored, but this time as a function of the acetone/Li^{+} mole ratio. The data are listed in Table 3. As noted above, the shift again smoothly progresses from that characteristic of lithium perchlorate in nitromethane to the limiting value of -1.05 ppm in neat acetone (Figure 4); however, this limiting shift is obtained in a solution in which the acetone/Li^{+} mole ratio is 15:1. Therefore, at this mole ratio nitromethane no longer contributes to the primary solvation shell of the Li^{+} ion, which again indicates that the lithium ion is preferentially solvated by acetone.

It is interesting to note that the isosolvation point (the composition at which the chemical shift is midway between two limiting values) occurs at an acetone/Li^{+} mole ratio of about 2:1. At the isosolvation point, it has been postulated that there is equal competition between the two solvents for sites in the cation solvation shell. In this case, the data may be indicative that when the primary solvation shell of Li^{+} is half-filled, it contains two molecules of acetone. Thus we might conclude that when the Li^{+} solvation shell is completely filled by acetone, four solvent molecules are present.

Additional information was obtained by studying the ^{35}Cl nuclear magnetic resonance in $LiClO_4$ solutions. Generally, the width at half height of the ^{35}Cl resonance in

Table 3. Variation of the Lithium-7 Resonance as a Function of the Acetone/$LiClO_4$ Mole Ratio for Solutions of $LiClO_4$ in Acetone-Nitromethane Mixtures.

Acetone/Li^+ Mole Ratio	Δppm
1	0.17
2	-0.11
3	-0.28
4	-0.43
5	-0.56
6	-0.66
7	-0.72
8	-0.80
9	-0.83
10	-0.86
15	-0.89
20	-1.05

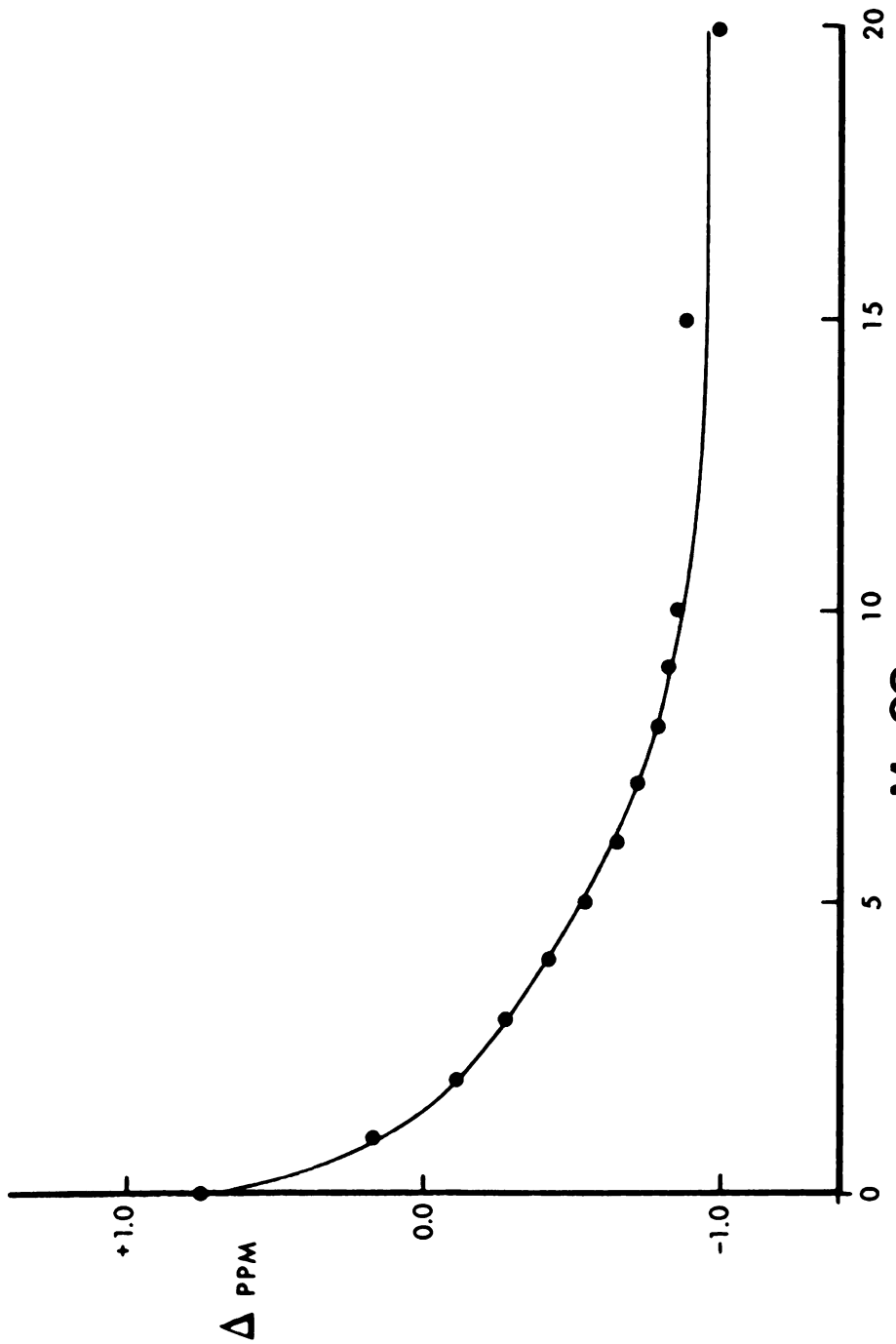


Figure 4. ^{7}Li chemical shifts vs acetone/$LiClO_4$ mole ratio. Concentration of $LiClO_4$ = 0.4 M; solvent: nitromethane.

perchlorate is quite narrow (10-20 Hz) due to the spherically symmetrical electric field around the chlorine nucleus. If this symmetry is perturbed, however, as would be the case if ClO_4^- formed a contact ion pair, then the linewidth of the ^{35}Cl resonance would be expected to become considerably broader. Reasons for this assumption have been previously discussed (56). In this investigation, the linewidth of the ^{35}Cl resonance was monitored as a function of the acetone/Li^+ mole ratio for solutions of 0.4 M $LiClO_4$ in nitromethane in which the acetone concentration was varied. The results are shown in Table 4 and in Figure 5. At high acetone/Li^+ mole ratios, when there is an excess of acetone, the linewidth is quite narrow, $\sim$20 Hz. However, as the acetone/Li^+ mole ratio decreases and the primary solvation shell of Li^+ becomes deficient in acetone, the ^{35}Cl resonance broadens very dramatically, which indicates that the ClO_4^- ion is penetrating into the Li^+ solvation sphere to form a contact ion pair. It is interesting to note that if the two linear portions of the curve shown in Figure 5 are extrapolated, they intersect at an acetone/Li^+ mole ratio of 4:1. This furnishes additional evidence that the solvation number of Li^+ by acetone is four.

The nuclear magnetic resonance data support the far-infrared and previously reported Raman data (9) in showing that acetone solvates the lithium ion much more strongly than does nitromethane, and that even when the Li^+-acetone

Table 4. Linewidths of ^{35}Cl Resonance for Solutions of $LiClO_4$ in Acetone-Nitromethane Mixtures.

Acetone/Li^+ Mole Ratio	$\nu_{1/2}$(Hz)
0.00	173±10
1.45	127±8
2.08	110±10
2.68	91±7
3.40	58±8
4.05	54±7
4.78	52±6
5.58	46±6
6 78	34±5
8.20	34±5
10.28	24±5
13.68	23±5
19.13	20±5

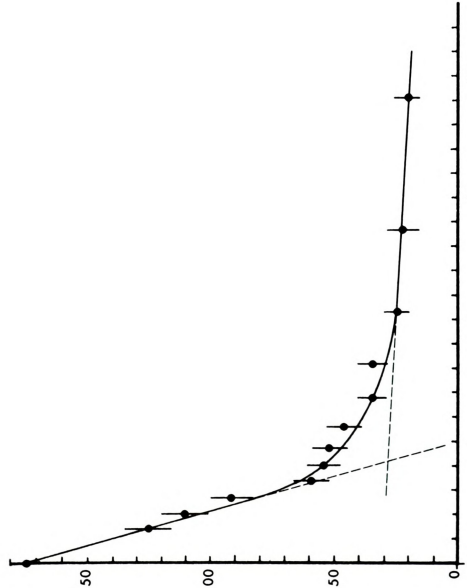


Figure 5. Band width at half height of ^{35}Cl resonance vs acetone/$LiClO_4$ mole ratio. Concentration of $LiClO_4$ = 0.4 M; solvent: nitromethane.

system is deficient in acetone, the perchlorate ion fills the vacant position in the solvation sphere of the lithium ion in preference to nitromethane. This conclusion is in agreement with other work (65,66) which has shown that the contact ion pair equilibrium strongly depends on the donor ability of the solvent as well as on the bulk dielectric constant of the medium. Although nitromethane has a high dielectric constant of 35.9, its donor ability is quite low, as shown by the Gutmann donor number of 2.7 (67). Acetone, on the other hand, has a lower dielectric constant, 20.7, but its donor number is 17.0 (67). Although it cannot be stated that nitromethane is devoid of all solvating ability, its relative inertness shows that it is a poor competitor of acetone for positions in the inner solvation shell of the lithium ion.

Laser Raman Spectra

In the investigation of solutions of $LiClO_4$ in acetone-nitromethane mixtures by Raman spectroscopy, a change in the appearance of the 789-cm^{-1} acetone band (methyl deformation) with the changing composition of the solvents was observed by Wong *et al.* (9). As the concentration of lithium perchlorate was increased, a new band, characteristic of the acetone molecule bound to the lithium ion, appeared at 803 cm^{-1}. The purpose of this study was to monitor carefully the behavior of these two bands to obtain some quantitative information on the strength of the

lithium-acetone interaction.

As can be seen in Figure 6, the 789-cm^{-1} and 803-cm^{-1} bands overlapped to an extent which could not be ignored if this investigation was to yield quantitative results. Therefore, digitized spectra were obtained and were analyzed by computer in order to resolve the overlapping spectral bands. The equation used for fitting the experimental data is the Lorentz-Gaussian product function described by Irish et al. (68):

$$I = I_o\{\exp[-(\bar{\nu}-\bar{\nu}_o)^2/2\sigma^2]\}\{1+(\bar{\nu}-\bar{\nu}_o)^2/\sigma^2\}^{-1} \qquad (2)$$

in which I is the intensity at frequency $\bar{\nu}$, $\bar{\nu}_o$ is the position of the line center with maximum intensity I_o, and σ is the variance which, when multiplied by the factor 1.46, gives the band width at half height. This equation was employed to fit the data by using a non-linear weighted least squares analysis, KINFIT (64). The procedure was to input the experimental $\bar{\nu}$ and I values and allow the computer to fit the data to the above equation, by calculating $\bar{\nu}_o$, I_o, and σ for each band. With these parameters, the area under each band could then be calculated.

To determine the concentration of free acetone in solutions of $LiClO_4$ in the acetone-nitromethane mixtures, the spectra of solutions of only acetone in nitromethane of known concentrations were obtained. Each spectrum was computer-fitted with the Lorentz-Gaussian product function,

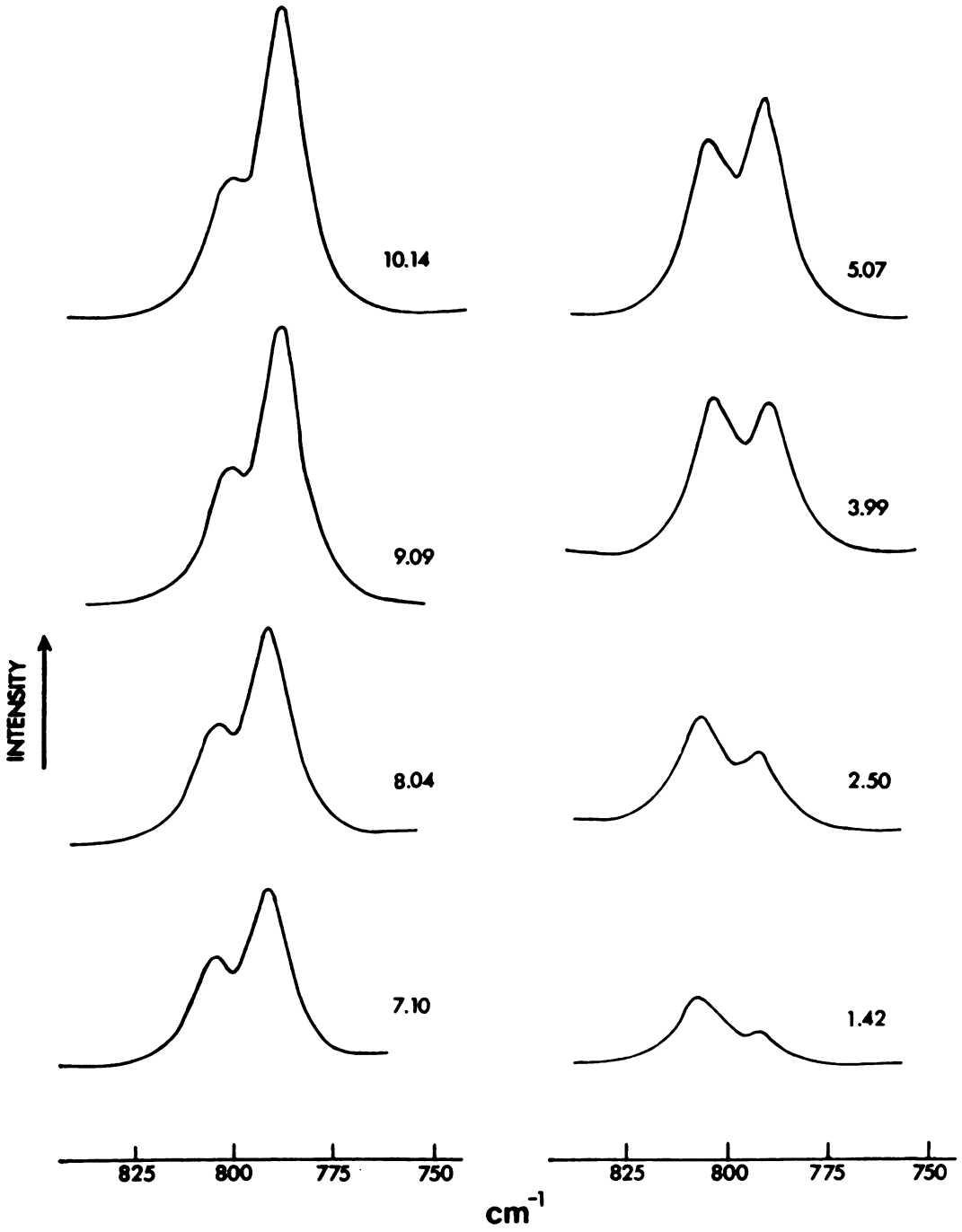


Figure 6. The 789-cm^{-1} and 803-cm^{-1} acetone Raman bands at various acetone/$LiClO_4$ mole ratios. Concentration of $LiClO_4$ = 0.4 M.

and the area of the 789-cm^{-1} band of acetone in these solutions was determined. A sample computer-fitted spectrum for an acetone standard solution is illustrated in Figure 7. The area was then plotted versus the concentration of acetone, and a linear working curve was obtained (Figure 8).

The Raman spectra were then obtained for the solutions of 0.4 M $LiClO_4$ in nitromethane in which the acetone concentration varied from 0.57 to 6.69 M. These spectra were then computer-fitted (Figure 9), and the concentration of free acetone in these solutions was then found from the area of the 789-cm^{-1} band and from the working curve. Thus, by knowing the total and free concentrations in each solution, the concentration of the complexed acetone could be determined.

The average solvation number, $\bar{n}$, of the lithium ion is defined as

$$\bar{n} = [(Ac)_t - (Ac)_f]/(Li^+)_t \qquad (3)$$

in which $(Ac)_t$ is the total concentration of acetone in solution, $(Ac)_f$ is the concentration of free acetone, and $(Li^+)_t$ is the formal $LiClO_4$ concentration. The values obtained for the concentration of free and bound acetone and the resulting $\bar{n}$ values are presented in Table 5. The plot of $\bar{n}$ versus the acetone/Li^+ mole ratio is shown in Figure 10. As the acetone/Li^+ mole ratio increases, $\bar{n}$ also increases until a limiting solvation number of four is obtained.

Figure 7. Computer-fitted 789-cm^{-1} acetone Raman band for a standard acetone solution in nitromethane. X means an experimental point, O means a calculated point, = means an experimental and calculated point are the same in the resolution of the plot.

