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# Correlation of Viscosity and Conductance with $^{23}Na^+$ NMR $T_1$ Measurements

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The relationship between rotational correlation time and the translational diffusion coefficient (Stokes-Einstein equation) of sodium ions dissolved in a series of water-glycerol solutions was studied. NMR longitudinal relaxation measurements gave an estimate of the rotational correlation time for the hydrated sodium ion. From this, using the Debye and Stokes-Einstein equations the translational diffusion coefficient was calculated. These results were compared with estimates of the sodium ion diffusion coefficient obtained using pulsed field gradient NMR. The simple, chemical system was chosen for this study so that the nature of the sodium relaxation would be unambiguous. Also, conductivity measurements were performed on each solution since the conductivity is intimately related to the rate of ionic diffusion. The results showed that due to the spherical nature of the hydrated sodium ion the Debye and Stokes-Einstein equations are well obeyed allowing translational diffusion coefficients to be calculated from rotational correlation times in good agreement with those measured using pulsed field gradient NMR.

It has been proposed that quadrupole relaxation could be used as a probe for investigating nearest neighbour interactions. However, contributions to the relaxation rate, caused by changes in the rotational correlation time ($\tau_c$) due to variations in viscosity need to be subtracted.[1] The relationship between rotational correlation time and solution viscosity, and the translational diffusion of a solute, has long been of interest.[2] With the advent of NMR and its applications to the study of cellular biological systems[3-5] it has become increasingly important to understand this connection. It is possible that the viscosity of a cell cytoplasm may play a significant role in diffusion control of certain enzymic reactions.[6]

It has been shown[7] for glycine in human erythrocytes that the translational diffusion coefficient, calculated from the correlation time using the Debye and Stokes-Einstein equations, is four times that measured by pulsed field gradient NMR (PFG NMR).[8] A direct measurement of the translational diffusion coefficient is obtained from PFG NMR. The NMR experiment labels nuclei and identifies them by their characteristic Larmor frequencies. The PFG NMR method is based on the labels being given a spatial dependence by the application of a well defined magnetic field gradient across the sample.[9] PFG NMR can be used to obtain precise diffusion coefficients over a large range of diffusion coefficient values and as a result is a useful tool for studying the microscopic theory of simple liquids.[9] Detailed expositions of the theory and applications of PFG NMR can be found in the reviews by Callaghan,[9] Kärger,[10] and Stilbs.[11] The correlation-time method assumes that the diffusing species is spherical and "sees" the solvent as a continuum. Thus for the diffusion coefficients, derived from correlation time measurements, to be valid requires that the restrictions on rotational motion of a solute by the solvent are the same as for translational motion. Monovalent ionic species in solution are spherical (irrespective of hydration sphere) and the translational diffusion coefficient is directly proportional to the electrical conductivity.[12] In contrast to the correlation-time method, it is not necessary to make any assumptions regarding the nature of solute-solvent interaction in the PFG NMR method.

We investigated the validity of the Stokes-Einstein equation for the sodium ion in aqueous glycerol solutions by studying the longitudinal relaxation and PFG NMR-determined diffusion coefficients of the ion. The sodium ion by itself, or when it is surrounded by a hydration sphere, is spherical. It may also "see" the solvent as a continuum as it is of dimensions similar to glycerol and $H_2O$. Therefore the sodium ion, in aqueous solutions of glycerol, is likely to conform to the assumptions of the Debye theory. Sugawara et al.[1] studied the relationship between linewidth ($\Delta\nu_{1/2}$), which is inversely proportional to the transverse or spin-spin relaxation time $T_2$, of the $^{35}Cl$ NMR resonance, and electrical conductivity ($\kappa$); they did this for a series of aqueous solutions of sodium chloride in polyethylene glycol of various molecular weights. They reported that the linewidth and the electrical conductivity are related to the microbut not the macroviscosity. In order to investigate further these relationships between solution physicochemical properties and rotational and translational correlation times we chose a particularly simple system, namely aqueous solutions of glycerol in a range of concentrations, but containing a constant concentration of sodium chloride. This system was chosen because of the unambiguous relaxation mechanism of the sodium ion. For each glycerol concentration the following NMR measurements for the sodium ion were performed: $T_1$, the longitudinal relaxation time; $T_2$ and; using PFG NMR the translational diffusion coefficient. Also the bulk viscosity ($\eta_B$) of each sample was determined using an Ostwald viscometer.

