can be bleached through its triplet state by exciting a sensitizer with light around 700 nm.[10]

The observation that upon cooling to 77°K radicals form only from the hydrosol suspension cannot be explained solely in terms of reducing the macroscopic diffusion coefficients. It seems that a special microcrystalline arrangement is essential to block the back reaction. This microcrystalline arrangement is provided by the hydrosol formation but not by cooling or by attaching the dye to a support like cotton wool or a protein.

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Conclusion

It was established in this work that CIDNP can be produced in macroscopic particles. The essential diffusional equilibrium is provided here by the relatively high mobility of the hydrogen atoms, while the observation of the polarization was made possible because of sufficiently fast exchange with the solvent compared to the spin lattice relaxation time.

Viscosity of the Hydrocarbon Region of Micelles. Measurement by Excimer Fluorescence[1]

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Abstract: The relative intensities of excimer and monomer fluorescence of pyrene are shown to be a simple linear function of the viscosity of a homologous series of solvents. This relationship may be represented by $\eta = kC'_h$, where C'_h is the pyrene concentration at which half the fluorescence intensity appears in both the excimer and monomer form. The constant k includes both instrumental and theoretical parameters and is determined empirically. These results have been used to measure the viscosity of the hydrocarbon region of micelles labeled with pyrene. The viscosities of alkyltrimethylammonium bromides with alkyl chain lengths from C_{10} to C_{18} were in the range of 125 to 200 cP.

Interest in the structure and function of biological membranes has been greatly intensified with the advent of probe systems for study of these systems. For example, spin-labeled compounds provide information about the rates of lateral diffusion of molecules in membranes, inside–outside transitions of phospholipid bilayers, and about strictures on molecular motion of the membrane components.[3-9] Changes in the spectral characteristics of fluorescent probes as the result of noncovalent interaction with macromolecules are frequently used to detect hydrophobic regions in proteins and membranes.[10-16] More recently, Shinitzky, *et al.*,[17] have employed fluorescent aromatic hydrocarbons to determine the viscosity of the hydrocarbon regions of synthetic micelles. In this method the rate of molecular rotation, but not fluorescence lifetime, is a function of the viscosity of the probe environment so that fluorescence depolarization as the result of rotational diffusion can be used to determine microviscosity.

In this report we describe a new probe technique which is based on the rate of translational diffusion of the fluorescent aromatic hydrocarbon, pyrene, measured by the relative intensities of monomer and excimer fluorescence at defined hydrocarbon concentrations. Pyrene can undergo the processes in Chart I.[18]

Chart I

	Rate	Process
$P + h\nu \rightarrow P^*$	I_a	Absorption, where I_a is the intensity of absorbed light
$P^* \rightarrow P + h\nu'$	$k_f P^*$	Monomer fluorescence
$P^* \rightarrow P$	$k_{r1} P^*$	Monomer radiationless transition
$P^* + P \rightarrow P^*_2$	$k_a(P)(P^*)$	Excimer formation
$P^*_2 \rightarrow P^* + P$	$k_d(P^*_2)$	Excimer decomposition
$P^*_2 \rightarrow P_2 + h\nu''$	$k'_f(P^*_2)$	Excimer fluorescence
$P^*_2 \rightarrow P_2$	$k'_{r1}(P_2^*)$	Excimer radiationless transition
$P_2 \rightarrow 2P$	$k'_d(P_2)$	Dimer decomposition

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According to this reaction scheme the concentration dependences of the fluorescence quantum efficiencies φ_M and φ_E for monomer and excimer fluorescence, respectively, have been shown[19] to be

$$\varphi_M = \varphi_M^{max}(1 + C/C_h)^{-1} \quad (1)$$

$$\varphi_E = \varphi_E^{max}(1 + C_h/C)^{-1} \quad (2)$$

where C_h represents the concentration at which half the fluorescence intensity appears in both the monomer and excimer fluorescence spectra, and C is the concentration at which the quantum efficiencies φ_M and φ_E are observed; φ_M^{max} and φ_E^{max} represent the maximum intrinsic fluorescence of each specie. The ratio of the quantum yield is

$$\frac{\varphi_E}{\varphi_M} = \frac{\varphi_E^{max}}{\varphi_M^{max}} \frac{C}{C_h} \quad (3)$$