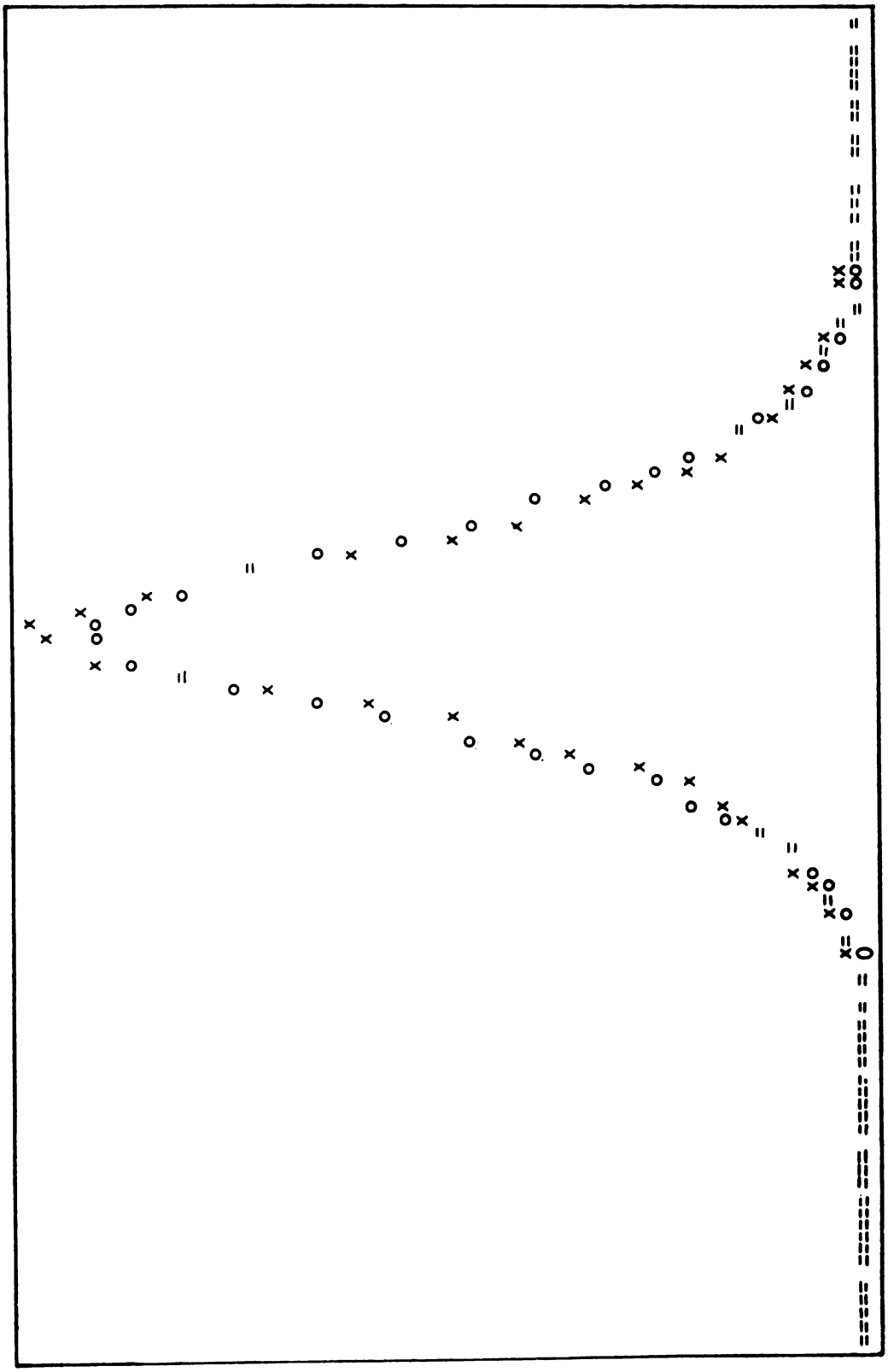


Figure 7

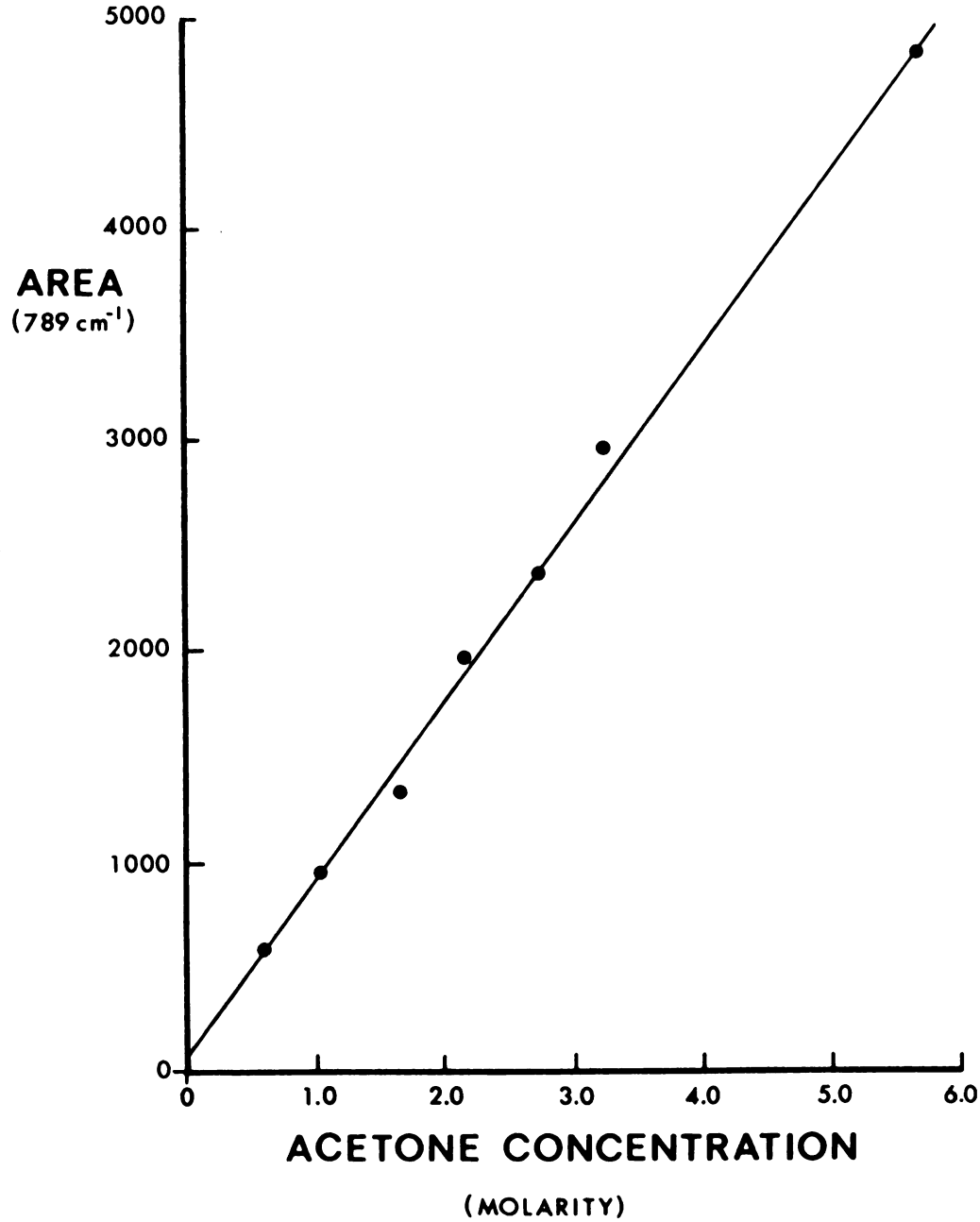


Figure 8. Plot of the area of the 789-cm^{-1} acetone Raman band vs concentration of acetone; solvent: nitromethane.

Figure 9. Computer-fitted 789-cm^{-1} and 803-cm^{-1} acetone Raman bands for a solution of 0.4 M $LiClO_4$ in an acetone-nitromethane mixture. X means an experimental point, O means a calculated point, = means an experimental and calculated point are the same in the resolution of the plot.

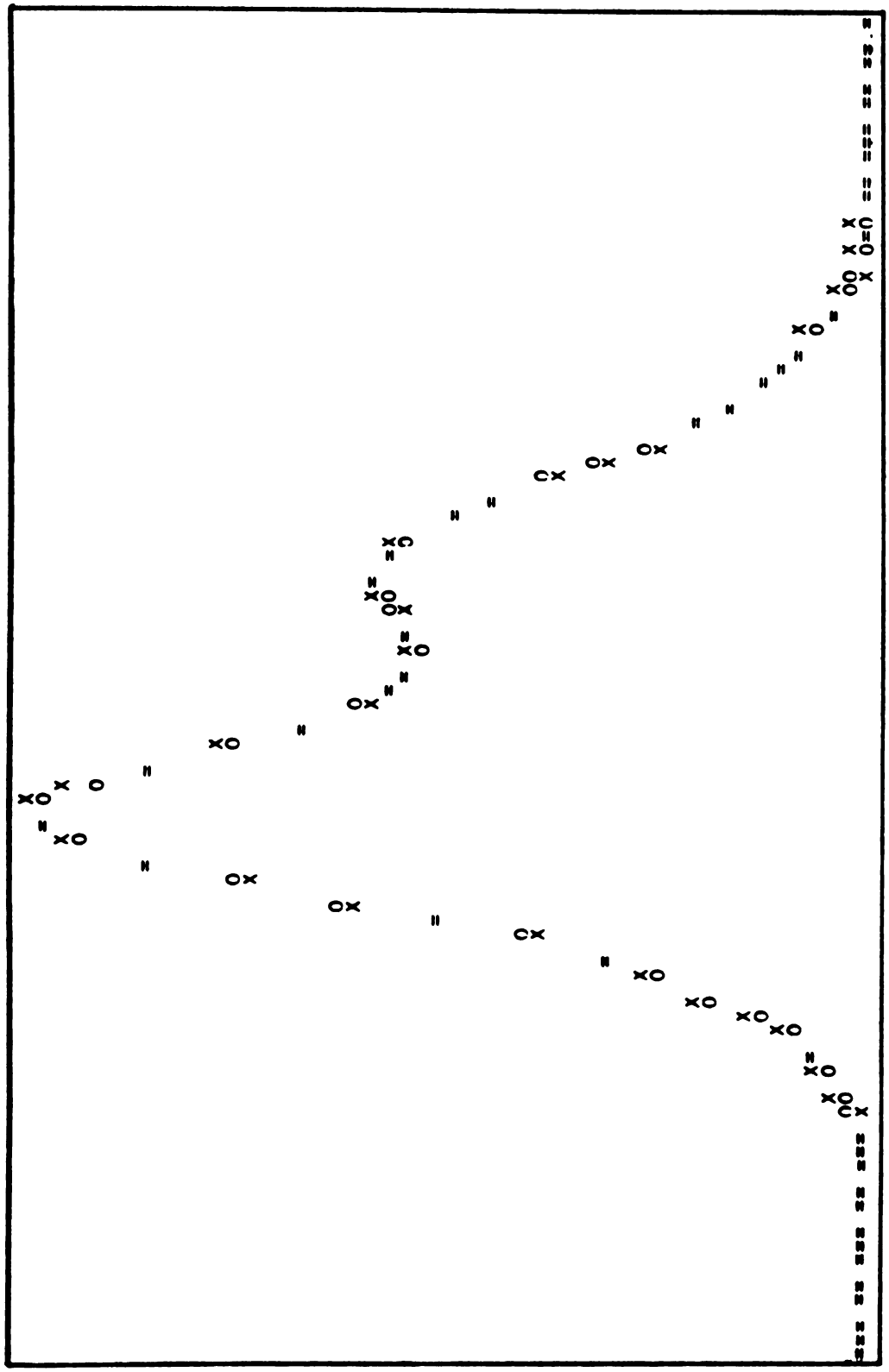


Figure 9

Table 5. Values Obtained for the Concentration of Free and Bound Acetone and $\bar{n}$ Values for the Raman Study of $LiClO_4$ in Acetone-Nitromethane Mixtures.

Acetone/Li^+ Mole Ratio	$[Acetone]^{free}$	$[Acetone]^{bound}$	$\bar{n}$
1.42	0.25 M	0.32 M	0.80
2.01	0.37	0.43	1.08
2.50	0.40	0.60	1.50
3.00	0.49	0.71	1.78
3.44	0.57	0.81	2.03
3.99	0.69	0.91	2.28
4.57	0.85	0.98	2.45
5.07	1.04	0.99	2.48
5.48	1.01	1.18	2.95
6.12	1.23	1.22	3.05
6.44	1.23	1.35	3.38
7.10	1.44	1.40	3.50
7.50	1.79	1.21	3.03
8.04	1.86	1.35	3.38
8.52	1.95	1.46	3.65
9.09	2.24	1.40	3.50
12.08	3.32	1.51	3.78
14.14	4.12	1.53	3.83
16.73	5.09	1.60	4.00

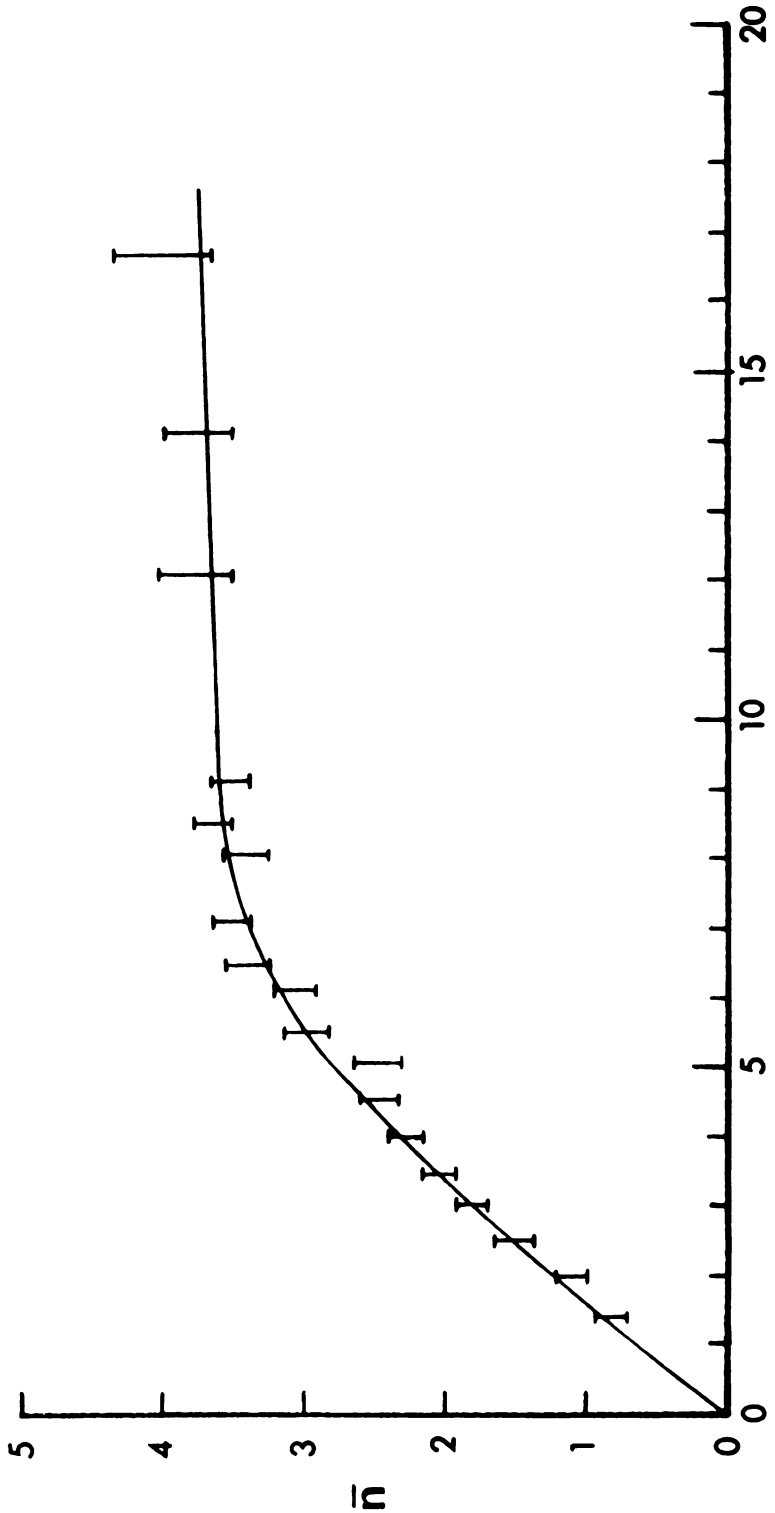


Figure 10. Plot of $\bar{n}$ vs the acetone/$LiClO_4$ mole ratio. Concentration of $LiClO_4$ = 0.4 M; solvent: nitromethane.

In his fundamental treatment of stepwise equilibria that give rise to species MA_1, MA_2,..., MA_n with corresponding equilibrium constants of K_1, K_2,...,K_n, Bjerrum (69) has shown that in a solution when $\bar{n} = n - \frac{1}{2}$, equal concentrations of $MA_{n-\frac{1}{2}}$ and MA_n must exist. Then it follows that,

$$K_n = 1/[(A)_f]_{\bar{n}=n-\frac{1}{2}} \qquad (4)$$

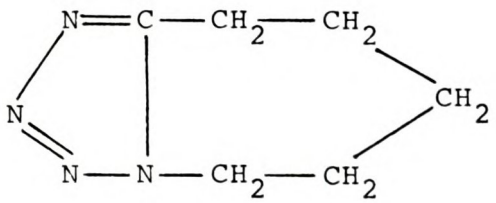
where $(A)_f$ is the concentration of free ligand at $\bar{n}=n-\frac{1}{2}$. Thus, the value of K_1 is given by $[1/(A)_f]_{\bar{n}=\frac{1}{2}}$, K_2 is given by $[1/(A)_f]_{\bar{n}=3/2}$, etc. By using this technique estimates of the stepwise stability constants for the complexation of the lithium ion by acetone have been made. The values obtained are: $K_1 = 19.1$, $K_2 = 2.5$, $K_3 = 1.3$, and $K_4 = 0.6$. It should be emphasized that due to the uncertainty inherent in this method, the calculated constants are probably only an indication of order of magnitude.

Effect of Complexing Agents

In previous work (9) it was noted that for solutions of $LiClO_4$ in acetone-nitromethane mixtures, the 935-cm^{-1} Raman band (ν_1, symmetric stretch) of the perchlorate ion ramained narrow and at constant frequency in solutions with acetone/Li^+ mole ratios of ≥ 4. As the acetone/Li^+ mole ratio became less than four, the band broadened and shifted to higher frequency. From these data and from the ^{35}Cl nuclear magnetic resonance results that were

discussed earlier in this chapter, it is evident that for lithium perchlorate-acetone solutions in nitromethane in which the acetone/Li^+ mole ratio is 1:1, the predominant solute species is the $Li(acetone)^+ClO_4^-$ ion pair. The purpose of this investigation was to add a weak complexing agent to solutions in which the acetone/Li^+ mole ratio was 1:1 and then observe if it displaced the perchlorate ion from the solvation shell of the lithium ion.

For this purpose, pentamethylenetetrazole (PMT) was used. The structure of PMT is shown below.



Previous studies (70) by ^7Li NMR have shown that in nitromethane solutions lithium forms a 1:1 complex with PMT with a formation constant of 4.85 $\underline{M}^{-1}$ (pentamethylenetetrazole will be discussed in detail in the second part of this thesis). Far-infrared spectroscopy was used to confirm the formation of this complex. For a nitromethane solution that was 0.5 $\underline{M}$ in $LiClO_4$ and 1.5 $\underline{M}$ in PMT, a broad band was observed at 408 cm^{-1} that could not be attributed to the salt, solvent, or complexing agent. Thus, this band was assigned to be due to a vibration of the Li^+-PMT complex.

To investigate the effect of PMT on the $Li^+ClO_4^-$ ion pair formation, the 935-cm^{-1} Raman band of perchlorate ion was monitored for solutions in which the acetone/Li^+ mole ratio was 1:1 as increasing amounts of pentamethylenetetrazole were added. From the results shown in Figure 11, it is seen that as the concentration of PMT is progressively increased, the band changes from a broad and fairly weak peak to a very sharp, intense peak which is indicative of the free perchlorate ion. Therefore, pentamethylenetetrazole displaces the ClO_4^- ion from the solvation shell of the lithium ion according to the equilibrium

$$Li(Acetone)^+ClO_4^- + PMT \rightleftarrows Li(Acetone)(PMT)^+ + ClO_4^- \quad (5)$$

At the same time only a very weak acetone band was observed at 789 cm^{-1}, while a larger band was located at 803 cm^{-1}, which indicated that most of the acetone was in the complexed form. Therefore, although PMT displaces ClO_4^- from the Li^+ solvation shell, it does <u>not</u> displace any acetone from the solvation shell of the lithium ion.

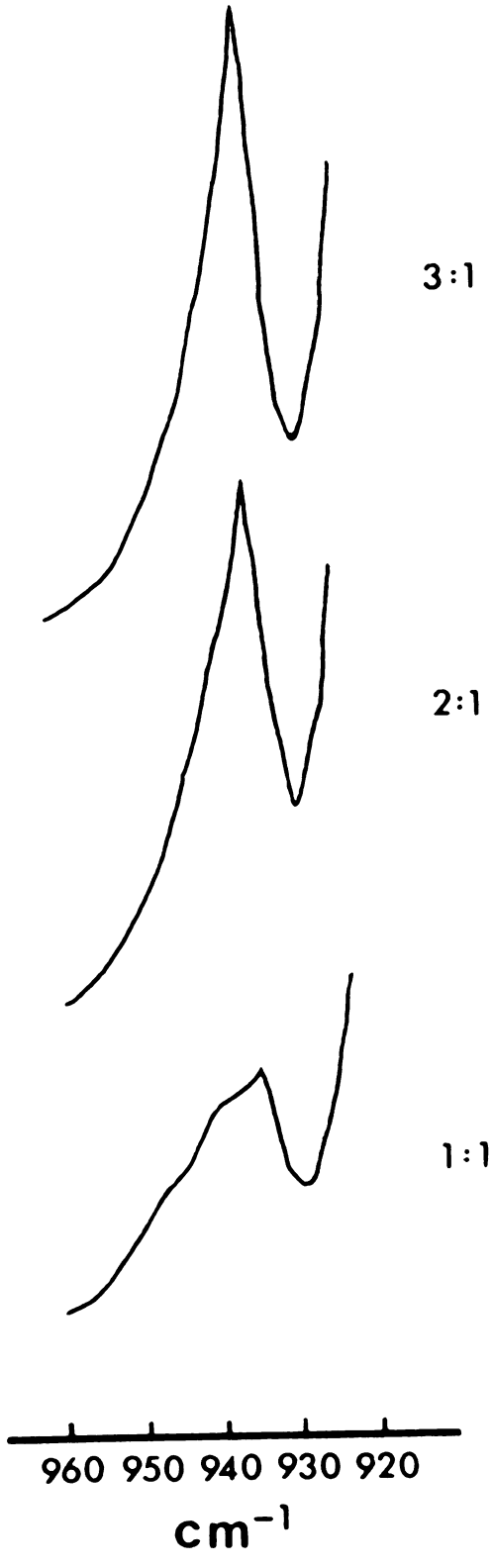


Figure 11. The 935-cm^{-1} ClO_4^- Raman band as a function of the PMT/$LiClO_4$ mole ratio.