## Experimental

**Materials.** Sodium chloride was obtained from Ajax Chemicals, Auburn, N.S.W. Australia; glycerol was

obtained from BDH Chemicals Port Fairy, Vic, Australia, and EDTA was from BDH Chemicals Poole, England.

**Solution Preparation.** Glycerol/saline solutions were prepared by mixing glycerol, water, NaCl, and EDTA to give a final NaCl concentration of 0.1 mol $dm^{-3}$ and an EDTA concentration of 1 mmol $dm^{-3}$. The EDTA was included to chelate paramagnetic impurities.

**NMR Measurements.** 0.5 ml of each sample was dispensed into flat bottom 8 mm NMR tubes and sealed with a Teflon vortex plug. These 8 mm tubes were then supported coaxially in 10 mm NMR tubes containing water. The vortex plug and outer layer of water were necessary to minimize susceptibility differences at the boundaries of the sample within the region of the radio frequency coils. The sample volumes were deliberately made small so that they fitted into the linear region of the field gradient generated by the PFG apparatus. All $^{23}Na$ spectra were recorded at 298.15 K on a Varian XL/VXR-400 NMR spectrometer at 105.8 MHz. $T_1$ measurements were made using an inversion recovery pulse-sequence[13] incorporating a composite 180° pulse. $T_2$ measurements were made using the Carr-Purcell-Meiboom-Gill sequence.[14]

PFG measurments were performed using a specially modified heteronuclear probe and home-built pulsed power supply[7]. For each experiment the amplitude of the field gradient, and the duration between the leading edges of the gradient pulses, were held constant while altering the gradient pulse width.

**Bulk Viscosity Measurements.** An Ostwald viscometer was used in a water bath controlled at 298.15 K. Deionized water served as a viscosity standard.[15] Samples were allowed to equilibrate thermally for at least 10 min prior to measurement. The results were the average of at least three measurements.

**Conductivity Measurements.** Conductivity measurements, corrected to 293.15 K, were performed using a Philips PW 9506 conductivity meter with a Philips PW 9510 electrode. 'Blanks' were prepared as for the samples described above except for the omission of sodium chloride.

## Theory

**$^{23}Na$ Relaxation.** Hubbard[16] has shown that for nuclei with spin 3/2, such as $^{23}Na$, both longitudinal and transverse relaxation of magnetization are described by the sum of two exponentially decaying functions. Consequently the relative proportions of the fast and slow processes are experimentally difficult to determine.[17] However, if the nucleus relaxes under 'extreme narrowing conditions' where $\omega_0\tau_c \ll 1$, ($\omega_0$ is the Larmor frequency of the nucleus) we have,

$$1/T_1 = 1/T_2 = \frac{(e^2qQ)^2}{10}\left[1+\frac{\theta^2}{3}\right]\tau_c, \qquad (1)$$

where $eq$ is the electric field gradient at the nucleus, $eQ$ is the nuclear quadrupole moment, and $\theta$ is the asymmetry parameter of the electric field gradient. The electric field gradient arises from the nuclear quadrupole moment interacting with the electrostatic point dipoles in the solvent molecules.[18] The relaxation process may be further complicated by binding, thus resulting in heterogeneous populations of nuclei each being subject to a different set of NMR parameters.[19,20] For the computation of correlation times the quadrupole coupling constant was set to $4.8\times10^6$ rad $s^{-1}$ [3] and the asymmetry parameter was set to zero, due to the high degree of symmetry of the sodium ion and its hydration sphere.

**Rotational Correlation Time, Diffusion, and Viscosity.** The rotational correlation time is related to the solution viscosity by way of the Debye equation,[21]

$$\tau_c = 4\pi\eta r_0^3/(3kT), \qquad (2)$$

where $\eta$ is the solution viscosity, $r_0$ the Stokes radius, $k$ is the Boltzmann constant, and $T$ is the absolute temperature. This model assumes that the solvent molecules surrounding the solute molecules can be treated as a continuum. Gierer and Wirtz[22] attempted to allow for the discontinuous nature of the solvent by including a coefficient of microfriction in their model.