Since the relative quantum yields are proportional to the relative fluorescence intensities of each specie, equivalent expressions utilizing the fluorescence intensity of each component can also be developed in which I_M^{max}

$$I_M = I_M^{max}(1 + C/C_h)^{-1} \quad (4)$$

$$I_E = I_E^{max}(1 + C_h/C)^{-1} \quad (5)$$

and I_E^{max} represent the maximum intrinsic fluorescence intensity. The ratio of the fluorescence intensities is

$$\frac{I_E}{I_M} = \frac{I_E^{max}}{I_M^{max}} \frac{C}{C_h} = \frac{C}{C'_h} \quad (6)$$

where $C'_h = (I_M^{max}/I_E^{max})C_h$. Inspection shows that changes in the ratio of monomer and excimer fluorescence intensity are proportional to the pyrene concentration, C. Provided the rate of excimer dissociation is negligible ($k_d \ll k'_f + k'_{r1}$),[21] eq 7 and 8 are valid so

$$\varphi_M^{max} = k_f(k_{r1} + k_f)^{-1} \quad (7)$$

$$\varphi_E^{max} = k'_f(k'_f + k'_{r1})^{-1} \quad (8)$$

that by appropriate substitution, in eq 3 and 6, the following relationships are obtained.

$$C_h = (k_f + k_{r1})k_a^{-1} \quad (9)$$

$$C_h' = (k_f + k_{r1})k_a^{-1}(I_M^{max}/I_E^{max}) \quad (10)$$

In this expression the half-intensity concentration, C_h', is a function of the rate of excimer formation, k_a, which has been shown to be diffusion controlled.[25]

The Einstein–Schmoluchowski diffusion theory[26-28] relates the rate of diffusion to the viscosity of the medium by the expression

$$k_a = 8(RT/3000\eta)(pa/b) \quad (11)$$

in which $pa/b = 1$ for pyrene.[19] The relationship of half-intensity concentration, C'_h, to the viscosity is apparent in the following equation.

$$C'_h = (k_f + k_{r1})(3000\eta/8RT)(I_M^{max}/I_E^{max}) \quad (12)$$

Inspection reveals that the C'_h value can be utilized as an index of viscosity. Thermal dissociation of the excimers ($k_d \neq 0$) at elevated temperatures can produce deviations from the predicted behavior. These are minimal at ambient temperature.[29]

The measurement of microviscosity by the determination of C'_h values appears to be an attractive method for obtaining this somewhat inaccessible information. The method presented in this paper requires only a conventional spectrofluorimeter. Pyrene is the probe of choice having a high quantum yield ($\varphi_f = 0.6$)[30] which permits the use of very small quantities. The small amount of pyrene required should introduce little or no perturbation of the system under investigation. In addition, the intense electronic absorption[30] of pyrene is in a spectral region which is accessible to commercial instruments. The emission spectrum of pyrene appears in a spectral region where the sensitivity of the photomultipliers on commercial instruments (for example, EMI 9558Q or RCA 1P28) is maximal. The insolubility of pyrene in water ensures that all of the probe in the system is present in the hydrophobic interior of the micelle.

Experimental Section

Materials. The following materials were used as received from the manufacturer: cyclohexane (Matheson Coleman and Bell, Spectroquality), 2-propanol (Matheson Coleman and Bell, ACS Reagent), 1-octanol (Fisher Certified Reagent), 1,2-propanediol (Matheson Coleman and Bell, 99+ mol %), 1,3-butanediol (Matheson Coleman and Bell, 99+ mol %), 1-dodecanol (East man), chloroform (Fisher, spectranalyzed), methanol (Baker Analyzed Reagent), and pyrene (Aldrich, zone-refined). All solvents were free of detectable absorption and emission above 300 nm. Buffer solutions were prepared from deionized water and tris(hydroxymethyl)aminomethane (Sigma Chemical Co.).

DTAB, LTAB, and MTAB, custom synthesized by Lachat Chemical, Inc., and CTAB (Eastman) and STAC (K & K Chemicals) were recrystallized from methanol–ether.[31]

Potassium dodecanoate was prepared by the addition of potassium hydroxide to dodecanoic acid. SDS was recrystallized from ethanol.[31]

Spectral Techniques. Before the fluorescence spectra were obtained, removal of oxygen from all solvents was accomplished by purging with dry nitrogen for 0.5–1.5 hr. For highly viscous solvents, longer times were required. Alternatively, and with similar results, the samples were heated to about 75° on a spinning band column at 0.05 mm of pressure for at least 1 hr. All transfers were performed under a nitrogen atmosphere. Failure to adequately remove oxygen from the samples produced spectra in which excimer fluorescence was quenched. The concentration of pyrene in the samples was determined by the dilution of an aliquot in methanol and the measurement of the absorbance at 334 nm. The Beer's law treatment of stock solutions of pyrene in methanol yielded an extinction coefficient of 50,000 l. mol^{-1} cm^{-1} at 334 nm. A Cary 14 or a Beckman Acta V spectrophotometer was employed at ambient temperatures to obtain absorption spectra and absorbance values. Fluorescence measurements were recorded on an Aminco-Bowman spectrofluorimeter.