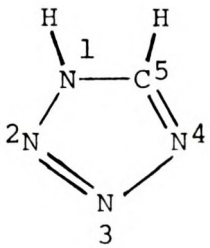
PART II

GAS CHROMATOGRAPHIC STUDIES OF CONVULSANT TETRAZOLES

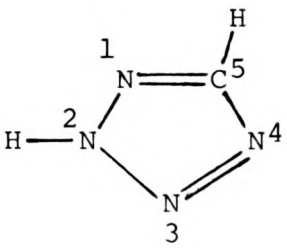
CHAPTER I

HISTORICAL

The chemistry of tetrazoles and substituted tetrazoles has been the subject of investigations for many years because of their strong stimulating action on the central nervous system. The parent compound, tetrazole, is composed of one carbon and four nitrogen atoms in a ring and can exist in two tautomeric forms I and II (71,72).



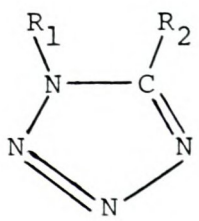
I



II

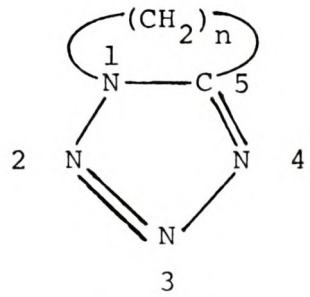
It has been shown that about 97% of the equilibrium mixture of I and II exists in form I (73). The numbering of the atoms in the ring starts with the nitrogen atom adjacent to the carbon (configuration I) and proceeds counterclockwise around the ring as shown above.

The hydrogen atoms in positions 1 and 5 can be easily replaced to give rise to two classes of 1,5-disubstituted tetrazoles. In the first of these, both hydrogens are replaced by aliphatic or aromatic groups as shown below.



1,5-disubstituted tetrazole

A special class of 1,5-disubstituted tetrazoles is composed of the cyclopolymethylenetetrazoles in which a hydrocarbon chain forms a second ring that is fused to the tetrazole ring.



n = 3,4,5,...

cyclopolymethylenetetrazole

The cyclopolymethylenetetrazoles and their derivatives are the primary subject of this investigation.

The cyclopolymethylenetetrazoles and their derivatives are known for their ability to cause epileptic convulsions. The convulsant activity varies with the number of methylenes in the hydrocarbon chain as well as with the nature and position of substituent groups (74). The convulsant activity and some physical properties of various tetrazoles are listed in Table 6.

As can be seen from Table 6, the insertion of methylene groups into the hydrocarbon ring significantly changes the convulsant property of the drug. A gradual monotonic increase in convulsant activity results as the number of methylene groups is increased. Thus, this series of compounds seems very useful for the study of possible

Table 6. Physical and Pharmacological Properties of some Cyclopolymethylenetetrazoles.

Tetrazole	Molecular Weight	Melting Point (°C)	Minimum Convulsant Dose* (74)
Trimethylenetetrazole	110.12	110	1000
Tetramethylenetetrazole	124.15	117	250
Pentamethylenetetrazole	138.18	60	50
Hexamethylenetetrazole	152.20	68	40
Heptamethylenetetrazole	166.23	42	30
Octamethylenetetrazole	180.26	117	insoluble
Nonamethylenetetrazole	194.28	90	insoluble
Undecamethylenetetrazole	222.34	66	insoluble
8-tert-butylpentamethylenetetrazole	194.28	133	3
8-sec-butylpentamethylenetetrazole	194.28	70	50

*The minimum convulsant dosage is the minimum amount of tetrazole necessary to cause the first symptoms of seizure. The dosage is given in units of milligrams of tetrazole per kilogram of body weight of the animal.

correlations between physicochemical properties and pharmacological activity.

Of the cyclopolymethylenetetrazoles, only pentamethylenetetrazole (PMT) has had some clinical applications. It has been used in patent medicines as a respiratory and cardiac stimulant, and in higher doses it has been used as an analeptic in barbiturate overdoses. Pentamethylenetetrazole has also been employed for screening anticonvulsant drugs and in veterinary medicine in hastening the recovery of animals from anesthesia.

Due to its use in chemotherapy, the chemistry of PMT has been studied in some detail. However, the analytical chemistry of PMT and the other cyclopolymethylenetetrazoles has not been thoroughly investigated.

A large number of studies have been devoted to the investigation of several PMT-transition metal complexes. These complexes have been discussed by Dister (75) and Popov (76), and as a result of these studies, considerable physical data pertaining to these compounds have been accumulated.

Complexes such as CuCl·PMT (77), $HgCl_2$·PMT (78), and $CdCl_2$·PMT (79) have been widely investigated. Because of their moderately low dissociation constants and solubilities, these complexes were employed in precipitation procedures for the quantitative analysis of pentamethylenetetrazole. There are some disadvantages, however, in using these complexes for the gravimetric determination

of PMT. The conditions of the reaction must be rigorously controlled so that the exact composition of the precipitate is known, as the ratio of PMT to metal can vary from one to two. Another drawback is that the solubility of the complexes seriously limits the analysis at low concentration levels.

Nonaqueous potentiometric determinations of PMT and substituted pentamethylenetetrazoles in formic acid were reported by Popov and Marshall (80,81). They determined the tetrazoles quantitatively and noted that the tetrazole with the greatest convulsant activity was the most basic and the most inactive compound was the least basic. However, the elaborate procedure used does not make this method practical for routine analytical determinations.

Beyrich and Schlaak (82) titrated PMT with perchloric acid-glacial acetic acid mixtures in a vessel containing benzene and acetic anhydride. They determined pentamethylenetetrazole in the presence of other drugs at concentration levels of 0.3 to 3.0 parts per thousand.

A spectrophotometric method for the determination of PMT in pharmaceutical preparations was described by Daoust (83). The analysis was based on the precipitation of the CuCl·PMT complex which was isolated and dissolved in nitric acid. The copper was then complexed with tetraethylenepentamine and the absorbance of the solution measured. The relative standard deviation was reported to be 8% at the 40 parts per million level.

Turczan and Goldwitz (84) measured the concentration of PMT in pharmaceutical preparations by proton nuclear magnetic resonance spectroscopy. Their reported detection limits were very poor as they found that the best results were obtained with solutions containing at least 3% by weight PMT.

Rylance and co-workers (85) investigated the use of thin layer chromatography in the determination of neutral drugs and found that PMT could not be determined due to its lack of an appreciable ultraviolet absorption near 254 nm. However, Guven (86) found that PMT could be spotted with a mixture of 10% copper sulfate and 2% ammonia solutions which gave blue spots upon drying.

Gas chromatography was first employed in the determination of pentamethylenetetrazole by Kawamoto (87) in 1962. His instrument was equipped with a thermal conductivity detector, and the baseline drift obtained for aqueous solutions was so severe that the determination of the peak areas was extremely difficult.

Several investigators have recently reported the use of gas chromatography with flame ionization detection systems for the analysis of PMT (88-90). Generally, liquid stationary phase coatings of 3-5% have been used. Pentamethylenetetrazole has been routinely determined in the 100 parts per million level with the technique capable of measuring concentrations as low as 10 ppm.

Clearly, this is the best technique thus far reported

for the quantitative determination of PMT since the analysis time is fast and the sensitivity is excellent. Although this method has been successfully applied to the analysis of PMT, there still are no reports on the analysis of the other cyclopolymethylenetetrazoles. Hence a portion of this research is involved with the quantitative determination of the cyclopolymethylenetetrazoles by using gas chromatography.

Numerous attempts to correlate physicochemical properties with physiological activities of the tetrazoles and other biologically active compounds have been made. In addition, several studies have centered around the determination of the nature of the tetrazole interaction in the biological system.

Popov and Holm (91) determined the dipole moments of pentamethylenetetrazole, 8-tert-butylpentamethylenetetrazole, and 8-sec-butylpentamethylenetetrazole in benzene solution to be 6.14, 6.20, and 6.18 D, respectively. They concluded that there was no correlation between their convulsant activities and the magnitude of their dipole moments.

Schueler et al. (92) studied correlations of the biological activity of some substituted tetrazoles with their ultraviolet absorptions. They found that alkyl-substituted tetrazoles of moderate activity generally showed little or no absorption down to 220 nm. However, aryl-substituted tetrazoles which act as depressants showed absorption bands

in the 290 and 225 nm spectral regions. Apparently, there is some correlation between ultraviolet absorptions of the tetrazoles and their physiological activities.

Erlich and Popov (93) determined basicity constants for six cyclopolymethylenetetrazoles varying from trimethylenetetrazole to undecamethylenetetrazole in formic acid solutions. It was shown that while the cyclopolymethylenetetrazoles do not have any detectable proton affinity in aqueous solutions, they do act as fairly strong monoprotic bases in formic acid solutions. The authors did not observe any correlation between the length of the hydrocarbon chain and the base strength of the tetrazole ring, as nearly all of the reported pK_b values were about 1.8.

The surface activity of PMT was investigated by Buchanan et al. (94) in a study of air-solution surface tension isotherms. They observed that central nervous system stimulants prefer the aqueous bulk phase, while drugs which exhibit depressant action collect at the air-solution interface. Recently, it has been shown that PMT can emulsify human cell membranes (95). This action weakens the membrane and causes it to rupture. Due to its solubility in lipid substances, PMT then diffuses rapidly through the membrane.

Gross and Woodbury (96) studied the effects of various cyclopolymethylenetetrazoles on ion transport in toad bladder membranes. They noted a strong correlation between the convulsant potency of the tetrazoles and the increase

of the short-circuit current produced in the isolated toad bladder. It was concluded that the cyclopolymethylenetetrazoles affect the potassium ion transport across the membrane. One possible explanation for this action is that the tetrazoles have an effect within the membrane. If this explanation is correct, the tetrazole must first pass into or through the membrane.

One theory for the passage of materials through a membrane postulates an actual dissolution of the material in the membrane. Thus an investigation of this phenomenon must involve studying the partitioning of the substance between an aqueous solution and the lipid membrane.

There have been no reports, however, on the partition coefficients of the cyclopolymethylenetetrazoles, although investigations have been carried out in which physicochemical properties have been related to the partitioning of other biologically active compounds between aqueous solutions and lipid solvents.

Meyer (97) and Overton (98) showed that the relative narcotic activities of drugs often paralleled their oil/water partition coefficients. They also noted that in a homologous series of compounds the partition coefficient increased by a factor of from two to four per methylene group. Recently, partition coefficients have been used as extrathermodynamic reference parameters for "hydrophobic bonding" in biochemical and pharmacological systems (99, 100).

Although partition coefficients have been tabulated for many biologically important compounds (101), very little is known about the distribution of the cyclopolymethylenetetrazoles between aqueous solutions and lipid solvents. Thus another section of this thesis is devoted to the investigation of the partition coefficients of the cyclopolymethylenetetrazoles.

CHAPTER II

EXPERIMENTAL

REAGENTS

Carbon tetrachloride (Fisher) was shaken with alcoholic sodium hydroxide and washed several times with water. It was then dried over calcium chloride and fractionally distilled. Water was doubly distilled in an all glass apparatus, once with potassium permanganate present in the charge to remove all oxidizable organic impurities. Chloroform (Fisher, Certified A.C.S.) was used without further purification.

Pentamethylenetetrazole (Aldrich) was recrystallized from diethyl ether and dried *in vacuo*. Trimethylenetetrazole (Aldrich) was purified by recrystallizing about 10 grams of the tetrazole from a solvent mixture of 50 ml of carbon tetrachloride and 10 ml of ethanol.

The other cyclopolymethylenetetrazoles were prepared and purified as described by D'Itri (102,103).

Analytical reagent grade sodium chloride (Mallinckrodt) was used without further purification.

SYNTHESIS OF 8-SEC-BUTYLPENTAMETHYLENETETRAZOLE

4-sec-butylcyclohexanol

To prepare 8-*sec*-butylpentamethylenetetrazole, 4-*sec*-butylcyclohexanone was needed as the starting material. This compound, however, is not commercially available, and the most similar compound that is commercially

available is p-sec-butylphenol. This phenol was used as the starting material for this synthesis.

The hydrogenation reaction was carried out in the Parr Series 3910 Hydrogenation Apparatus which can withstand pressures of up to five atmospheres. The experimental procedure was similar to that reported by Somerville and Theimer (104) for the hydrogenation of p-tert-butylphenol.

One hundred grams of p-sec-butylphenol (Eastman Organic Chemicals, Practical Grade) were dissolved in 100 ml of absolute ethanol in the reaction bottle, and 4 grams of 5% rhodium on alumina catalyst (Pfaltz & Bauer) were added. The reaction bottle was mounted in the Parr apparatus and subjected to a pressure of 50 psi of hydrogen. The reaction vessel was shaken and heated to about 80°C.

As the pressure in the reservoir decreased, more hydrogen was added to maintain a pressure of 50 psi. The reaction was allowed to proceed for several days until there was no further uptake of hydrogen.

The apparatus was then disassembled and the reaction mixture was fractionally distilled at reduced pressure. The product was collected at 120-130° at 20 mm pressure [lit. (105) 128° at 20 mm]. The yield of 4-sec-butylcyclohexanol was about 50%.

4-sec-butylcyclohexanone

The oxidation of the 4-sec-butylcyclohexanol was carried out by using the procedure for the preparation of

menthone from menthol (106).

A solution of 30 ml of concentrated sulfuric acid in 340 ml of water was added to 68 grams of sodium dichromate in a one liter round-bottomed flask. Fifty grams of 4-sec-butylcyclohexanol were then added in three portions while the mixture was stirred. Heat was evolved and the temperature of the reaction mixture increased to approximately 55°. After the mixture cooled to room temperature, the oil was mixed with an equal volume of ether, separated in a separatory funnel, and washed with three 100-ml portions of 5% sodium hydroxide solution. The ether was removed and the residue distilled under reduced pressure. The product was a colorless liquid which distilled at 110-120° at 30 mm [lit. (105) 104-106° at 13 mm]. Twenty-five grams of the 4-sec-butylcyclohexanone were obtained.

4-sec-butylcyclohexanone Oxime

The cyclohexanone was converted into the oxime by treatment with an aqueous solution of hydroxylamine as described by Herbst and co-workers (107).

A mixture of 15.4 grams of 4-sec-butylcyclohexanone and 8.4 grams of hydroxylamine hydrochloride (Matheson, Coleman, and Bell) was added to 70 ml of a 10% sodium carbonate solution. The mixture was stirred for a few hours and the oxime was extracted with ether. The ether was removed and the product was distilled at 140-145° at 28 mm. The yield of the product, a colorless liquid, was

approximately 85%.

8-sec-butylpentamethylenetetrazole

The 8-sec-butylpentamethylenetetrazole was prepared by using the method described by Herbst and co-workers (107) for the preparation of 8-isopropylpentamethylene-tetrazole.

A suspension of 14.6 grams of powdered sodium azide (Eastman Organic Chemicals, Practical Grade) in 250 ml of 1,2-dichloroethane was added to a 2-liter three-necked flask equipped with a stirrer, dropping funnel with the tip immersed in the reaction mixture, exit tube, and a long-stemmed alcohol thermometer with the bulb immersed in the reaction mixture.

CAUTION: The following procedure involves the generation of hydrazoic acid. Hydrazoic acid vapors are highly toxic, and all reactions in which it is involved should be carried out in an efficient hood. Heavy metals, such as mercury from a broken thermometer, must be excluded because of the explosive nature of mercury (II) azide which could form.

While stirring the suspension, 120 grams of chlorosulfonic acid were added at such a rate that the temperature did not rise above 35°. After the addition of the acid was completed, a solution of 16 grams of 4-sec-butylcyclohexanone oxime in 125 ml of 1,2-dichloroethane was added with continuous, vigorous stirring so that the

temperature remained between 35 and 45°. Upon complete addition of the oxime, stirring was continued until the reaction mixture had cooled to room temperature. Water was then slowly added to the mixture to decompose the excess chlorosulfonic acid (external cooling was required). The aqueous layer was separated and the acid neutralized with aqueous sodium hydroxide. The neutral solution was extracted with four 100-ml portions of 1,2-dichloroethane. The extracts were then combined with the 1,2-dichloroethane solution, dried over sodium sulfate, and the solvent removed by evaporation. The residue was boiled with 100 ml of 10% aqueous hydrochloric acid for three hours, and the product was extracted with several portions of 1,2-dichloroethane.

After washing the combined extracts with water and drying over sodium sulfate, the solvent was removed by evaporation. The product, a dark brown oil, was then cooled for several hours in an ice bath in order to facilitate crystallization. The crude product was purified by recrystallizing it a few times from a mixture of heptane and 1,2-dichloropropane. The melting point of the purified 8-sec-butylpentamethylenetetrazole was 69-70° [lit. (107) 70-71°]. The yield based on 4-sec-butylcyclohexanone oxime was approximately 50%. The steps in the synthesis of this compound are summarized in Figure 12.

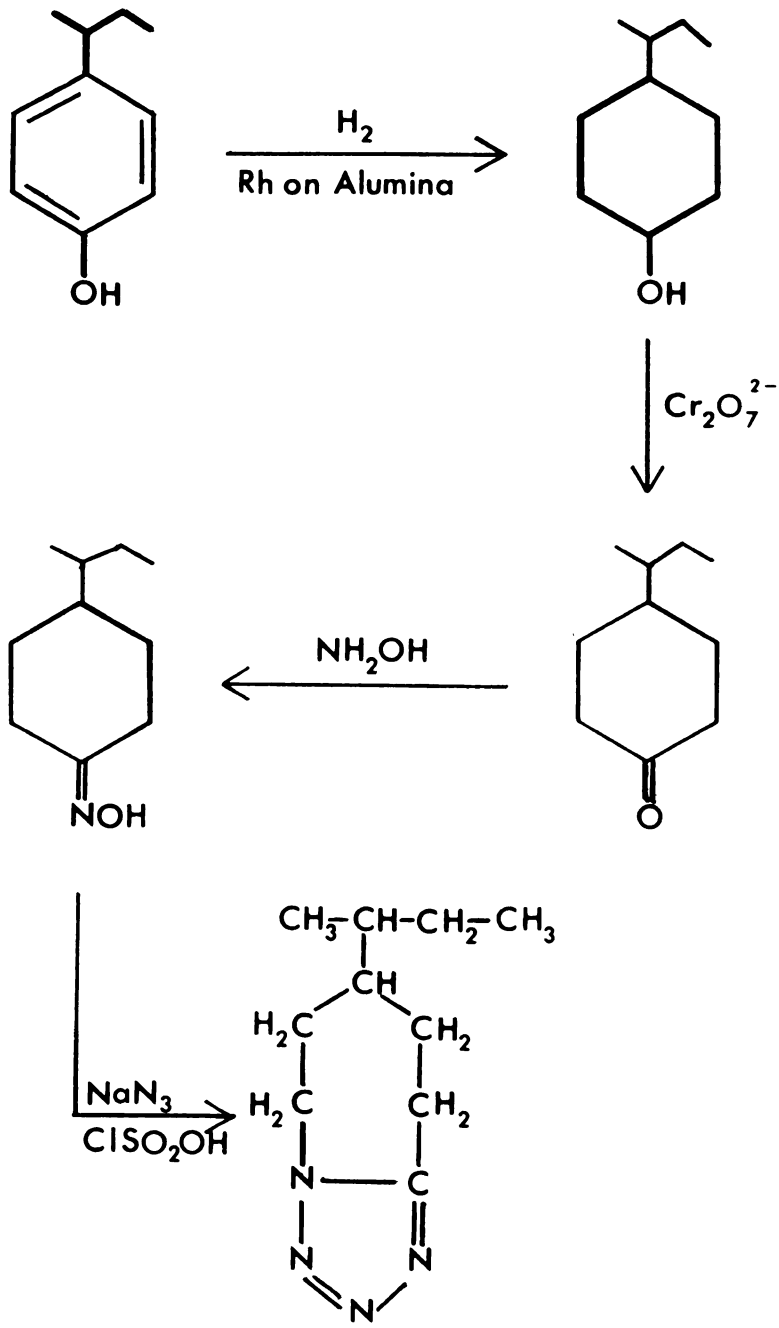


Figure 12. Synthesis of 8-sec-butylpentamethylenetetrazole.