By rearranging Eq. 2 it is possible to calculate the solution viscosity if the correlation time is known. However, for the equation to remain valid the molecular size and shape need to be independent of concentration. Further, if the probe molecule is rotating under 'extreme narrowing condition' then viscosity is inversely proportional to the longitudinal relaxation time.[21] With a knowledge of $r_0$ and $\eta$ it is possible to calculate the translational diffusion coefficient of the probe molecule using the Stokes-Einstein relationship,[2,23]

$$D = k\,T/(6\,\pi\eta r_0). \qquad (3)$$

Conversely, it is easily seen that from a knowledge of the Stokes radius and the translational difusion coefficient we can calculate the solution viscosity.

**PFG NMR Diffusion Measurements.** The attenuation of the spin-echo signal due to the gradient pulses, assuming free-diffusion of the nuclei being observed is given by,[8]

$$S(G)/S(0) = \exp[-D\,\gamma^2 G^2 \delta^2 (\Delta - \delta/3)] \qquad (4)$$

where $S(G)$ and $S(0)$ denote the echo amplitude with and without gradient pulses, respectively; $D$ is the diffusion coefficient, $\gamma$ is the magnetogyric ratio, $\delta$ is the duration of the field gradient pulses and $\Delta$ is the separation between the leading edges of the field gradient pulses.

**Solution Viscosity and Conductance.** The electrical conductivity and microviscosity of a solution are related by the Walden product,[24]

$$\kappa\eta = \text{constant}. \qquad (5)$$

## Results

The $^{23}Na$ spin lattice relaxation times, corresponding correlation times and PFG NMR-determined diffusion coefficients are summarized in Table 1. Examples of PFG NMR spectra are shown in Fig. 1;

the experimental parameters are given in the figure legend.

Table 2 summarizes the conductivity, bulk viscosity, NMR-determined viscosities (from correlation time, $\eta_{\tau_c}$; from PFG diffusion measurement, $\eta_{PFG}$). In calculating the NMR-determined viscosities, it was assumed that the water solution gave the same result when measured by NMR as by Ostwald viscometry. This allowed a value of the Stokes radius for the sodium ion to be calculated as 0.2009 nm from $\tau_c$ and 0.2152 nm from the PFG measurements. This method of determining the Stokes radius took into account the hydration of the ion, otherwise the radius of the ion itself would have been used. Figure 2 is a plot of the three different viscosity estimates versus $1/\kappa$. Figure 3 is a plot of diffusion coefficients estimated from rotational correlation times versus measured diffusion coefficients.

## Discussion

PFG NMR measurements of diffusion in sodium chloride solutions were made on the sodium ion in preference to the chloride ion because of its inherently larger $T_2$ value, thus facilitating the measurements. The PFG NMR method allows diffusion measurements to be conducted on species with shorter $T_2$ values than the steady gradient NMR method.[25] Both $T_1$ and $T_2$ measurements were performed on the sodium nuclei to verify that the 'extreme narrowing condition' held in all samples and thus justify analysis of the relaxation time courses as single exponentials. This was consistent with the results of Lerner and Torchia.[26]

Sutter and Harmon[27] calculated that for the quadrupolar nucleus lithium, when present in an aqueous solution of LiCl, that the electric field gradient produced at the lithium nucleus thus causing the relaxation, is an order of magnitude smaller than the field gradient caused by the electric dipole moment of a water molecule in the lithium hydration shell. It seems reasonable to assume that a similar situation exists for the sodium ion in an aqueous environment. In calculating the nuclear quadrupolar coupling constant it was assumed that the quadrupole coupling arises from randomly fluctuating distortions in the hydration sphere caused by movement of the water molecules. Although this model may be incomplete, because water molecules moving into and out of the hydration sphere may affect the nature of the electric field gradient,[3] calculations emulating those of Sutter and Harmon[27] that allowed for this possibility, gave a similar answer. Thus it may also be necessary to consider the effects of the monovalent ions on water structure. As the $Na^+$ ion is neither a 'structure breaker' nor an electrostrictive 'structure maker,' the sodium ion is probably a reasonable probe of the conditions of the surrounding water molecules.

The Stokes radii used in the $\eta_{\tau_c}$ and $\eta_{PFG}$ calculations were computed from the viscosity of the water solution, as measured by Ostwald viscometry. This, therefore, includes the contribution of the hydration sphere to the "effective" dimensions of the ion. Also, the physical interpretation of what constitutes the actual rotor in the relaxation measurements is unclear, but by calculating $r_0$ in the present manner, an actual physical interpretation of the nature of the rotor is unnecessary.