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(31) Abbreviations used are DTAB, decyltrimethylammonium bromide; LTAB, lauryltrimethylammonium bromide; MTAB, myristyltrimethylammonium bromide; CTAB, cetyltrimethylammonium bromide; STAC, stearyltrimethylammonium chloride; and SDS, sodium dodecyl sulfate.

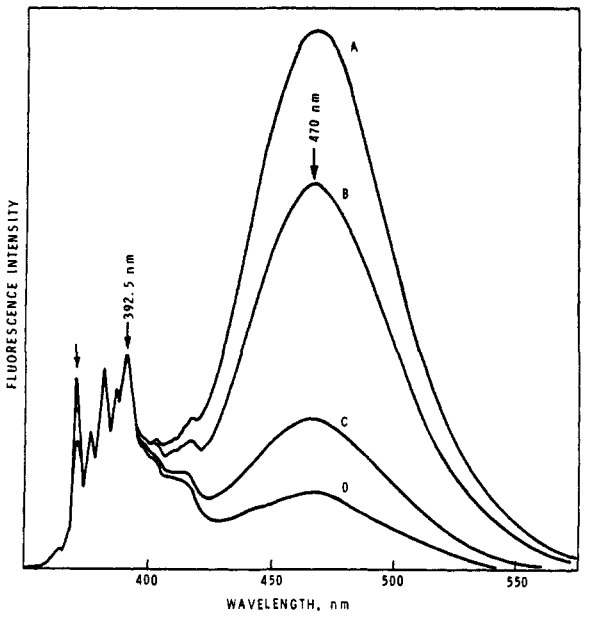

Figure 1. The pyrene fluorescence in 2-propanol at 22° as a function of concentration. Pyrene concentrations are: (A) 6.15 × 10^{-3} *M*; (B) 4.65 × 10^{-3} *M*; (C) 1.72 × 10^{-3} *M*; (D) 0.9 × 10^{-3} *M*. (The unlabeled arrow indicates the region of strong self-absorption.)

Viscosity Measurements. The viscosity of the solvents was determined from the average of at least three measurements with a calibrated Cannon-Manning semimicro viscometer in a thermostated bath at 23°.

Preparation of Labeled Micelles. A solution of 10 m*M* Tris, pH 7.0, was purged with nitrogen for about 1 hr. Under a nitrogen atmosphere, for this and subsequent operations, surfactant was added in an amount at least five times greater than the critical micelle concentration (cmc). Aliquots of this solution were combined with varied amounts of pyrene. The pyrene was solubilized in the micelles by heating to *ca.* 90° with sonication. The samples were then centrifuged to sediment any undissolved pyrene and passed through a 5 μ Millipore filter. The resulting optically clear solutions were transferred to a 2 × 3 × 20 mm quartz microcell. The fluorescence spectrum was measured with 4-mm excitation slits and excitation at 335 nm; analyzing slits were 0.2 mm. The concentration of the sample was calculated from the absorbance at 334 nm after appropriate dilution in methanol.

Results

Viscosity of Pure Solvents. The total fluorescence spectrum of pyrene in 2-propanol at various concentrations is shown in Figure 1. All spectral intensities were normalized at 392.5 nm to demonstrate the proportional increase of excimer fluorescence with pyrene concentration. Values for I_M and I_E were obtained at 392.5 and 470 nm, respectively. It is noteworthy that the strong self-absorption which distorts the spectrum near the 0–0 band (unlabeled arrow in Figure 1) at high pyrene concentrations did not interfere with the spectral intensity at 392.5 nm. In addition, the two maxima at which I_M and I_E were measured are well separated. This spectrum is representative of those obtained in other solvents although the concentration range of pyrene in each solvent was different.