SYNTHESIS OF 8-TERT-BUTYLPENTAMETHYLENETETRAZOLE

4-tert-butylcyclohexanone Oxime

4-tert-butylcyclohexanone (Aldrich) was converted into its oxime by treatment with an aqueous hydroxylamine solution as described previously for the preparation of 4-sec-butylcyclohexanone oxime. In this preparation, though, the product was a solid which melted at 135-137° [lit. (107) 137.5-138.5°].

8-tert-butylpentamethylenetetrazole

This compound was prepared as described for the 8-sec-butylpentamethylenetetrazole. The crude product was recrystallized from isopropanol. The colorless crystals which were obtained melted at 133° [lit. (107) 132.5-133°].

MELTING POINTS

The melting points of the tetrazoles were measured on a Fisher-Johns melting point apparatus.

CONSTANT TEMPERATURE BATH

A Waco refrigerated constant temperature bath designed by the Wilkens-Anderson Company, Chicago, Illinois, was used. It was modified by the addition of a shaker unit so that solutions could be shaken while submerged in the bath.

GAS CHROMATOGRAPHIC MEASUREMENTS

Two gas chromatographs were used. Most of the measurements were made on a Varian 1440-10 Gas Chromatograph. The other instrument employed in these studies was a Hewlett Packard Series 5700A Gas Chromatograph. Both instruments were equipped with flame ionization detectors.

The columns were constructed of 6 ft. x 1/8 in. lengths of stainless steel tubing. They were packed with 3% Hi-Eff 8-BP (Applied Science Laboratories, Inc.) on 100/120 mesh Gas-Chrom Q (Applied Science Laboratories, Inc.). The 8-BP liquid phase is a polyester produced from cyclohexanedimethanol succinate which can tolerate a maximum operating temperature of 250°. Gas-Chrom Q is a high quality silane-treated support used for steroids, pesticides, alkaloids, pharmaceuticals, and other compounds that require very inert supports.

Helium was used as the carrier gas, and the flow rate was monitored daily with a 10-ml soap film flowmeter and a stop watch. The flow rates of the hydrogen and compressed air to the flame unit were monitored in the same fashion.

The readout was a Houston Instruments model 5113-4 single pen recorder with a sensitivity of one millivolt full scale.

For most measurements the operating conditions were as listed below.

Injector temperature: 220°

Column oven temperature: 210°

Detector oven temperature: 300°

He flow rate: 55-65 ml/min.

H_2 flow rate: 20-25 ml/min.

Air flow rate: 300-350 ml/min.

In making any quantitative determinations, at least three injections were made for each sample.

ANALYTICAL STUDIES

Solutions

For each cyclopolymethylenetetrazole quantitatively determined, working curves were obtained by analyzing a series of solutions prepared by the appropriate dilution of a stock solution.

Chromatographic Response

The average detector response was determined in units of peak height and peak area. In the case of peak height, the average response was determined from the product of the chart deflection and the attenuation factor. When peak area was used, the average detector response was determined from the product of the chart deflection, the attenuation factor, and the peak width at half height.

Retention Times

The retention times were measured with a stop watch as the time from the leading edge of the solvent peak to the peak maximum of the sample.

DISTRIBUTION STUDIES

Two methods were used in studying the distribution of the cyclopolymethylenetetrazoles between water and carbon tetrachloride. In some cases, the tetrazole was extracted from an aqueous solution with carbon tetrachloride. In cases where the tetrazole was only sparingly soluble in water, the tetrazole was extracted from a carbon tetrachloride solution with water. A typical extraction procedure is described in the following paragraph.

Several aqueous stock solutions of the tetrazole were prepared (usually 0.1-0.5M). Five milliliters of carbon tetrachloride were then added to 5 ml of a stock solution in a glass stoppered 50-ml Erlenmeyer flask. The flask was placed in a constant temperature bath maintained at 25° and shaken for 30 minutes. The mixture was then transferred to a glass stoppered 15-ml centrifuge tube and centrifuged for a period of 5 minutes. The phases were separated by using a 5-ml syringe and stored in small snap-cap vials. The concentration of the tetrazole in each phase was then determined by gas chromatography.

It was established that the 30 minute shaking period

was sufficient time for the system to reach equilibrium by performing the reverse extraction (i.e., the tetrazole was extracted from a carbon tetrachloride solution with water). The results were the same as those obtained by extracting the tetrazole from an aqueous solution into carbon tetrachloride.

SOLUBILITY STUDIES

Enough of a given tetrazole was added to 5 ml of water to create a saturated solution. The solution was placed in the constant temperature bath at 25° and was gently shaken for a few hours. The temperature was then increased to 35°, and the solution was shaken overnight. The following morning the temperature was lowered to 25°, and solution was allowed to equilibrate. The solution was then analyzed by gas chromatography.

For the tetrazoles that were only sparingly soluble in water, the concentrations were determined by comparing the chromatographic response of the saturated solution with that of a standard solution of the given tetrazole. For the compounds that showed appreciable water solubility, the concentrations were determined in a similar manner; however, prior to the analysis it was necessary to dilute quantitatively the saturated solutions.

CRYOSCOPIC MEASUREMENTS

Molecular weight determinations were made by the usual freezing point depression method (108). A Beckmann thermometer calibrated to 0.001° was used, and a slush of dry ice and ethylene glycol was used as the cooling medium.

CHAPTER III

ANALYTICAL STUDIES OF THE CYCLOPOLYMETHYLENETETRAZOLES AND THEIR MIXTURES BY GAS CHROMATOGRAPHY

INTRODUCTION

Even though PMT has been used in anticonvulsant screening and in chemotherapy for many years, analytical techniques for the determination of small amounts of this drug fall short of the desired precision and accuracy (77-85).

The most sensitive methods thus far developed for the quantitative determination of PMT are based on gas chromatography. One of the first studies using this technique was reported by Kawamoto (87), who used a phenylmethylsilicone column with a thermal conductivity detector. The lowest concentrations of PMT detected were on the order of 1000 ppm. The most recent investigations employing gas chromatography for the determination of PMT have utilized flame ionization detection systems (88-90). Marcucci et al. (88) reported a method for determining brain levels of PMT which could detect as low as 50 ng of this drug. Recently, Stewart and Story (89) described a method for the determination of PMT in biological fluids. They used a column packed with 5% polyethylene glycol 20,000 on 80/100 mesh diatomaceous earth, and they were able to detect as little as 1 ppm of PMT.

Although this technique has proved successful for the analysis of PMT, there have been no reports on the quantitative determination of the other cyclopolymethylenetetrazoles. To study possible correlations between the physicochemical properties and convulsant activities of these compounds, it is necessary to have a method for

the analysis of these tetrazoles. Thus, the purpose of this investigation was to determine the chromatographic characteristics of several cyclopolymethylenetetrazoles and to determine the conditions for the routine quantitative determination of these compounds by gas chromatography.

RETENTION TIMES AND SENSITIVITIES

The retention time or volume is a property that is characteristic of the sample and the mobile phase of the column packing, and can therefore, be used to identify the sample. However, according to a simple approximation made by Giddings (109), the absolute retention time doubles for every 30° decrease in the column temperature. Thus it is necessary that retention times be reported relative to a standard. In these studies PMT was chosen as the standard, and all of the reported retention times have been made relative to that of PMT. The actual retention time of PMT is 210 seconds at a carrier gas flow rate of 60 ml/min. and an oven temperature of 210°.

The relative retention times were determined for single component solutions of the cyclopolymethylenetetrazoles and some cyclopolymethylenetetrazole derivatives in chloroform. The values obtained are listed in Table 7. A plot of the relative retention times versus molecular weight of the compounds studied is shown in Figure 13. The relative retention times increase exponentially as the length of

Table 7. Relative Retention Times and Sensitivities for the Cyclopolymethylenetetrazoles.

Tetrazole	Relative Retention Time	Relative Sensitivities Peak Height	Peak Area
Trimethylenetetrazole	0.99	0.16	0.16
Tetramethylenetetrazole	1.03	0.71	0.78
Pentamethylenetetrazole	1.00	1.00	1.00
Hexamethylenetetrazole	1.20	1.05	1.28
Heptamethylenetetrazole	1.59	0.93	1.41
Octamethylenetetrazole	1.96	0.92	1.71
Nonamethylenetetrazole	2.41	0.93	1.91
Undecamethylenetetrazole	4.95	0.50	2.13
8-tert-butylpentamethylenetetrazole	2.77	0.73	1.80
8-sec-butylpentamethylenetetrazole	3.09	0.66	1.75

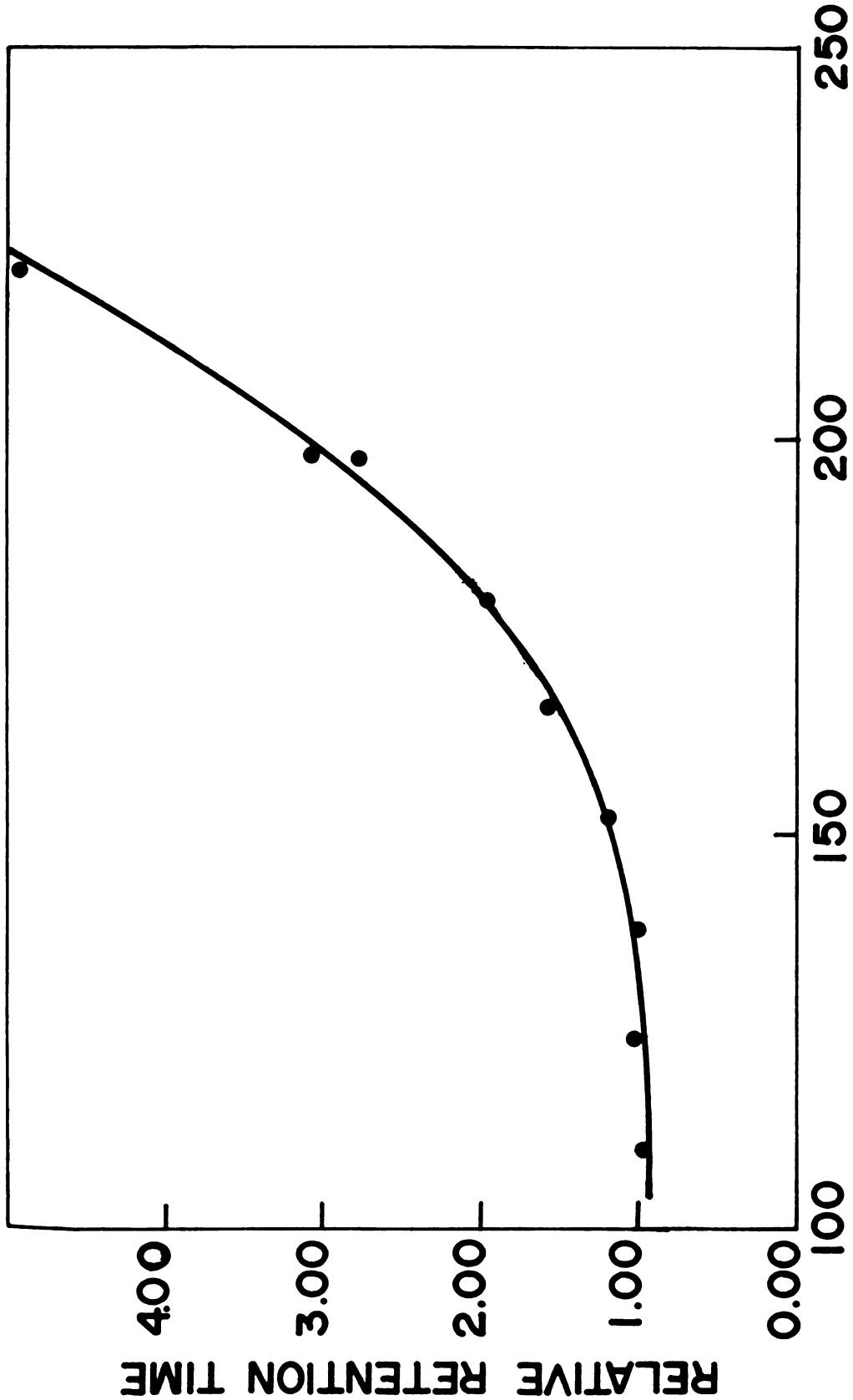


Figure 13. Plot of the relative retention time vs the molecular weight of the cyclopolymethylenetetrazoles.

the hydrocarbon chain increases. This same observation has been made for members of other homologous series such as paraffins, ketones, and esters (110). In this respect, therefore, the behavior of the cyclopolymethylenetetrazoles is that which is expected of any regular homologous series of compounds.

The chromatographic sensitivities of the cyclopolymethylenetetrazoles were measured for single component solutions in chloroform. These values, listed in Table 7, were determined by dividing the average detector response by the number of nanomoles of sample injected. Since changes in the instrumental parameters can greatly affect the detector response, the sensitivities are also reported relative to that of PMT. However, the relative detector response can be measured either in terms of peak height or peak area; but since the concentration of the sample is proportional to the peak area, one should probably give more significance to the values determined on this basis.

Upon examining the relative sensitivities based on peak area in Table 7, a near linear increase is observed as methylene groups are added to the hydrocarbon chain. The glaring exception is trimethylenetetrazole which has a very low value of 0.16. This compound was investigated in more detail, and it was determined that trimethylenetetrazole was undergoing thermal decomposition. By lowering the temperature of the injection port to 175°, this

decomposition was reduced, but was not completely eliminated.

RESOLUTION OF A MIXTURE OF TETRAZOLES

A solution containing a mixture of the cyclopolymethylenetetrazoles, each present at a concentration of 0.0138 grams per 10 ml of chloroform, was prepared to determine how well the individual components could be resolved. As can be seen in Figure 14, baseline resolution is achieved in most cases, and the only problem encountered is that trimethylenetetrazole, tetramethylenetetrazole, and pentamethylenetetrazole are all eluted at the same time. This behavior was not unexpected since the relative retention times of these compounds are very similar. Thus, to separate these three tetrazoles, one must either use a different column packing or reduce the temperature of the column oven.

The other cyclopolymethylenetetrazoles are quite well resolved, however, and the relative retention times measured for the components in the mixture are identical to those measured for the single component solutions previously discussed. Thus, the retention times are not influenced by the presence of other species in solution and can be used to identify qualitatively these cyclopolymethylenetetrazoles.

Figure 14. Chromatogram of a mixture of cyclopolymethylenetetrazoles. Sample: 2.0 μl of C_3 - C_{11} tetrazoles, each present at a concentration of 0.0138 g/10 ml of chloroform. Injector: 220°; Column: 210°; Detector: 300°; He at 60 ml/min; Sensitivity: 128 x 10^{-11} a.f.s.

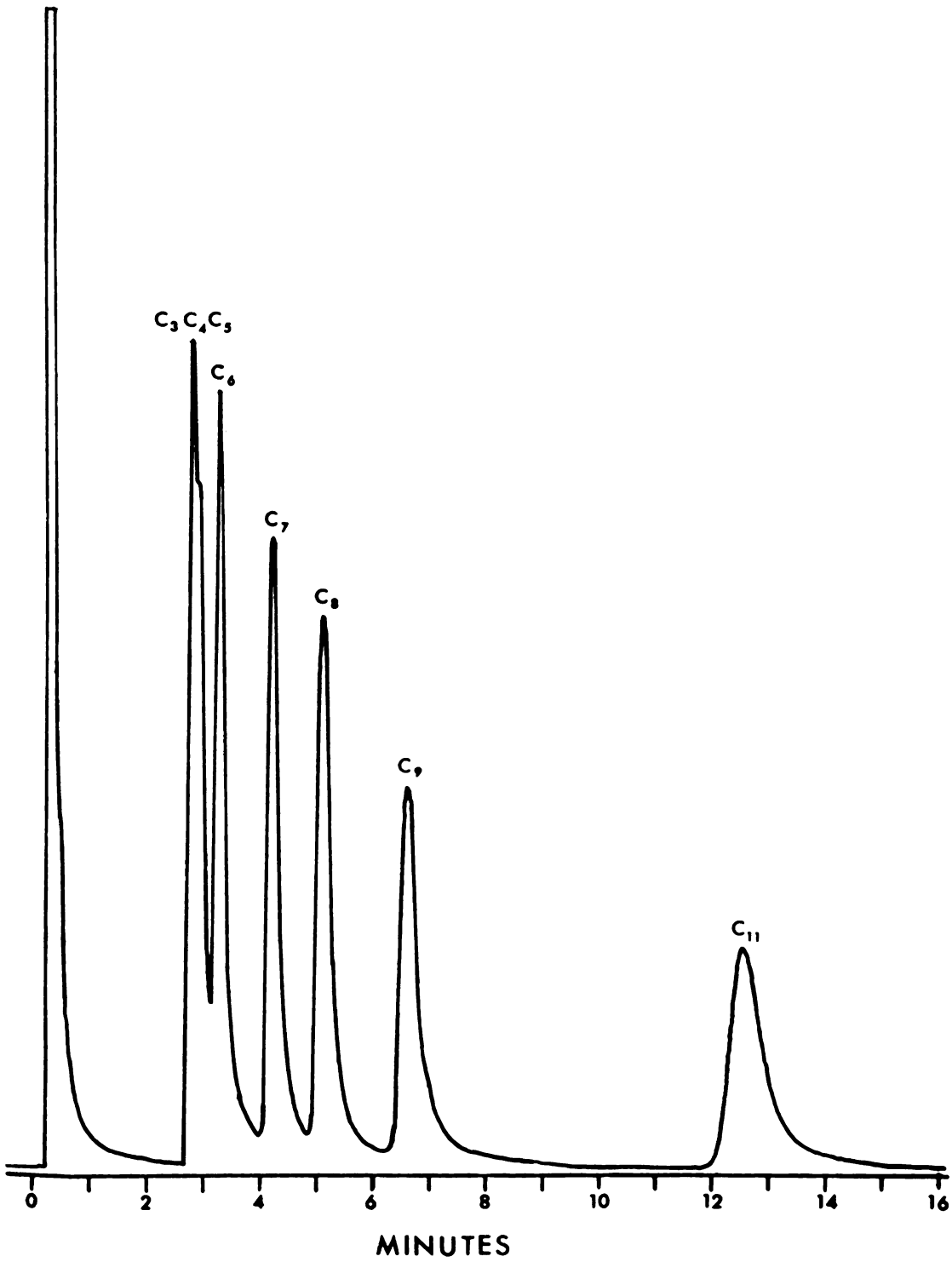


Figure 14

WORKING CURVES

To determine the cyclopolymethylenetetrazoles quantitatively, it was necessary to construct working curves for each of these compounds. It was observed that for tetrazole solutions up to a concentration of 0.1 M, the peak shape was symmetric and reproducible, with no broadening at the higher concentrations. Thus, the calibration curves were prepared by plotting the average detector response (in units of peak height) versus the concentration of the tetrazole.

A typical working curve is illustrated in Figure 15. In this case the average detector response was monitored as a function of the PMT concentration. The PMT concentration ranged from millimolar to 0.1 M, and as can be observed in the figure, the plot is linear over this concentration range. Similar working curves have been obtained for the other cyclopolymethylenetetrazoles in this concentration range.