Because the plot of $D_{\tau_c}$ versus $D_{PFG}$ (Fig. 3) is linear it is reasonable to conclude that the Stokes radius of the hydrated ion does not alter in the different glycerol solutions.

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sion coefficient from relaxation times of the sodium nucleus it is necessary to assume that the nuclear electric field gradients are modulated in time by isotropic molecular diffusion. However, ionic nuclei in electrolyte solutions, probably because of the hydration sphere, experience environments different from those of the bulk solution. Thus, there is some doubt about the validity of relating bulk viscosity to $^{23}Na$ NMR correlation times.[29]

Our results shown in Fig. 2 indicate that, at least in low viscosity glycerol solutions, the Walden product is obeyed for the different electrical conductivities. However, of the three methods of viscosity determination, bulk viscosity gave the worst correlation with the Walden product, while the PFG-determined viscosity gave the best. This was as expected since the conductivity is directly proportional to translational diffusivity. Thus, in simple systems, containing constant concentrations of electrolyte species, conductivity can be used to determine the micro-viscosity or diffusion coefficient.

Of the two methods used for determining the sodium ion diffusion coefficient, the PFG values are more accurate since no assumptions need be made concerning the connection between molecular reorientation and translational diffusion. In this particular system the two methods give surprisingly similar results (within 11%); this is most likely the result of two phenomena. First, the sodium ion is spherical, irrespective of its hydration state. Second, the solvent molecules in this solution are of a similar size to the probe molecule. Whereas in studies of viscosity of the red cell cytoplasm carried out using glycine as the probe molecule, the ratio of the estimates from the two methods for determining the diffusion coefficient, the $T_1$-method and the PFG method, was approximately four.[7] This can be attributed to the non-sphericity of glycine, and the fact that hemoglobin (occupying much of the red cell cytoplasm) is too large to be considered as a solvent continuum.

Table 1. $^{23}Na$ $T_1$ Values, Corresponding Correlation Times, and the Diffusion Coefficient Determined by PFG NMR

| Glycerol | $T_1$ | $\tau_c$ | $D_{\tau_c}$ | $D_{PFG}$ |
|---|---|---|---|---|
| g/100 g | ms | $\times 10^{12}$ s | $m^2 s^{-1} \times 10^9$ | $m^2 s^{-1} \times 10^9$ |
| 0 | 58.0 | 7.48 | 1.200 | 1.120±0.022 |
| 5 | 52.3 | 8.30 | 1.082 | 1.022±0.027 |
| 10 | 46.4 | 9.35 | 0.960 | 0.890±0.020 |
| 15 | 40.7 | 10.66 | 0.842 | 0.804±0.022 |
| 20 | 36.0 | 12.06 | 0.744 | 0.735±0.042 |
| 25 | 30.7 | 14.14 | 0.635 | 0.615±0.023 |
| 30 | 25.8 | 16.82 | 0.534 | 0.545±0.026 |

All measurements were performed at 105.8 MHz and 298.15 K. The $T_2$ measurements (not shown) were, within experimental error, the same as the $T_1$ values. $D_{\tau_c}$ was calculated using $r_0$=0.2009 nm.