In Figure 2 the ratio of relative intensity of excimer and monomer fluorescence, I_E/I_M, is plotted against pyrene concentration in 2-propanol and shows the good linearity obtained with all solvents. The C'_h value is obtained from the reciprocal of the slope of these plots. In Table I the C'_h values for a variety of solvents at 23° are compiled with the corresponding kinematic viscosities. The relationship between these C'_h values and solvent viscosity was expected to be linear, and the data shown in Figure 3 substantiate this prediction. The slope of this line, 0.73 × 10^{-3}, is equal to the quantity $[(k_f + k_{r1})I_M^{max}/I_E^{max}](3000/8RT)$. From this calibration curve, the viscosity of a solvent containing the pyrene probe can be determined by measurement at C'_h and evaluation with the expression $\eta = C'_h/0.73 \times 10^{-3}$.

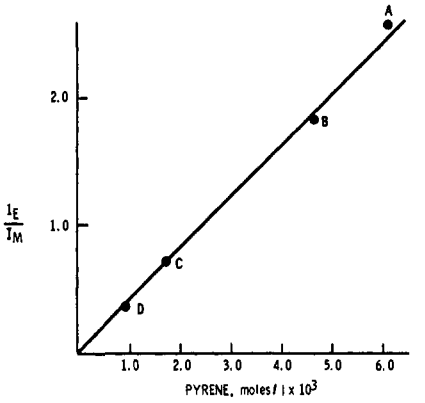

Figure 2. A plot of I_E/I_M *vs.* concentration of pyrene in 2-propanol at 22°. Pyrene concentrations are: (A) 6.15 × 10^{-3} *M*; (B) 4.65 × 10^{-3} *M*; (C) 1.72 × 10^{-3} *M*; (D) 0.9 × 10^{-3} *M*.

Table I. Kinematic Viscosity and Half-Intensity Concentration Values, C'_h

Solvent	Kinematic viscosity, cP	C'_h × 10^3, mol/l.	Lit. value, cP	Ref
Cyclohexane	0.94	1.1	$0.980^{20°}$	32
2-Propanol	2.33	2.5	$2.86^{15°}$	32
1-Octanol	8.35	8.0	$10.6^{15°}$	32
1-Dodecanol	17.3	11.3	$17.4^{25°}$	33
1,2-Propanediol	48.3	37.5	$56.0^{20°}$	32
1,3-Butanediol	117.0	78.0	$130.0^{20°}$	32

The procedures for measurement of fluorescence intensity are described in Methods and were performed at 23° except for 1-dodecanol which was done at 24°.

Viscosity of Micellar Interiors. The microscopic concentration, C_μ, of pyrene within the hydrocarbon core of the micelle is given by

$$C_\mu = C_b/(C_s \bar{V}_{hc})$$

where C_s is the surfactant concentration, C_b is the bulk phase concentration of pyrene, and $\bar{V}_{hc}$ is the partial molal volume of the hydrocarbon region of the micelle. The pyrene is assumed to be exclusively in the micelle interior. The estimated value of $\bar{V}_{hc}$ of the various surfactants was calculated from the expression $\bar{V}_{hc}$ = MW(hc)/MW(s)1.1 $\bar{V}_s$, where $\bar{V}_s$ is the partial molal volume of the surfactant, and MW(hc) and MW(s) are the respective molecular weights of the hydrocarbon chain and the surfactant. The factor 1.1 is included to correct for a 10% increase in partial molal volume of a surfactant accompanying transition from the mono-

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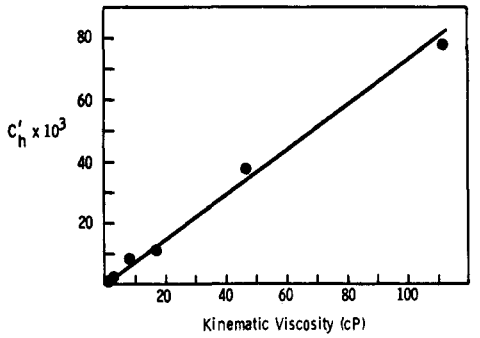

Figure 3. A plot of C'_h *vs.* kinematic viscosity. In order of increasing viscosity the solvents are: cyclohexane, 2-propanol, 1-octanol, dodecyl alcohol, 1,2-propanediol, and 1,3-butanediol.

meric to the micellar phase.[34] An alternative method assigns a partial molal volume of 18 ml/mol for each methyl or methylene group in the aliphatic chain[34] and gives a result which is within 5% of the value obtained by the first method.