At concentrations greater than 0.1 M, however, the curves begin to deviate from linearity. This behavior is illustrated in Figure 16, where a working curve for heptamethylenetetrazole is shown. In this study the concentration ranged from 0.02 to 0.5 M. As can be seen in the figure, at the higher concentrations, the curve bends towards the concentration axis. Despite this deviation from linearity, these calibration plots are still useful

Figure 15. Plot of average detector response vs concentration of pentamethylenetetrazole in aqueous solution.

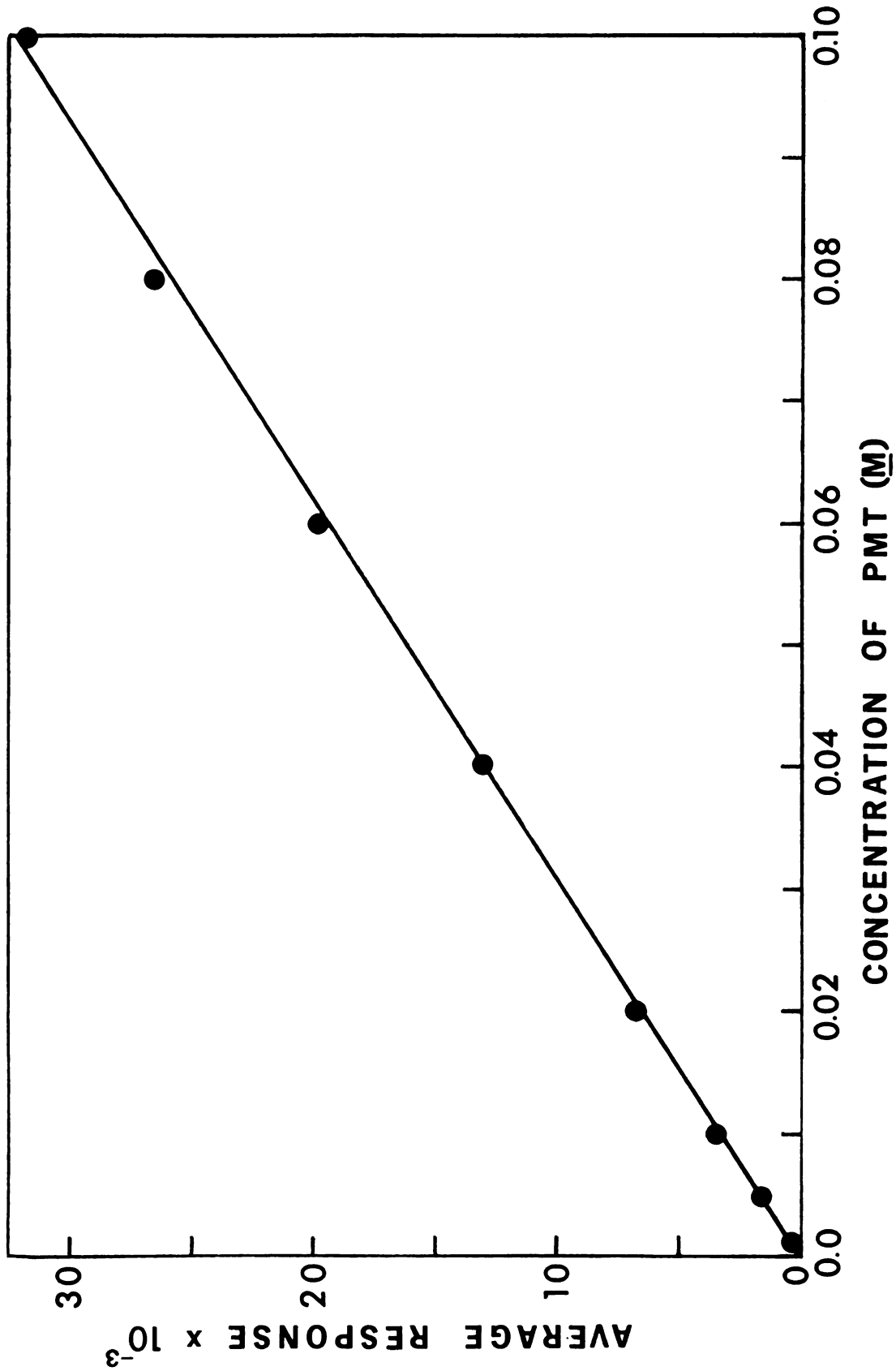

AVERAGE RESPONSE x 10^{-3}
0
10
20
30
0.0
0.02
0.04
0.06
0.08
0.10
CONCENTRATION OF PMT (M)

Figure 15

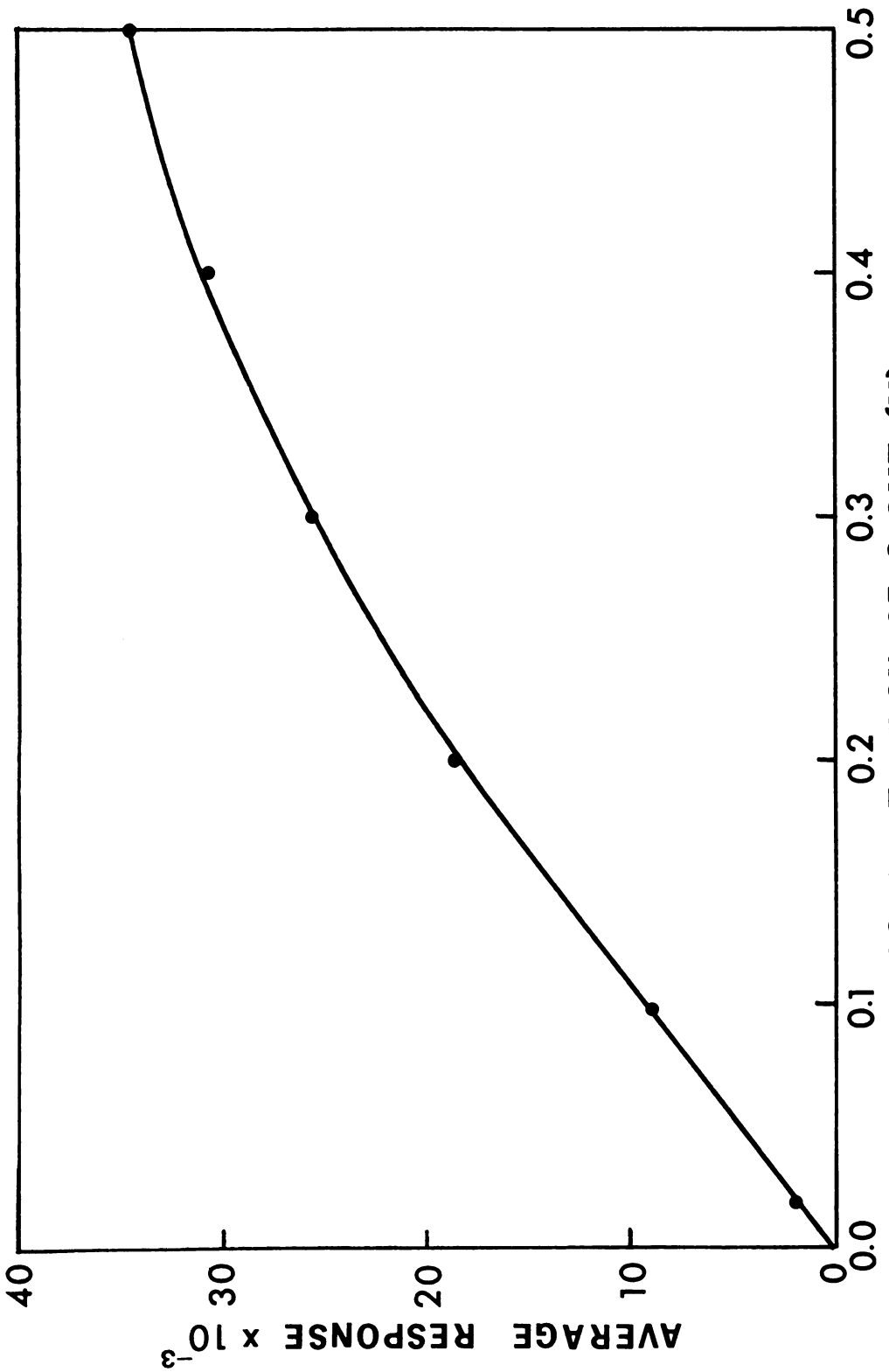


Figure 16. Average detector response vs concentration of heptamethylenetetrazole in carbon tetrachloride solution.

for the analysis of tetrazoles at these higher concentrations.

As a result of this investigation, a gas chromatographic method for the routine analysis of the cyclopolymethylenetetrazoles at the 50-100 ppm level has been developed, and the technique is capable of determining concentrations of less than 10 ppm.

CHAPTER IV

SOLUBILITY AND DISTRIBUTION STUDIES OF THE CYCLOPOLYMETHYLENETETRAZOLES

SOLUBILITY STUDIES

The solubilities of several cyclopolymethylenetetrazoles were determined in aqueous solutions, and the data are presented in Table 8. As expected, an increase in the number of carbon atoms in the polymethylene chain generally decreases the solubility in aqueous solution. The glaring exception, however, is pentamethylenetetrazole which is soluble in water up to a concentration of 5.0 *m*.

Table 8. Solubilities of some Cyclopolymethylenetetrazoles

Tetrazole	Molal Solubility	Solubility in g/ml
Trimethylenetetrazole	1.4 *m*	0.16
Pentamethylenetetrazole	5.0	0.69
Heptamethylenetetrazole	0.18	0.031
8-*sec*-butylpentamethylenetetrazole	0.0052	0.0010
8-*tert*-butylpentamethylenetetrazole	0.0029	0.00057

It has been reported previously that PMT is essentially completely miscible with water, as viscous solutions with concentrations up to 750 grams of PMT per 100 grams of water were obtained (111). The present study indicates that aqueous solutions of PMT have a strong tendency to supersaturate.

It is also interesting to note the solubilities of the two isomeric pentamethylenetetrazole derivatives, namely 8-sec-butylpentamethylenetetrazole (8-sec-butyl PMT) and 8-tert-butylpentamethylenetetrazole (8-tert-butyl PMT). While the structural differences between the two compounds are very minor, the water solubility of 8-sec-butyl PMT is nearly twice that of the other isomer.

DISTRIBUTION STUDIES

Since there is a possibility that the physiological action exhibited by the cyclopolymethylenetetrazoles may be due to an interaction of the tetrazole with a lipid membrane, it was decided to study the distribution of these tetrazoles between aqueous solutions and lipid solvents.

In addition to investigating the series of cyclopolymethylenetetrazoles, it was also of interest to study some pentamethylenetetrazole derivatives. The compounds 8-sec-butyl PMT and 8-tert-butyl PMT were of particular interest because the convulsant activities of these two tetrazoles were reported by Gross and Featherstone (112) to be 750 milligrams per kilogram of body weight for 8-sec-butyl PMT and only 3 milligrams per kilogram of body weight for 8-tert-butyl PMT. Thus a small change in the nature of the substituent group greatly affects the pharmacological activity of these compounds. However, it was

recently shown that the convulsant activity of 8-sec-butyl PMT is 50 milligrams per kilogram of body weight rather than the 750 milligrams that was reported previously (Appendix II).

An appropriate solvent for these distribution studies should have the solvent properties of lipids and have a very low water content on saturation. Carbon tetrachloride fits these requirements. In addition it is readily available and can be purified easily. Thus carbon tetrachloride was selected as the nonaqueous solvent for these studies.

The distribution ratios of pentamethylenetetrazole, hexamethylenetetrazole (C_6CMT), and heptamethylenetetrazole (C_7CMT) between water and carbon tetrachloride solutions were determined at several concentrations. The distribution ratio, D, is defined as the total concentration of the tetrazole in the organic phase divided by the total concentration of the tetrazole in the aqueous phase. The results are listed in Table 9.

In all three cases the distribution ratio is somewhat concentration dependent. Thus for all three of these compounds, this concentration dependence may be an indication that some reaction such as dimerization is occurring in one or both of the phases. As can be seen in Figure 17, the distribution ratio for PMT increases gradually from 0.18 to 0.33 over the concentration range of 0.1 to 0.5 M. Linear plots of the distribution ratio versus the concentration of the tetrazole are obtained for C_6CMT and C_7CMT

Table 9. Distribution Ratios of Pentamethylenetetrazole, Hexamethylenetetrazole, and Heptamethylenetetrazole at Various Concentrations.

Tetrazole	Conc. Before Extraction	Conc. in CCl_4 Phase	Conc. in H_2O Phase	Distribution Ratio
Pentamethylenetetrazole	0.1 M	0.0146	0.0838	0.17±0.01
	0.2	0.0365	0.165	0.22±0.01
	0.3	0.0608	0.239	0.25±0.01
	0.4	0.0935	0.306	0.30±0.02
	0.5	0.125	0.370	0.33±0.01
Hexamethylenetetrazole	0.1	0.0410	0.057	0.72±0.04
	0.2	0.100	0.095	1.05±0.04
	0.3	0.175	0.123	1.42±0.05
	0.4	0.250	0.140	1.79±0.04
	0.5	0.345	0.161	2.14±0.05
Heptamethylenetetrazole	0.1	0.0758	0.0223	3.40±0.3
	0.2	0.175	0.0320	5.47±0.2
	0.3	0.264	0.0376	7.02±0.3
	0.4	0.345	0.0430	8.02±0.1
	0.5	0.433	0.0459	9.43±0.3

Figure 17. Plot of the distribution ratio vs the concentration of pentamethylenetetrazole for the water-carbon tetrachloride system.

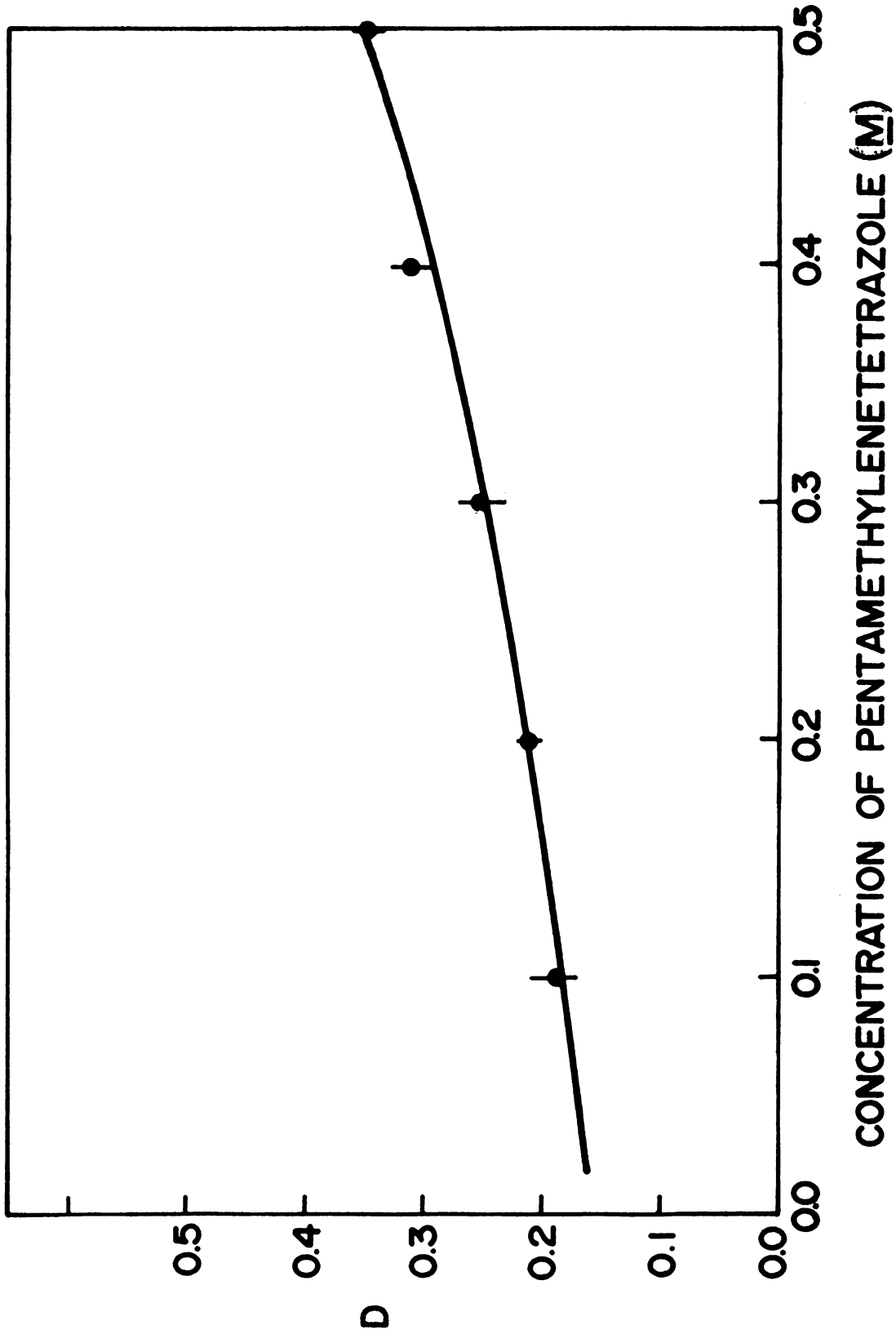

D
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.1
0.2
0.3
0.4
0.5
CONCENTRATION OF PENTAMETHYLENETETRAZOLE (M)

Figure 17

(Figures 18 and 19).

Distribution studies were also carried out on some other cyclopolymethylenetetrazoles. In the case of 8-tert-butyl PMT, the compound was only soluble to a concentration of 0.05 M in carbon tetrachloride and only slightly soluble in water. Due to this limited solubility it was very difficult to determine the concentrations of the tetrazole in the aqueous and organic phases. In this case the distribution ratios were determined from the chromatograms as the average detector response of the organic phase divided by the average detector response of the aqueous phase. The results are listed below in Table 10, and a plot of the distribution ratio versus concentration is shown in Figure 20. Once again, the distribution ratio is dependent on the concentration of the tetrazole.