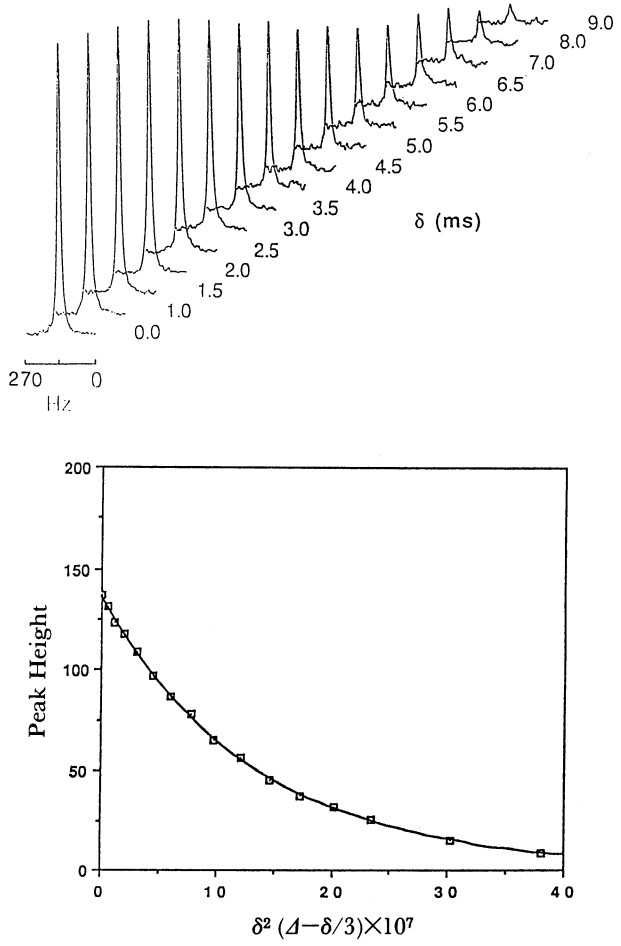


Fig. 1. $^{23}Na$ ion diffusion measurement using PFG NMR. (A) Spin-echo spectra from the least viscous of the glycerol solutions (0 g/100 g). Experimental parameters were $\Delta$=50 ms, $\tau$ (echo time)=50 ms and gradient strength=36.36 G cm$^{-1}$. Seven seconds were allowed between transients to minimize heating from the radio frequency pulses. Spectra were recorded at 298.15 K and 105.8 MHz and are presented in phase sensitive mode. Typical linewidth (no line broadening) is ca. 13 Hz. (B) Analysis of the PFG data: the solid line is the result of regression of Eq. 4 onto the data resulting in $D\gamma^2G^2$=7.424±0.145×10$^5$ s$^{-3}$.

Table 2. Conductivity, Bulk Viscosity and NMR-determined Viscosities

| Glycerol | $\kappa$ | $\eta_B$ | $\eta_{\tau_c}$ | $\eta_{PFG}$ |
|---|---|---|---|---|
| g/100 g | mS cm$^{-1}$ | mPas | mPas | mPas |
| 0 | 9.045 | 0.906 | 0.906 | 0.906 |
| 5 | 8.122 | 1.021 | 1.005 | 0.993 |
| 10 | 7.181 | 1.153 | 1.132 | 1.140 |
| 15 | 6.488 | 1.317 | 1.291 | 1.262 |
| 20 | 5.899 | 1.541 | 1.461 | 1.381 |
| 25 | 5.350 | 1.786 | 1.713 | 1.650 |
| 30 | 4.523 | 2.137 | 2.037 | 1.862 |

All measurements were performed at 298.15 K. $\eta_{\tau_c}$ and $\eta_{PFG}$ were calculated using $r_0$=0.2009 and 0.2152 nm, respectively.

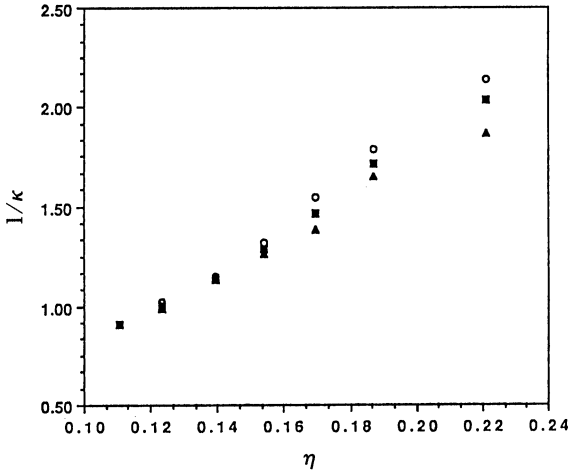


Fig. 2. The inverse of electrical conductivity (mS$^{-1}$ cm) versus the three sets of viscosity (mPas) estimates given in Table 2, (○ $\eta_B$; ■ $\eta_{\tau_c}$; ▲ $\eta_{PFG}$) each made at 298.1 K. The more linear the relationship the closer is Walden's principle obeyed.