In pyrene-labeled micelles of cetyltrimethylammonium bromide, CTAB, the measured viscosity of the hydrocarbon core was essentially constant in the concentration range 10^{-2}–2.75×10^{-1} *M* CTAB. Inspection of Figure 4 reveals that the slope of the I_E/I_M *vs.* C_μ plot is essentially identical at three different concentrations of CTAB. The average C'_h value for these three samples is 110×10^{-3} *M*, from which a microscopic viscosity of 151 ± 6 cP was calculated. Microviscosities of all the alkyltrimethylammonium bromides were nearly constant at concentrations which greatly exceeded the surfactant cmc. In contrast, the C'_h value of labeled SDS micelles was not as reproducible when different concentrations of detergent were used. The value of $\eta = 193 \pm 25$ cP can be considered only a fair estimate of the true microscopic viscosity of the micelle. In Table II the microscopic viscosities of other micelles are compiled in addition to the values for SDS and CTAB.

Table II

Compound	Viscosity, cP
Sodium dodecyl sulfate (SDS)	193 ± 25
Potassium dodecanoate	150 ± 20
Octadecyltrimethylammonium chloride (STAC)	130 ± 15
Hexadecyltrimethylammonium bromide (CTAB)	151 ± 6
Tetradecyltrimethylammonium bromide (MTAB)	125 ± 15
Dodecyltrimethylammonium bromide (LTAB)	150 ± 15
Decyltrimethylammonium bromide (DTAB)	155 ± 15

Discussion

The results presented in this communication illustrate that the viscosity of micellar interiors can be determined rapidly and simply by measurement of pyrene excimer fluorescence. This method should also be applicable to other systems with hydrophobic regions, such as membranes and lipoproteins. Certain advantages of this method over depolarization methods exist. Results from excimer fluorescence are not affected by the depolarizing effect of micellar rotation. Corrections necessary but difficult to quantitate for turbid solutions which scatter and depolarize fluorescence are not required. In addition, the concentration of surfactant does not appear to be important in the dynamics of excimer fluorescence when the concentration is greater than the critical micellar concentration. This observation is consistent with those of Förster, *et al.*,[35] who found no effect on I_E/I_M ratios of detergent solutions when diluted with water.

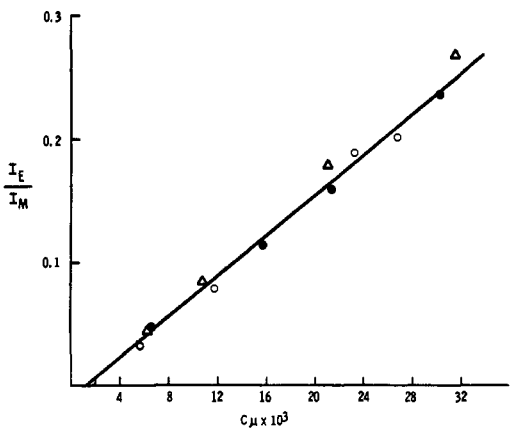

Figure 4. A plot of I_E/I_M *vs.* microscopic concentration of pyrene in hexadecyltrimethylammonium bromide, CTAB. CTAB concentrations employed were: (▲) 2.75×10^{-1} *M*; (●) 3.32×10^{-2} *M*; (○) 1×10^{-2} *M*.

The relatively constant microviscosity of CTAB over a large concentration range indicates that the rate of transfer of pyrene from one micelle to another is quite slow compared to the rate of diffusion of the micelle. If excimer formation as the result of transfer were to occur at a diffusion controlled rate, arbitrarily low viscosity values would be obtained and the C'_h values would depend on surfactant concentration. Even at CTAB concentration of 0.275 *M* no decrease in C'_h or η is noted. There are two aspects of this observation. The collision rate between micelles is $k_d(M)^2$. A diffusion controlled rate constant of 10^8 l./(mol sec^{-1}) and a micelle concentration 3×10^{-3} *M* dictates a collision rate of only about 10^3 mol/(l. sec) micelles, a rate which is too infrequent to account for the observed excimer fluorescence. Both the rate of diffusion of pyrene to the exterior of the micelle at the point of impact and the necessary activation energy to move from the aliphatic environment of the interior through the ionic outer layer provide additional energy barriers to transfer. Further, the rate of diffusion to the ionic layer will be slow because of the highly viscous nature of the micelle interior. It has been reported that oxygen does not penetrate the outer layer of the micelle to quench pyrene fluorescence in the hydrophobic core.[36] In these experiments the I_E/I_M ratios of pyrene in micelles have been constant, even after prolonged exposure to air.