Table 10. Distribution Ratios for 8-tert-butylpentamethylenetetrazole between Water and Carbon Tetrachloride

Concentration Before Extraction	Distribution Ratio
0.005 M	26.1
0.02	28.1
0.04	31.9
0.05	35.6

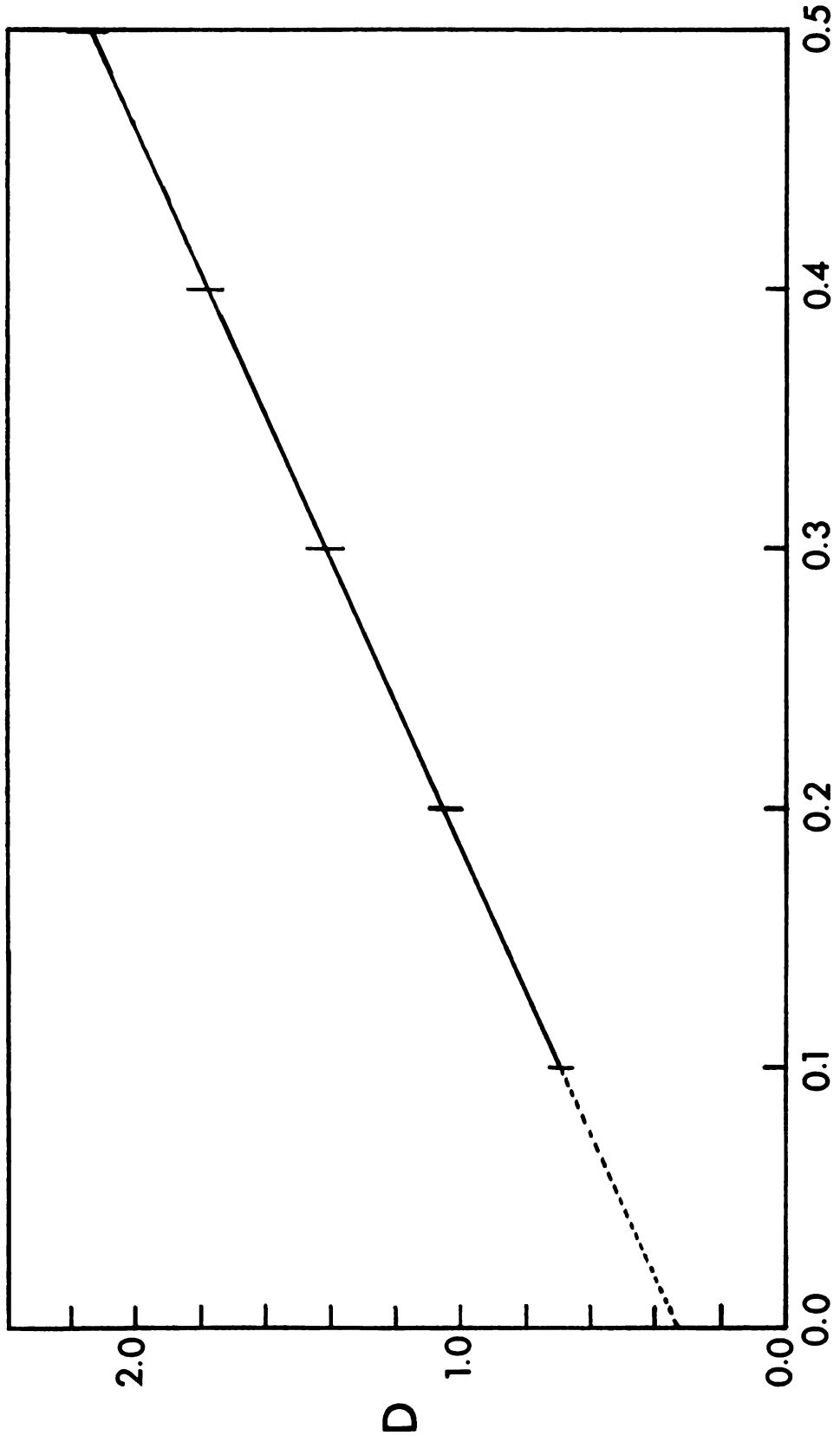


Figure 18. Distribution ratio vs concentration of hexamethylenetetrazole for the water-carbon tetrachloride system.

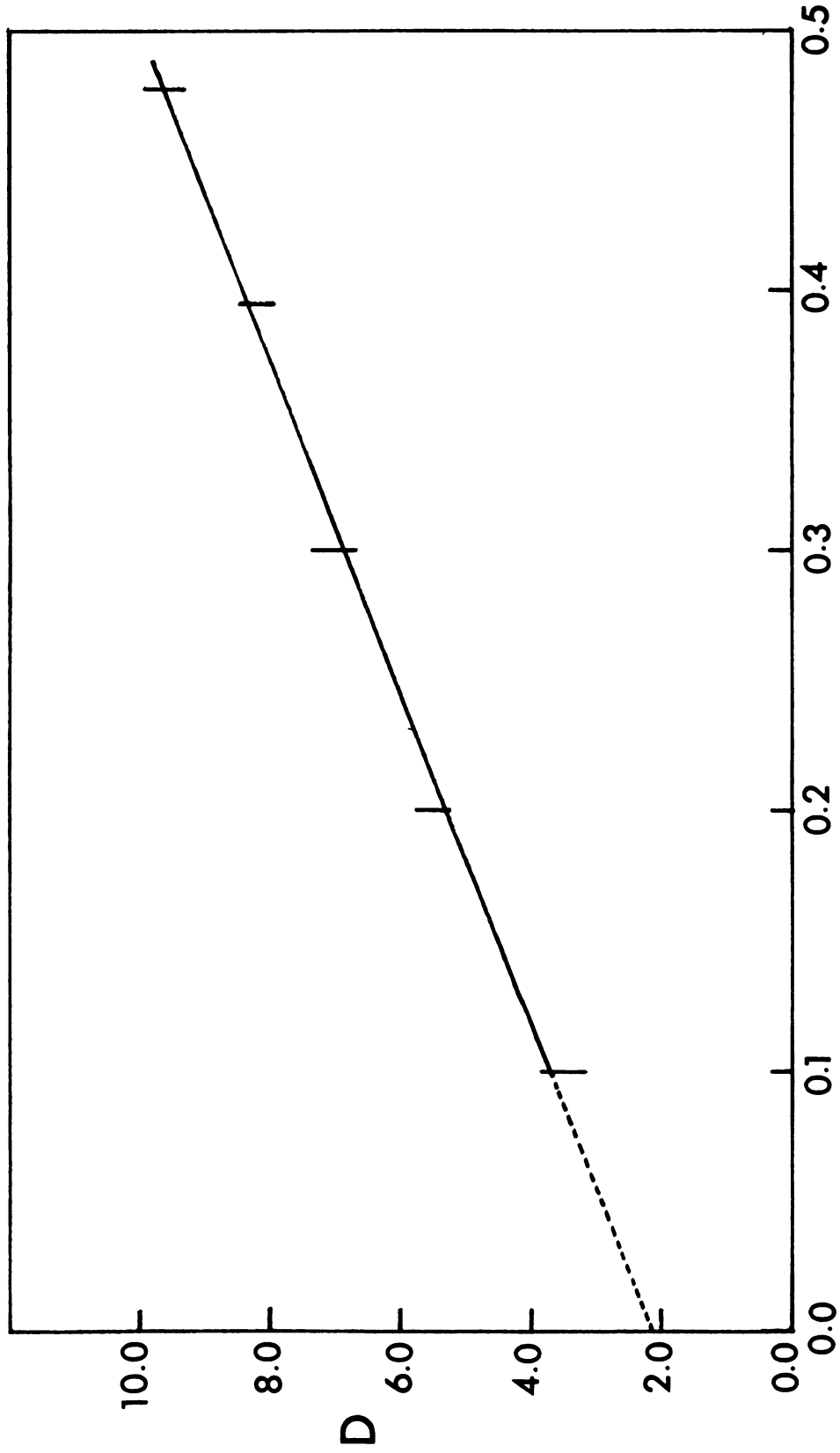


Figure 19. Distribution ratio vs concentration of heptamethylenetetrazole for the water-carbon tetrachloride system.

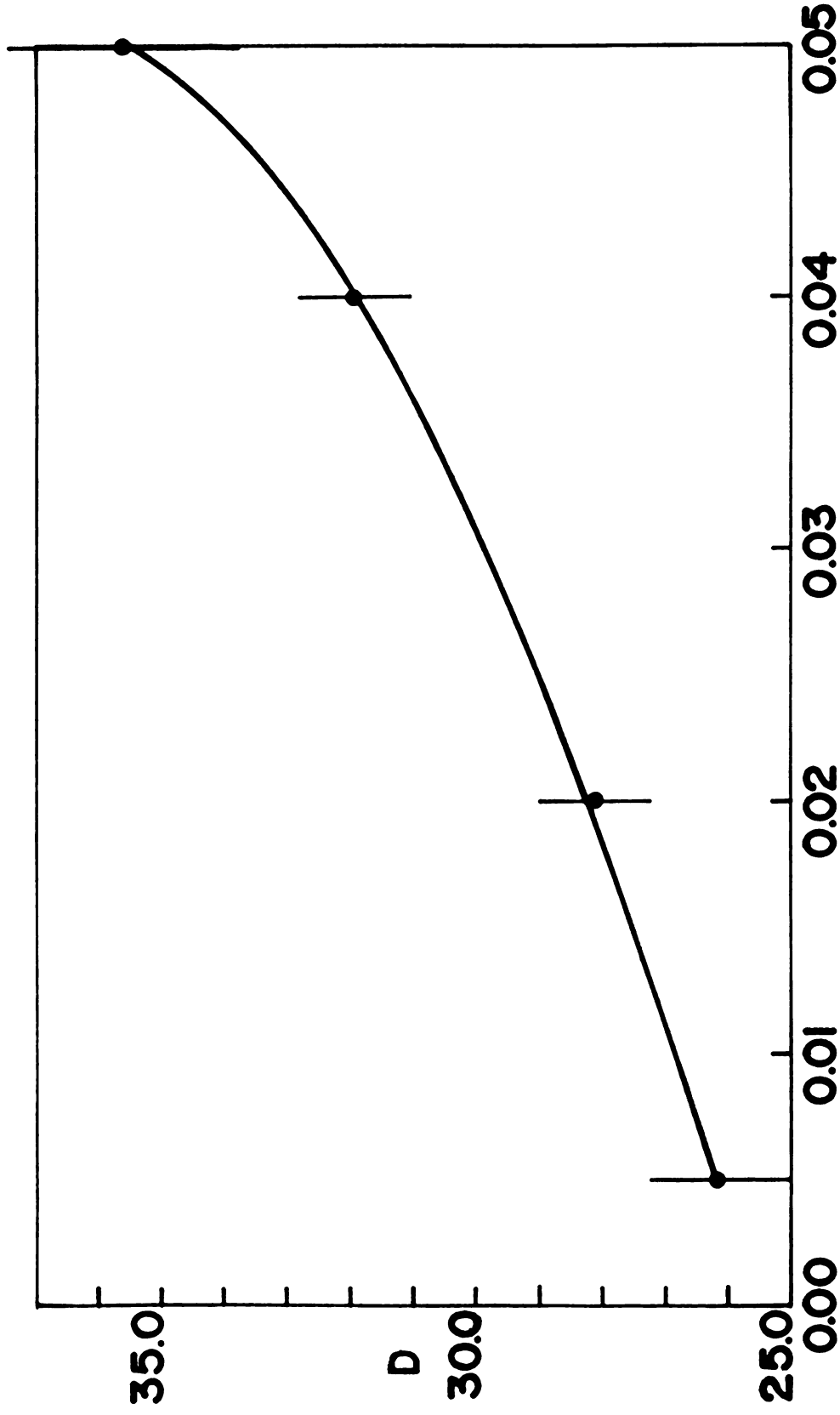


Figure 20. Distribution ratio vs concentration of 8-tert-butylpentamethyleneteterazole for the water-carbon tetrachloride system.

Attempts were also made to determine the distribution ratios for trimethylenetetrazole (C_3CMT) and 8-sec-butyl PMT. In the case of C_3CMT, the distribution ratio appeared to be extremely small, which, when coupled with the very low chromatographic sensitivity of trimethylenetetrazole, made it very difficult to analyze the organic phase. However, from the studies performed it was estimated that the distribution ratio for C_3CMT is $\sim$0.0014 at a concentration of 0.20 M.

On the other hand, the distribution ratio for 8-sec-butyl PMT was so large that it was difficult to analyze the aqueous phase. It was estimated that the distribution ratio for 8-sec-butyl PMT is on the order of 220 at a concentration of 0.20 M.

DISCUSSION OF RESULTS

A summary of the distribution ratios of the various cyclopolymethylenetetrazoles for the water-carbon tetrachloride system is shown in Table 11. In comparing the values for trimethylenetetrazole through heptamethylenetetrazole, it is observed that the distribution ratio increases as methylene groups are added to the hydrocarbon chain. This relationship was not unexpected since it was previously reported that in a homologous series of compounds, the partition coefficient usually increases by a factor of two to four for each additional methylene group (97,98).

It is also interesting to note that at least for PMT,

Table 11. Distribution Ratios of some Cyclopolymethylene-tetrazoles between Water and Carbon Tetrachloride.

Tetrazole	Conc. Before Extraction	Distribution Ratio
Trimethylenetetrazole	0.20 M	0.0014
Pentamethylenetetrazole	0.20	0.22
Hexamethylenetetrazole	0.20	1.05
Heptamethylenetetrazole	0.20	5.47
8-tert-butylpentamethylenetetrazole	0.05	35.6
8-sec-butylpentamethylenetetrazole	0.20	220

C_6CMT, and C_7CMT, the distribution ratio depends on the concentration of the tetrazole. Thus for all three compounds, it appears that some kind of association reaction may be occurring. In order to describe quantitatively the partitioning of these compounds, it was of interest to determine the nature of this reaction.

The possibility of an acid-base reaction in the aqueous phase can be excluded since the addition of cyclopolymethylenetetrazoles to an aqueous solution does not result in any change of the pH of the solution (93). Therefore, the only possible reaction involves the formation of dimers or higher aggregates in one or both of the liquid phases.

To simplify the calculations it was first assumed that the variation of the distribution ratio with concentration was due to the formation of dimers in the organic phase. It was also assumed that only the tetrazole monomer was being transferred from one phase to the other.

The dimerization reaction can be represented by the equilibrium

$$2(Tz)^{org} \rightleftarrows (Tz)_2^{org} \qquad (6)$$

in which $(Tz)^{org}$ and $(Tz)_2^{org}$ represent the concentration of the tetrazole monomer and dimer, respectively, in the organic phase. The equilibrium conconstant for this reaction is given by

$$K_{dimer.}^{org} = \frac{(Tz)_2}{(Tz)^2} \qquad (7)$$

From the assumption that dimers are formed only in the organic phase, the distribution ratio, D, which is defined as the total concentration of tetrazole in the organic phase (C_t^{org}) divided by the total concentration of tetrazole in the aqueous phase (C_t^{aq}), can be represented by the equation

$$D = \frac{C_t^{org}}{C_t^{aq}} = \frac{(Tz)^{org} + (Tz)_2^{org}}{(Tz)^{aq}} \qquad (8)$$

The actual partition coefficient, P, which is defined as the concentration of the tetrazole monomer in the organic phase divided by the concentration of the tetrazole monomer in the aqueous phase and which should be independent of concentration, is given by

$$P = \frac{(Tz)^{org}}{(Tz)^{aq}} \qquad (9)$$

From the above equations, the following relation can be derived easily,

$$D = 2\ K_{dimer.}^{org}\ P^2\ (Tz)^{aq} + P \qquad (10)$$

which relates the distribution ratio to the dimerization equilibrium constant and to the partition coefficient. If the assumptions are valid, a plot of the distribution

ratio versus the concentration of the tetrazole in the aqueous phase should be a straight line. The intercept should yield the partition coefficient, and from the slope, the value of $K^{org}_{dimer.}$ can be calculated.

In the case of PMT, the plot of D versus the concentration of PMT in the aqueous phase yields a straight line as can be seen in Figure 21. From the intercept the value of the partition coefficient was determined to be 0.12, and from the slope, $K^{org}_{dimer.}$ was calculated to be 21.

However, in the cases of C_6CMT and C_7CMT, the plots were non-linear. It appears, therefore, that a different explanation for this concentration dependence of the distribution ratio must be sought.

The state of aggregation of the solute species in aqueous solutions was determined by cryoscopic measurements. The results of these studies are shown in Table 12.

The molecular weights obtained for PMT in aqueous solutions indicate that some association is occurring in solutions in which the PMT concentration is $\geq$ 0.5 m. Thus it appears that in this case the assumption that dimerization occurs only in the organic phase is not a valid one. These results agree with those previously reported by Erlich (111). In studying solutions of PMT by vapor phase osmometry, he noted that polymers, or at least dimers, were formed in aqueous solution. Thus the partitioning of PMT between water and carbon tetrachloride is most

Figure 21. Plot of the distribution ratio vs the concentration of pentamethylenetetrazole in the aqueous phase for the water-carbon tetrachloride system.

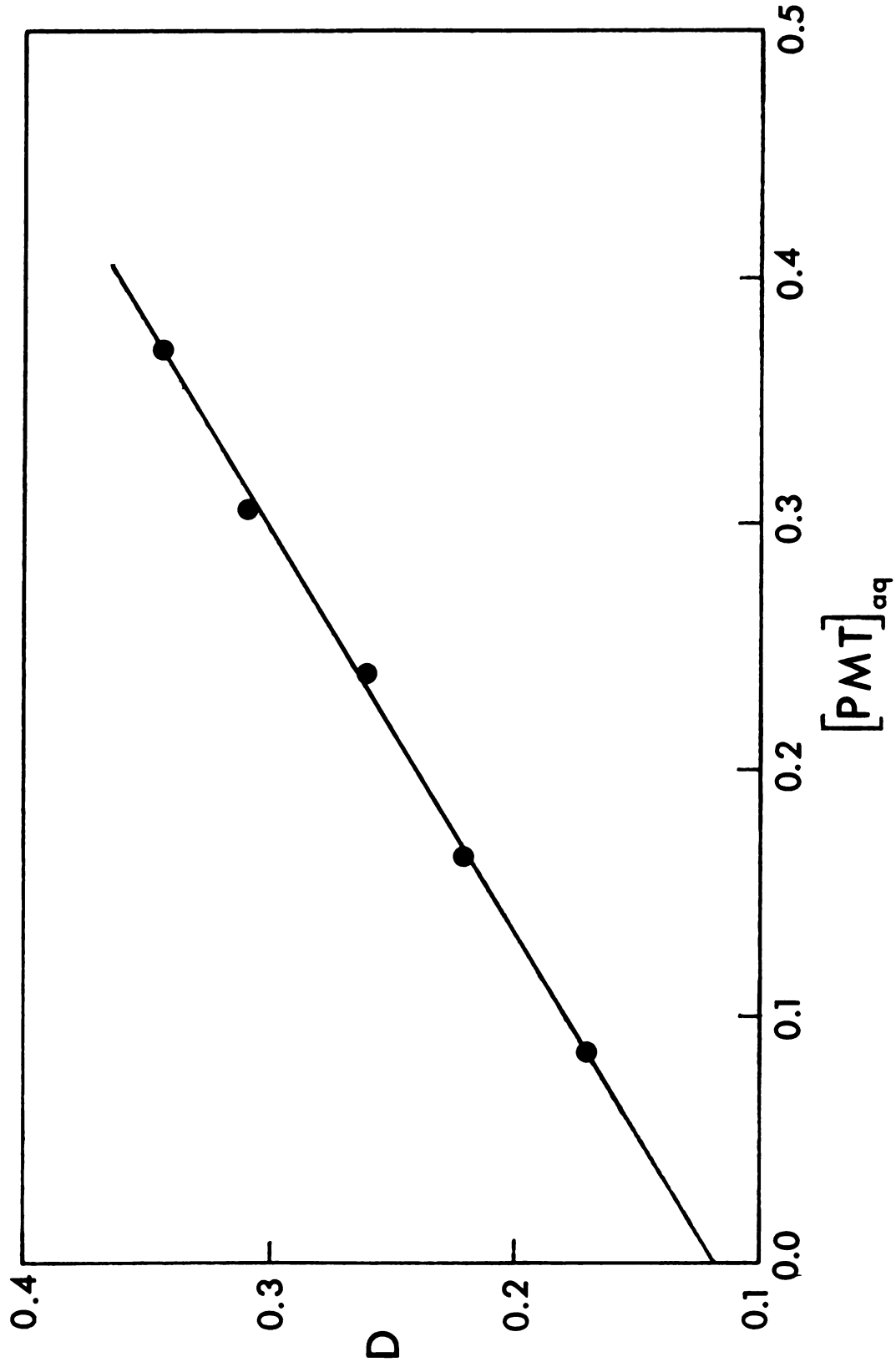


Figure 21

Table 12. Molecular Weights of some Cyclopolymethylenetetrazoles in Aqueous Solution as Determined by Cryoscopic Measurements.

Tetrazole	Conc. (g/kg H_2O)	ΔT_f (°C)	Observed Molality (m)	Observed Molec. Wt.	Actual Molec. Wt.
Trimethylenetetrazole	55.67	0.888	0.477	117	110.10
	109.25	1.463	0.787	139	
Pentamethylenetetrazole	14.02	0.198	0.106	132	138.18
	68.87	0.772	0.388	177	
	71.07	0.777	0.418	170	
	71.18	0.754	0.405	176	
	137.10	1.165	0.626	219	
Hexamethylenetetrazole	24.79	0.326	0.175	142	152.20
	24.79	0.302	0.162	153	
Heptamethylenetetrazole	7.77	0.090	0.0484	161	166.23

likely a very complicated system which involves several complex equilibria, however the calculated partition coefficient of 0.12 is probably a reasonable estimate of the actual partition coefficient.