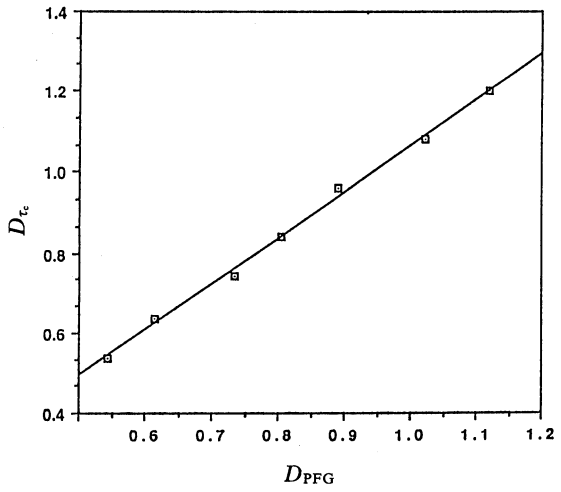


Fig. 3. The diffusion coefficient ($m^2 s^{-1} \times 10^9$) calculated for the sodium ion from the NMR-derived rotational correlation time versus that measured using PFG NMR.

Calculations of diffusion coefficients from the rotational correlation times of non-spherical molecules can be therefore erroneous.

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### References

1) T. Sugawara, M. Yudaska, K. Takahashi, R. Tamamushi, H. Iwamura, and T. Fujiyama, *Bull. Chem. Soc. Jpn.*, **55**, 1959 (1982).
2) A. Einstein, "Investigations on the Theory of the Brownian Movement," ed by R. Furth, Dover Publications, New York (1956).
3) M. M. Civan and M. Shporer, in: "Biological Magnetic Resonance," ed by L. J. Berliner and J. Reuben, Plenum Press, New York (1978), Vol. 1, p. 1.
4) W. S. Price, P. W. Kuchel, and B. A. Cornell, *Biophys. Chem.*, **33**, 205 (1989).
5) Z. H. Endre, B. E. Chapman, and P. W. Kuchel, *Biochem. J.*, **216**, 615 (1983).
6) R. A. Alberty and G. G. Hammes, *J. Phys. Chem.*, **62**, 154 (1958).
7) W. S. Price, B. E. Chapman, B. A. Cornell, and P. W. Kuchel, *J. Magn. Reson.*, **83**, 160 (1989).
8) E. O. Stejskal and J. E. Tanner, *J. Chem. Phys.*, **42**, 288 (1965).
9) P. T. Callaghan, *Aust. J. Phys.*, **37**, 359 (1984).
10) J. Kärger, H. Pfeifer, and W. Heink, *Adv. Magn. Resn.*, **12**, 1 (1988).
11) P. Stilbs, *Prog. Nucl. Magn. Reson. Spectrosc.*, **19**, 1 (1987).
12) R. A. Robinson and R. H. Stokes, "Electrolyte solutions," Butterworths, London (1965), p. 302.
13) R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, **48**, 3831 (1968).
14) S. Meiboom and D. Gill, *Rev. Sci. Instrumen.*, **29**, 688 (1958).
15) R. C. Weast, "Handbook of Chemistry and Physics," CRC Press, Cleveland, Ohio (1984).
16) P. S. Hubbard, *J. Chem. Phys.*, **53**, 985 (1970).
17) G. L. Mendz, P. W. Kuchel, and G. R. Wilcox, *Biophys. Chem.*, **30**, 81 (1988).
18) H. G. Hertz, *Ber. Busen-Ges. Physik. Chem.*, **77**, 531 (1973).
19) A. G. Marshall, *J. Chem. Phys.*, **52**, 2527 (1970).
20) M. Goldberg and H. Gilboa, in: "Nuclear Magnetic Resonance in Molecular Biology," ed by B. Pullman, D. Reidel Co. Dordrecht, Holland (1978), p. 481.
21) N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, **73**, 679 (1948).
22) A. Gierer and K. Wirtz, *Z. Naturforsch.*, **8a**, 532 (1953).
23) G. G. Stokes, *Trans. Cambridge Phil. Soc.*, **9**, 8 (1856).
24) P. Atkins, "Physical Chemistry," Oxford University Press, (1978), p. 836.
25) B. M. Braun and H. Weingärtner, *J. Phys. Chem.*, **92**, 1342 (1988).
26) L. Lerner and D. A. Torchia, *J. Am. Chem. Soc.*, **108**, 4264 (1986).
27) E. J. Sutter and J. F. Harmon, *J. Phys. Chem.*, **79**, 1958 (1975).
28) K. L. Yam, D. K. Anderson, and R. E. Buxbaum, *Science*, **241**, 330 (1988).
29) M. St. J. Arnold and K. J. Packer, *Molec. Phys.*, **14**, 241 (1968).