The microviscosity values for these micelles, obtained by excimer fluorescence, are consistently much higher than those obtained by fluorescence depolarization. The value reported by Shinitzky, *et al.*,[17] for the microviscosity of CTAB is between 19 and 30 cP at 27°; our value is 151 ± 6 cP at 23°. The difference in temperature, though small, may be important. The mobility

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within the micelle would be expected to increase as the temperature is raised toward the Krafft point, the hypothetical melting point of a hydrated solid.[37] A second source of depolarization in labeled micelles and consequently low value for microviscosity is that which results from rotation of the micelle itself.[38] If the rate of micelle rotation is of the same order of magnitude as rotation within the micelle itself, serious error in the microviscosities obtained by this method can be introduced. The degree of depolarization is defined by $r_0/r = 1 + (kT\tau)/(\eta V)$ where k is the Boltzmann constant, T the temperature, η the viscosity, V the effective volume of the fluorescent rotor (micelle and/or the fluorescent label), and τ the average lifetime of the fluorophore.[17] Comparison of the term $1/\eta V$ for both micelle and fluorophore rotation indicates that rotational retardation of the micelle ($V \sim 5000$ Å^3) in water ($\eta = 1.0$ cP) becomes competitive with that of the label in highly viscous micelle interiors.

Other considerations are necessary to account for the discrepancy between microviscosities based on depolarization and those obtained by excimer fluorescence. Additional information regarding structure of anisotropy of the micelle are important. The homogeneous distribution of the label throughout the micelle interior is probably not entirely valid.

The model presented here is based on collision theory which assumes a large number of pyrene molecules for each micelle interior. This assumption implies that the majority of the pyrene collisions are with other pyrene molecules within the micelle. This is not entirely accurate. For example, a microscopic concentration of 0.16 M pyrene in CTAB corresponds to an average value of six pyrene molecules per micelle if an aggregation of 85 CTAB molecules/micelle is assumed.[39] If a large number of pyrene molecules were present in each micelle, pyrene–pyrene collisions would be far more frequent than pyrene–micelle wall collisions and the Einstein–Schmulochowski equation would be valid. With a small number of pyrene molecules in each micelle, the excimer fluorescence is less than predicted by diffusion theory since the number of pyrene–micelle wall collisions is the same order of magnitude as pyrene–pyrene collisions. Therefore, the intensity of excimer fluorescence is less than predicted and high values of viscosity can be obtained.

We are now in the process of developing expressions to account for both the small number of pyrene molecules per micelle and the population distribution of pyrene molecules in a collection of labeled micelles. The magnitude of the differences introduced by this treatment cannot be estimated at this time. However, it should not greatly affect the relative viscosity values of these surfactants.

Although the variations in I_E/I_M with viscosity do not always follow a linear relationship,[19] I_E/I_M ratios were not affected by other solvent properties other than viscosity. In the calibration curve with structurally similar alcohols and diols, I_E/I_M values were a linear function over a wide range of viscosities. Although absolute microviscosities may not be measurable with a high degree of accuracy, this method is adequate for studying changes in phase or viscosity affected by temperature, pH, ionic strength, and other variables. For these experiments, pyrene excimer fluorescence is suitable as a microviscometric probe system.

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Mechanism of Ionic Self-Acylation in the Gas Phase by Ion Cyclotron Resonance Spectroscopy

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Abstract: A general survey of the ion–molecule reactions of ketones by ion cyclotron resonance spectroscopy shows that self-acylation is a common reactive channel of the molecular ion. Unsymmetrical ketones with no γ hydrogens, RCOR′, are shown to yield preferentially the acylated product, $RCO^+(RCOR')$, which results from a cleavage of the molecular ion in an analogous fashion to the preferred fragmentation in mass spectroscopy (RCO^+). Pressure studies are used to show that these reactions proceed through a complex intermediate, which is usually stabilized above 10^{-5} Torr as a clustered dimer ion. Acetone is the only case where this cluster is not observed. Ketones with a γ hydrogen undergo an ion–molecule reaction reminiscent of the McLafferty rearrangement rather than self-acylation. The relative ability of acetone, butanone, and 3-pentanone to stabilize an acyl cation (acyl affinity) is shown to follow the increasing polarizability of these compounds.

The ion chemistry of acetone at low pressures ($\sim 10^{-5}$ Torr) reveals a condensation–elimination reaction leading to an acetylated product

$$CH_3CO^+CH_3 + CH_3COCH_3 \longrightarrow CH_3CO^+(CH_3COCH_3) + \cdot CH_3 \quad (1)$$

The rate constant for this reaction has been recently reported by Futrell[1] in a comprehensive study of the ion–molecule processes in acetone using ion cyclotron

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