For hexamethylenetetrazole and heptamethylenetetrazole, however, the cryoscopic data do not indicate that any association is occurring in the aqueous phase. Thus for these compounds, the assumption that no association is occurring in the aqueous phase appears to be valid; but as noted previously, however, the distribution data for C_6CMT and C_7CMT do not fit the derived equation that involved the formation of dimers in the organic phase (Equation (10)). It was then assumed that these compounds formed primarily trimers in the organic phase. The trimerization reaction is given by

$$3(Tz)^{org} \rightleftarrows (Tz)_3^{org} \qquad (11)$$

with the corresponding equilibrium constant

$$K^{org}_{trimer.} = \frac{(Tz)_3}{(Tz)^3} \qquad (12)$$

From these equations and from Equations (8) and (9), the following relationship was derived.

$$D = 3\, K^{org}_{trimer.}\, P^3\, (Tz)^2_{aq} + P \qquad (13)$$

If Equation (13) is valid, a plot of the distribution ratio versus the square of the concentration of the tetrazole in the aqueous phase should produce a straight line. The intercept should be equal to the partition coefficient, P, and the value for $K^{org}_{trimer.}$ could be calculated from the slope.

For both C_6CMT and C_7CMT, plots of D versus $(Tz)^2_{aq}$ yield very good straight lines as can be observed in Figures 22 and 23, respectively. In the case of C_6CMT the partition coefficient was determined to be 0.5 and $K^{org}_{trimer.}$ was calculated to be 170; while for C_7CMT the partition coefficient was found to be 1.6 and the trimerization equilibrium constant was determined to be 310.

For these two tetrazoles, since the distribution ratio increases linearly with increasing tetrazole concentrations (Figures 19 and 20), there is another method by which the partition coefficient could be estimated. This method involves the extrapolation of the line back to zero concentration. At this point the distribution ratio and the partition coefficient, P, should be equal.

In this manner, the partition coefficients were determined to be 0.34 for C_6CMT and 2.1 for C_7CMT. These values compare quite favorably to those obtained by using Equation (13), as can be seen in Table 13.

Figure 22. Plot of the distribution ratio vs the square of the concentration of hexamethylenetetrazole in the aqueous phase for the water-carbon tetrachloride system.

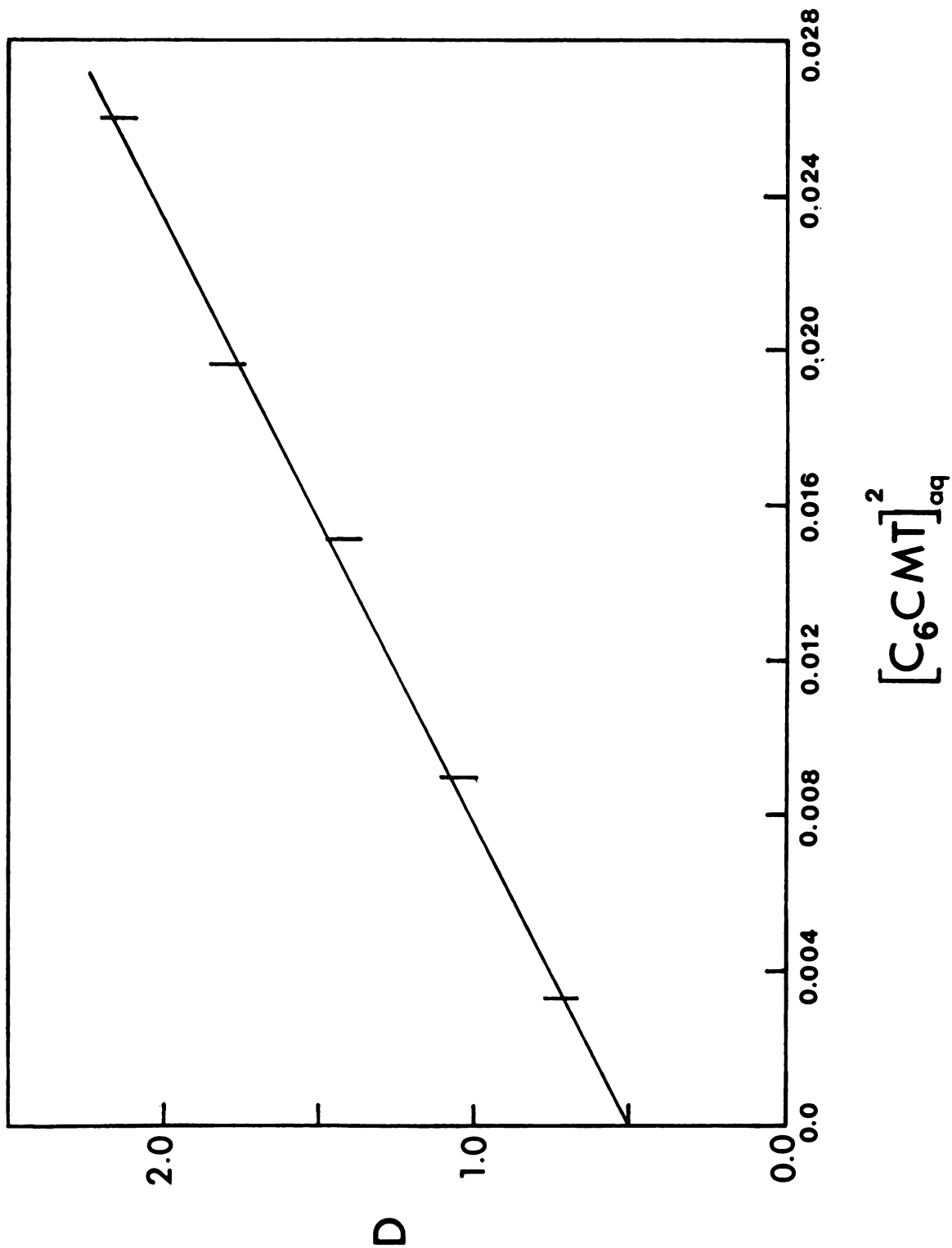


Figure 22

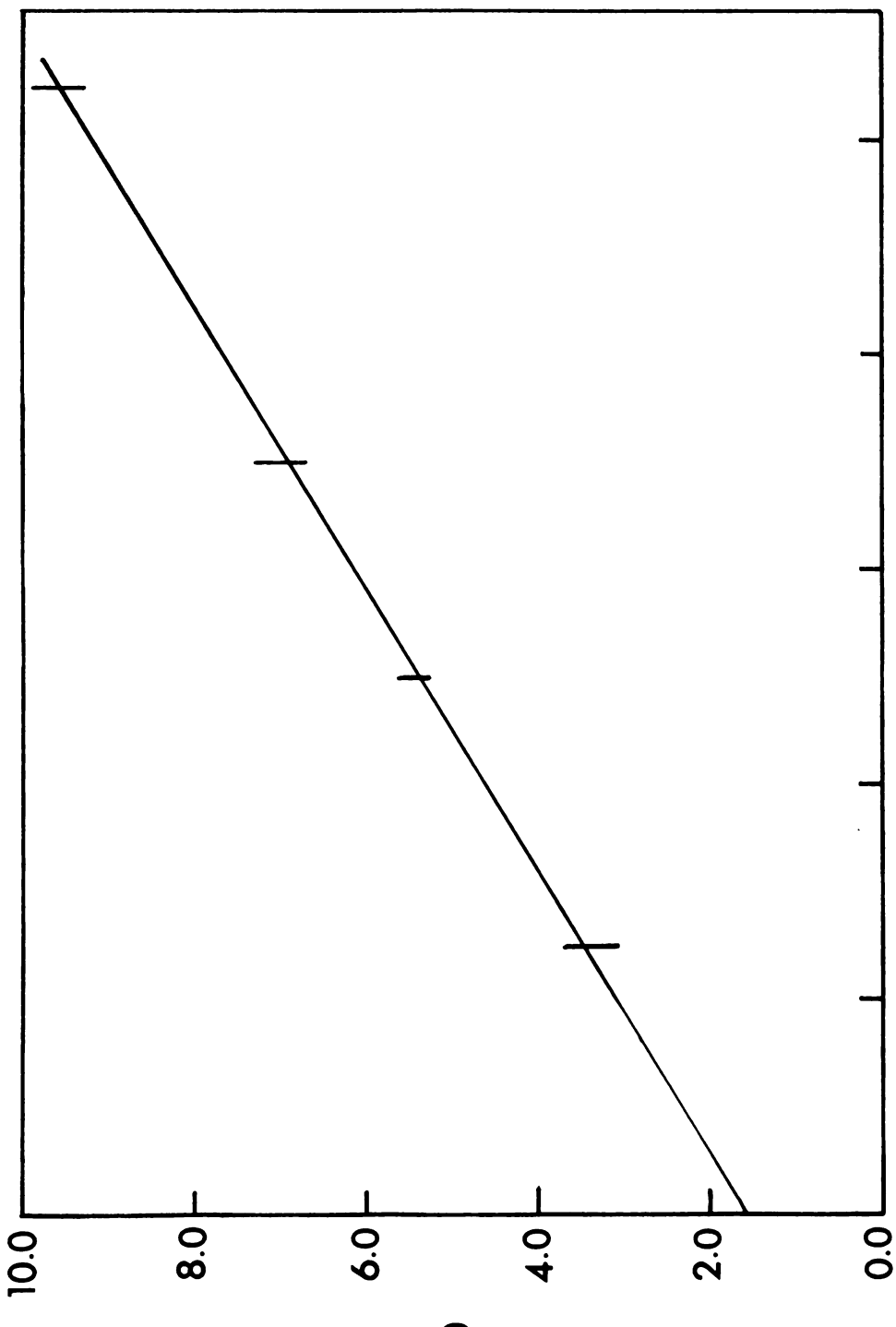


Figure 23. Distribution ratio vs the square of the concentration of heptamethyleneetetrazole in the aqueous phase for the water-carbon tetrachloride system.

Table 13. Partition Coefficients for Hexamethylenetetrazole and Heptamethylenetetrazole.

Tetrazole	P Obtained by Extrapolation	P Obtained by Equation (13)
Hexamethylenetetrazole	0.34	0.5
Heptamethylenetetrazole	2.1	1.6

Since these results are in fairly good agreement, it would seem that the values of the partition coefficients for C_6CMT and C_7CMT between water and carbon tetrachloride obtained in this investigation are, indeed, good estimates of the actual partition coefficients.

However, whether or not these compounds actually form trimers in the organic phase is still not entirely clear, as no supporting data have been obtained. Moreover, a mechanism has yet to be postulated for the formation of trimers. Thus additional investigations are in order so that the nature of the solute species in carbon tetrachloride solutions may be determined.

Although the results obtained in this investigation did not lead to any definite correlations with the pharmacological properties of the cyclopolymethylenetetrazoles, it would be interesting to extend these distribution studies to include other solvent systems to determine if any such correlations exist.

APPENDICES

APPENDIX I

DESCRIPTION OF PROGRAM RAMAN AND THE ACCOMPANYING INTERFACE FOR THE SPEX RAMALOG 4 RAMAN SPECTROMETER

DESCRIPTION OF PROGRAM RAMAN AND THE ACCOMPANYING INTERFACE FOR THE SPEX RAMALOG 4 RAMAN SPECTROMETER

RAMAN is a program written in FORTRAN and SABRE designed to acquire data from the Spex Ramalog 4 Laser-Raman spectrometer equipped with the appropriate interface. The program was written and the interface designed by Patrick M. Kelly of this department. The program features include:

1. acquisition of up to 500 data points at 0.1 or 1.0 wavenumber intervals;
2. signal averaging of each data point;
3. correction for baseline drift;
4. an option of integrating any spectral bands;
5. presentation of data in tabular form.

INTERFACE

Triggering Interface

The Spex Ramalog 4 spectrometer has a wavenumber encoder which makes a 2.5 v pulse available at a rate of one pulse per tenth wavenumber. The frequency of the pulses during a scan is further divided by using a series of decade counters so a pulse may be obtained every ten

or one hundred wavenumbers. This interface taps the appropriate decade counter in order that a pulse can be obtained either at a rate of one per wavenumber or one per tenth wavenumber. These pulses are then used to trigger a Schmidt trigger which initiates A/D conversion at the appropriate time. The Schmidt trigger is then reset under software control. The two encoder signals are used to trigger data acquisition in the following manner:

Mode 1: Data acquisition at 0.1 cm^{-1} intervals

Since the spectrometer is usually scanned at a rate of 20 wavenumbers per minute, encoder pulses will never come faster than a frequency of 3.3 Hz in this mode of operation. At each pulse a positive going signal will fire the Schmidt trigger which initiates A/D conversion. The program accepts the conversion and loops back through the conversion routine 24 additional times and finally computes the average. The Schmidt trigger is then reenabled and the program is readied for the next set of 25 points to be taken. Each conversion takes about 100 μsec. Consequently, the total acquisition time is approximately 2.5 msec, thus leaving over 300 msec for the computer to average and process the data and get set for the next set of 25 points.

Mode 2: Data acquisition at 1.0 cm^{-1} intervals

In this mode, the triggering pulse comes at a frequency of 0.33 Hz. The method of data acquisition is identical

to that used in Mode 1.

ADC Interface

Since the largest analog signal available at the recorder is approximately 10 mv, the signal must be amplified to give a significant ADC reading. This is accomplished through the use of a 141A operational amplifier with a gain of approximately 75. This supplies a 0.75 v signal to the ADC for a 10 mv signal at the recorder.
CAUTION: The voltage input to the ADC must fall between -1.00 v and +1.00 v. Voltages outside these limits will result in meaningless data points.

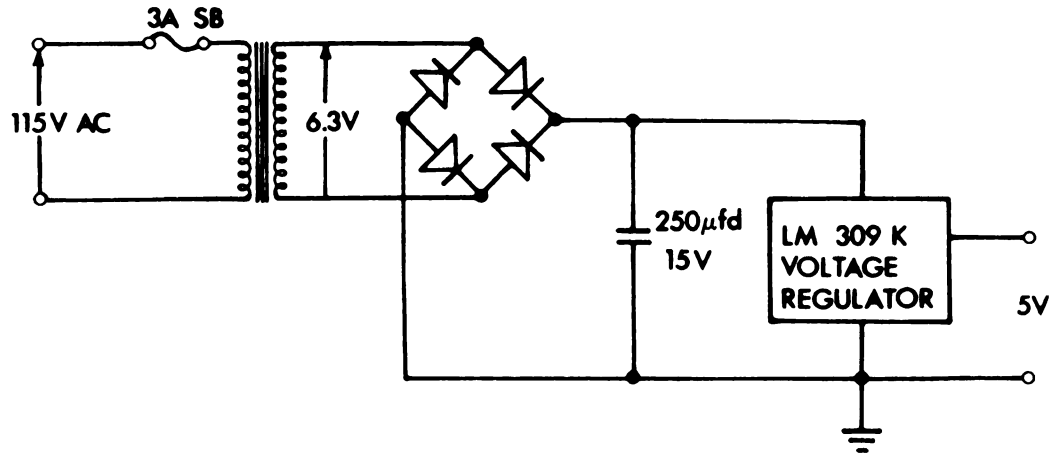
Since sufficient digital noise filtering cannot be accomplished in the program, the amplifier is fitted with a low pass filter to eliminate the large amount of noise produced in the photon counting process.

The triggering interface and its power supply are shown in Figure A1, while the ADC interface and its power supply are illustrated in Figure A2.

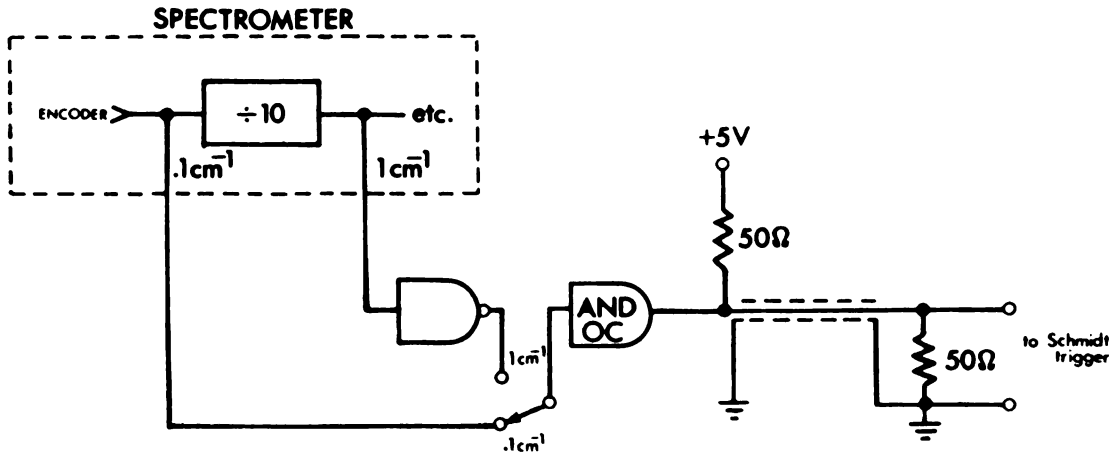
OPERATION OF PROGRAM RAMAN

Enabling OS/8

1. Mount the DECtape containing program RAMAN on the tape unit and roll about 10 ft. of tape onto the empty roll.
2. Place the tape unit in the WRITE LOCK and REMOTE mode and turn the teletype to LINE.

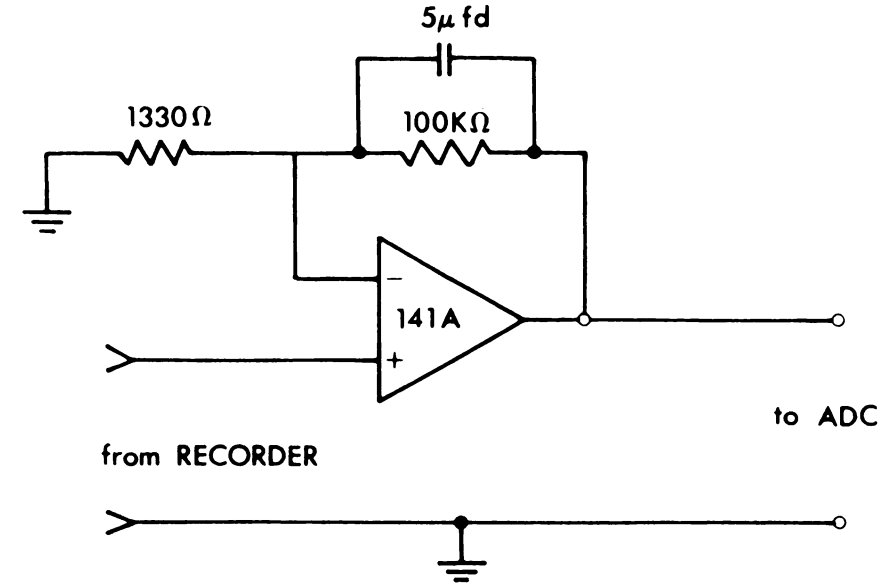


5V REGULATED POWER SUPPLY

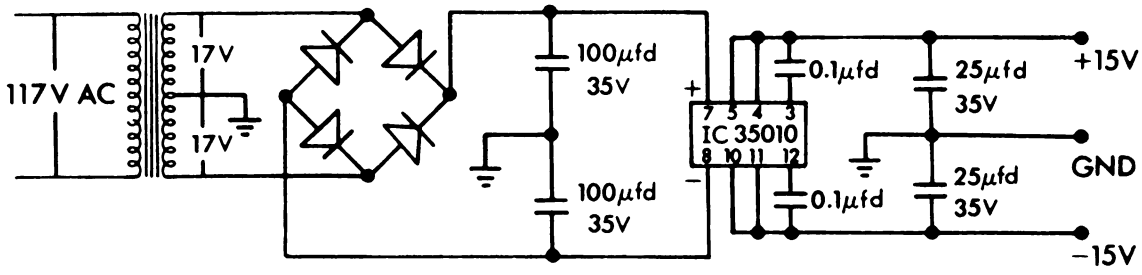


TRIGGERING INTERFACE

Figure A1. Triggering interface and 5 volt regulated power supply.



ADC INTERFACE



±15V REGULATED 50 mA POWER SUPPLY

Figure A2. Analog-digital converter and power supply.

3. Set the switch register on the CPU to 7470, 111 100 111 000.
4. Press EXTD ADDR LOAD, ADDR LOAD, CLEAR, and CONTinue on the CPU in that order.
5. If OS/8 is loaded, the teletype will respond with a dot (.).
6. Turn on both the wavelength encoder and the amplifier.
7. Plug the cable from the wavelength encoder into Schmidt trigger two (2).
8. Check the voltage output range of the amplifier for a full-scale deflection of the recorder. This range must fall between -1.00v and +1.00v. The output voltage range of the amplifier may be changed by using the zero suppress control on the spectrometer.
9. Plug the cable from the amplifier into channel zero (0) of the A/D converter.

Running Program RAMAN

1. On the teletype, type R RAMAN and RETURN.
2. The teletype will respond with the question, "INITIAL WAVENUMBER?"
3. Type in the initial wavenumber or tenth of a wavenumber minus one (i.e., if the scan were to go from 950 cm^{-1} to 1250 cm^{-1} at 1.0 cm^{-1} intervals, the user would type 949 or 949.0). The user then types the RETURN key. At this time the

spectrometer should be set at exactly 949.0 wavenumbers on the indicator dial, and the interval switch on the interface set to the proper position.

4. The teletype then quizzes,
   "FINAL WAVENUMBER?"
5. The user then types in the final wavenumber (i.e., 1250 or 1250.0) and then RETURN.
6. The teletype then replies,
   "INTERVAL BETWEEN DATA POINTS"
7. The user then types the interval between data points (i.e., 0.1 or 1.0) and then RETURN.

CAUTION: If the interval between data points is 0.1 cm^{-1}, the scan can only cover a maximum of 50 cm^{-1}. Likewise, if the interval is 1.0 cm^{-1}, the scan can cover a maximum of 500 cm^{-1}. If these conditions are violated the program will give spurious results.

8. The user now begins the scan in a positive direction.
9. When the scan is finished the raw intensities are printed out and the teletype responds,
   "CHOOSE TWO WAVENUMBERS AS A BASELINE"
10. The user then types in two numbers to be used as a baseline (i.e., 950 or 950.0 RETURN, and then 1250 or 1250.0 RETURN).

CAUTION: The user should never use the initial wavenumber (i.e., 949.0) as a point for baseline calculation.

NOTE: The baseline calculated is a straight line and is automatically subtracted from the raw data.

11. The teletype responds,
    "DO YOU WISH TO INTEGRATE A PEAK?"
12. The user replies with a zero (0) and RETURN for no and a one (1) and RETURN for yes.
13. If the answer is yes, the teletype will respond with, "CHOOSE YOUR INTEGRATION LIMITS"
14. The user then types in the limits (i.e., 950 or 950.0 RETURN and 1250 or 1250.0 RETURN).

CAUTION: The initial wavenumber (i.e., 949 or 949.0) may not be used as a limit.

15. Once the band has been integrated the program gives two more chances for integrating two additional spectral bands by repeating,
    "DO YOU WISH TO INTEGRATE A PEAK?"
16. The user can reply as before.
    NOTE: If the user replies with a no to the question above, the question is not repeated.
17. The teletype then responds,
    "THE AREA OF PEAK 1 IS number"
    "THE AREA OF PEAK 2 IS number"
    "THE AREA OF PEAK 3 IS number"
    NOTE: If only one band is integrated the data for peaks 2 and 3 will not appear.
18. The teletype then responds,
    "TYPE A 1 FOR PEAKS OUTPUT:A Ø FOR KINFIT OUTPUT"
19. The user replies with a one (1) or a zero (0) and then RETURN.

20. If the KINFIT output was selected, the teletype asks, "WAVENUMBER VARIANCE?"
21. The user types in the variance and RETURN.
22. The teletype then quizzes, "INTENSITY VARIANCE?"
23. The user again types in the variance and RETURN.
24. The teletype responds with, "YOU HAVE 20 SECONDS TO TURN ON THE PUNCH!"
25. At this time the user may punch a leader on the paper tape and prepare for the punching process.
26. Upon completion of the punching process, the program will wait an additional 20 seconds to allow the user to punch a trailer on the paper tape.
27. At the end of this waiting period the program will return to its beginning.

    NOTE: If an error is made during a run and the user wishes to restart the program, he toggles the bootstrap into the CPU as described in "Enabling OS/8". This returns computer control to MONITOR and the teletype will type a period (.). The user then types R RAMAN and proceeds again.

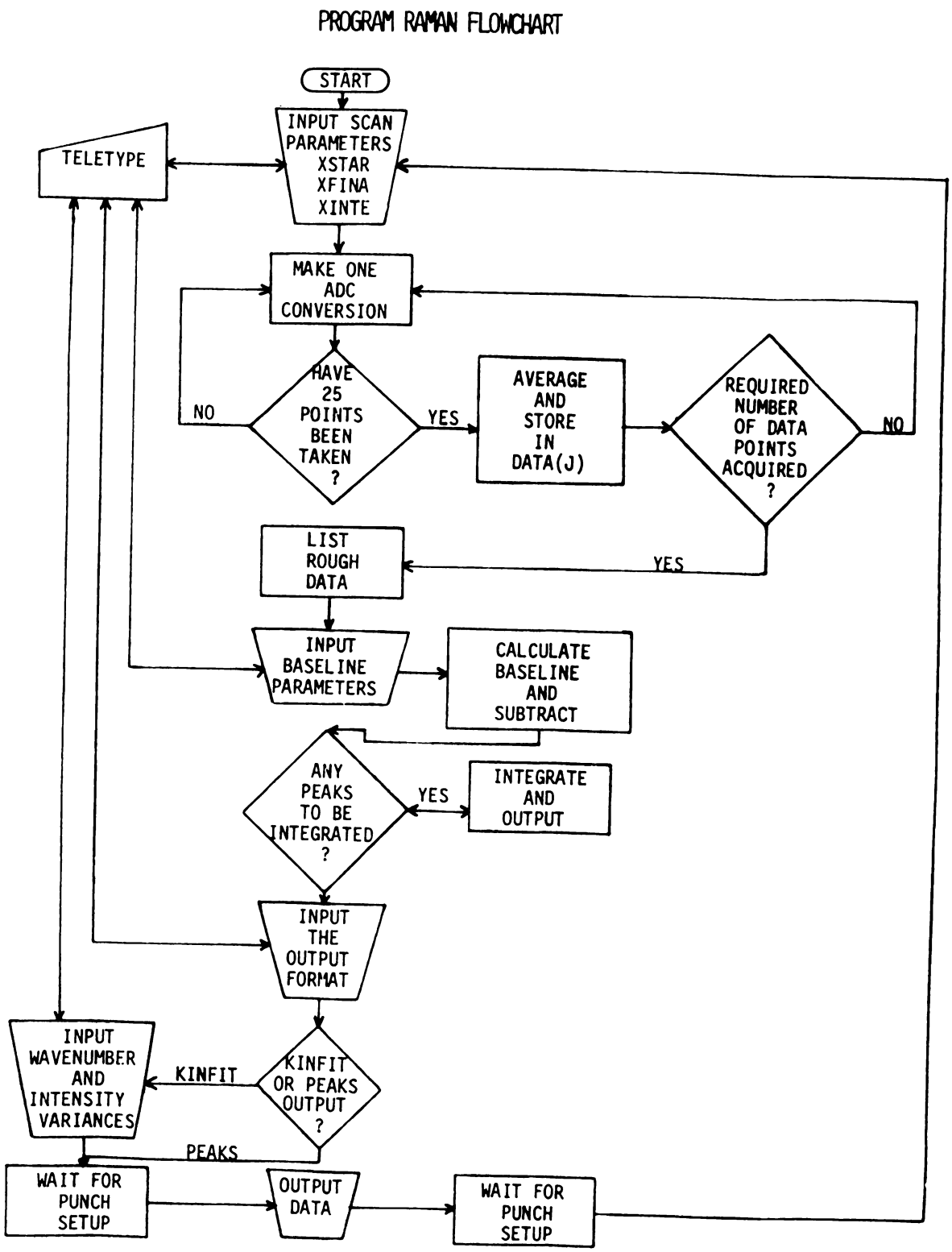


Figure A3. Flow chart for Program Raman.

PROGRAM RAMAN

```
      DIMENSION DATA(500), IPTS(100), AREA(3)
800   MORE = 1
      M = 2
      I = 0
      WRITE (1,1)
1     FORMAT ('INITIAL WAVENUMBER?')
      READ (1,2) XSTAR
2     FORMAT(1F6.0)
      WRITE (1,3)
3     FORMAT ('FINAL WAVENUMBER?')
      READ (1,4) XFINA
4     FORMAT (1F6.0)
      WRITE (1,5)
5     FORMAT ('INTERVAL BETWEEN DATA POINTS?')
      READ (1,6) XINTE
6     FORMAT (1F6.3)
      INTER = (XFINA - XSTAR)/XINTE
      DO 10 J = 1, INTER
      M=1
      DATA(J) = 0.
S     CPAGE 22
S     START,  CLA CLL          /CLEARS ACC,LINK & STATUS REG
S             6135
S             TAD STOP
S             6130
S             CLA CLL
S             TAD ROT          /SET CLOCK ENABLE REGISTER
S             6132
S             CLA CLL
S     AGAIN,  6131             /HAS SCHMITT TRIGGER FIRED?
S             JMP AGAIN        /NO, WAIT
S     FOUR,   CLA CLL
S             6532             /START CONVERSION
S     DONE,   6534             /CONVERSION DONE?
S             JMP DONE         /NO, WAIT
S             6533             /YES, READ ADC INTO ACC
S             DCA \I           /ASSIGNMENT TO VARIABLE
S             SKP
S     STOP,   7777
S             SKP
S     ROT,    0002
      IPTS(M) = I
      IF (M-25) 7,8,8
7     M = M + 1
S     JMP AGAIN
8     PTS = 0
      DO 600 IQ = 1,25
      PTS = IPTS(IQ) + 512
      DATA(J) = DATA(J) + PTS
600   CONTINUE
```

```
      DATA(J) = DATA(J)/25.
10    CONTINUE
      WRITE(1,31)
      A = XSTAR + XINTE
      DO 83 L =1,INTER
      WRITE(1,9) A, DATA(L)
9     FORMAT(/, 1F10.1, 10X, 1F10.4)
      A = A + XINTE
83    CONTINUE
      WRITE ( 1,11)
11    FORMAT ('CHOOSE TWO WAVENUMBERS AS A BASELINE')
      READ (1,12) BASE1
12    FORMAT (1F6.0)
      READ (1,35) BASE2
35    FORMAT (1F6.0)
      ICAL1 = (BASE1-XSTAR)/XINTE
      ICAL2 = (BASE2-XSTAR)/XINTE
      BAS1Y = DATA(ICAL1)
      BAS2Y = DATA(ICAL2)
      SLOPE = (BAS2Y-BAS1Y)/(BASE2-BASE1)
      YINTE = BAS1Y-SLOPE*BASE1
      A = XSTAR + XINTE
      DO 13 N = 1, INTER
      Y = SLOPE*A + YINTE
      DATA(N) = DATA(N) - Y
      IF (DATA(N)) 34,60,60
34    DATA(N) = 0
60    A = A + XINTE
13    CONTINUE
22    WRITE (1,23)
23    FORMAT('DO YOU WISH TO INTEGRATE A PEAK?')
      READ (1,24) KANS
24    FORMAT(I1)
      KANS = KANS + 1
      GO TO (26,19), KANS
19    WRITE (1,20)
20    FORMAT('CHOOSE YOUR INTEGRATION LIMITS')
      READ(1,21) ALIM1
21    FORMAT(1F6.0)
      READ(1,76) ALIM2
76    FORMAT(1F6.0)
      ILIM1 = (ALIM1-XSTAR)/XINTE
      ILIM2 = (ALIM2-XSTAR)/XINTE
      SUM = 0.0
      DO 25 M = ILIM1, ILIM2
      SUM = SUM + DATA(M)*XINTE
25    CONTINUE
      AREA(MORE) = SUM
      MORE = MORE +1
      GO TO 22
26    GO TO (342,27,27,27), MORE
```

```
27      MORE = MORE + 1
        MARK = MORE - 2
        DO 28 L = 1, MARK
        WRITE (1,29) L, AREA(L)
29      FORMAT('THE AREA OF PEAK ',III,' IS ',1F10.2)
28      CONTINUE
342     WRITE(1,343)
343     FORMAT(//,'TYPE A 1 FOR PEAKS OUTPUT; A 0 FOR KINFIT OUTPUT')
        READ (1,344) NOUT
344     FORMAT(III)
        IF (NOUT-0) 30,30,345
30      WRITE(1,103)
103     FORMAT('WAVENUMBER VARIANCE?')
        READ(1,104) WAV
104     FORMAT(1F6.0)
        WRITE(1,105)
105     FORMAT('INTENSITY VARIANCE?')
        READ(1,106) VAR
106     FORMAT(1F6.0)
345     WRITE(1,69)
69      FORMAT(//,'YOU HAVE 20 SEC. TO TURN ON THE PUNCH!',///)
        DO 68 MY = 1,700
        MZ = 0
        DO 67 NJ = 1, 2000
        MZ = MZ + 1
67      CONTINUE
68      CONTINUE
        IF (NOUT-0) 346,346,347
31      FORMAT(//,' WAVENUMBER',10X,'INTENSITY')
346     A = XSTAR + XINTE
        DO 32 M = 1,INTER,2
        A1 = A + XINTE
        IF (M-INTER) 107,108,107
107     MM = M + 1
        WRITE(1,33) A,WAV,DATA(M),VAR,A1,WAV,DATA(MM),VAR
33      FORMAT(4(F10.0,F10.4))
        A = A + 2. * XINTE
32      CONTINUE
        GO TO 999
108     WRITE(1,109) A,WAV,DATA(M),VAR
109     FORMAT(2(F10.0,F10.4))
        GO TO 999
347     WRITE(1,349) (DATA(M), M=1,INTER)
349     FORMAT(7(1F10.0))
999     DO 112 JY=1,700
        JZ=0
        DO 113 IJ=1,2000
        JZ=JZ+1
113     CONTINUE
112     CONTINUE
        GO TO 900
        END
```

PROGRAM RAMAN PARAMETERS*

DATA(N) - real dimension used to store data points

IPTS(N) - integer dimension used to store a set of 25 unaveraged points.

AREA(N) - real dimension used to store peak areas.

MORE - integer variable used to index the peak areas.

M - integer variable used to index the number of data points to be averaged.

I - integer variable used to represent each data point which is transferred into IPTS(N).

XSTAR - real input variable representing the initial wavenumber.

XFINA - real input variable representing the final wavenumber.

XINTE - real input variable representing the interval between data points.

XINTER - integer variable used to represent the number of data points to be taken.

J - integer variable used as an index in data acquisition routine.

PTS - real variable used to sum the 25 unaveraged data points and convert them to a real number.

IQ - integer variable used as an index in the summing process.

A - real variable used to index the appropriate wavenumber upon output of data.

*in order of appearance in program listing

L - integer variable used as an index when listing the rough data.

BASE 1 - real variable used to represent one point on the baseline.

BASE 2 - real variable used to represent one point on the baseline.

ICAL1 and ICAL2 - integer variables used for indexing in the baseline calculation routine.

BAS1Y and BAS2Y - real variables representing the intensities at BASE 1 and BASE 2.

SLOPE - real variable representing the slope of the baseline.

YINTE - real variable representing the Y-intercept of the baseline.

Y - real variable used to represent corrected intensity.

KANS - integer variable used as an index for integrating peaks.

ALIM1 and ALIM2 - real variables used to represent the integration limits.

ILIM1 and ILIM2 - integer variables used to index the intensities at the integration limits.

SUM - real variable representing a transient integral.

MARK - integer variable used as an index for the output of the area of each peak.

NOUT - integer variable used as an index for KINFIT or PEAKS output.

WAV - real variable representing the wavenumber variance.

VAR - real variable representing the intensity variance.

MY, MZ, and NJ - integer variables used as indices in a waiting routine

A1 - real variable used as wavenumber counter in KINFIT output.

MM - integer variable used to index intensity output.

JY, JZ, and IJ - integer variables used as indices in second waiting routine.

APPENDIX II

DETERMINATION OF PHARMACOLOGICAL PROPERTIES OF 8 -TERT-BUTYLPENTAMETHYLENETETRAZOLE AND 8-SEC-BUTYLPENTAMETHYLENETETRAZOLE AS QUOTED FROM COMMUNICATIONS RECEIVED FROM PROFESSOR WILLIAM E. STONE

DETERMINATION OF PHARMACOLOGICAL PROPERTIES OF 8-TERT-BUTYLPENTAMETHYLENETETRAZOLE AND 8-SEC-BUTYLPENTAMETHYLENETETRAZOLE AS QUOTED FROM COMMUNICATIONS RECEIVED FROM PROFESSOR WILLIAM E. STONE

Professor William E. Stone, Department of Physiology, University of Wisconsin, Madison, Wisconsin, determined the pharmacological properties of the pentamethylenetetrazole derivatives. The tests were made on Swiss-Webster white mice, and the convulsants were administered intraperitoneally. Dose-response curves were calculated by the method of least squares, and for each compound the dose which induced a generalized seizure in 50% of the animals (CD_{50}) was determined. The test results are given below.

8-tert-butylpentamethylenetetrazole

The solubility of this compound in 0.85% sodium chloride solution appeared to be slightly less than 0.85 mg/ml. For these tests it was found advantageous to use 50% glycerol in water as the solvent. A solution which contained 1.6 mg of 8-tert-butyl PMT per ml of solution was prepared. The compound dissolved completely on warming to 56°, but crystallization occurred overnight at room temperature. For lower doses, the solution that was used contained 1.2 mg/ml. There was also some crystallization from this solution

on standing overnight at room temperature.

The CD_{50} was found to be 5.25 mg per kg of body weight, with 95% confidence limits of 3.97 to 6.93 (log CD_{50} = 0.7198±0.1207).

The "approximate minimum convulsant dose" previously reported was 9 mg/kg (74). This falls at the point CD_{80} on the dose-response curve.

In animals that had seizures, the latent periods were about 1 to 5 minutes.

8-sec-butylpentamethylenetetrazole

The solubility of this compound seemed to be slightly greater than that of 8-tert-butyl PMT in 0.85% sodium chloride solution, and considerably greater in 50% glycerol. The solution that was used contained 5 mg/ml of 8-sec-butyl PMT in 50% glycerol. This amount dissolved completely on warming to 56°, but crystallized on cooling. The warm solution was used for injection. (It sometimes crystallized in the injection needle and plugged it.)

The CD_{50} was found to be 48.6 mg/kg, with 95% confidence limits of 34.1 to 69.4 (log CD_{50} = 1.6870±0.1544).

The latent periods in animals having seizures were about 2 to 4 minutes.

Pentamethylenetetrazole

These results are included for comparison with the above tetrazoles.

The CD_{50} was 45.1 mg/kg, with 95% confidence limits of 40.3 to 50.2 (log CD_{50} = 1.6542±0.0488).

The "approximate minimum convulsant dose" previously reported was 50 mg/kg (74). This corresponds to the point CD_{69} on the dose-response curve.

In animals that had seizures, the latent periods were about 1.5 to 9 minutes.

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