ESR STUDY OF ROTATIONAL MOTIONS OF SPIN-LABELED LONG-CHAIN NITROXIDES IN SOLUTION

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Information on the rotational motion of various types of spin-labelled long-chain nitroxides was obtained from the anisotropic ESR signals. By varying the position of the nitroxide moiety along the chain, the intrinsic flexibility of molecular chain was examined. The rotational correlation times (τ_c) of spin-labelled long-chain nitroxides were about 10^{-10} s. The activation parameters for rotational motion were estimated from the temperature and pressure dependences of τ_c. It was found that the activation entropy observed for methyl 16-doxyl stearate is obviously large compared with those for methyl 5- and methyl 12-doxyl stearates. The results suggest an increase in the molecular motions at the end of the hydrocarbon chain. The viscosity dependence of τ_c is discussed in terms of the difference in the strength between solute–solvent and solvent–solvent interactions.

INTRODUCTION

Spin labelling is a convenient technique for acquiring knowledge of the structure of biological membranes, multilayers and micelles.[1] Furthermore, studies via the spin-label technique provide useful information about the types and rates of motions at a molecular level. The anisotropy observed in an ESR spectrum is directly related to the rotational mobility of spin labels, and this technique is sensitive to molecular motions with rates faster than 10^7 S^{-1}.

Conformations of long-chain molecule in a liquid medium are continually changing. This motion is determined by the length of the chain, temperature viscosity of the solvent, etc. By varying the position of the nitroxide moiety along the chain, it is possible to examine the segmental motion of the part of the chain to which the nitroxide moiety is attached.

The correlation time for the rotational diffusion of molecules is generally correlated with the macroscopic viscosity of the solvent according to the Stokes–Einstein–Debye equation. In many cases, the relationship has been used successfully for a macroscopic sphere rotating in a continuous medium. However, as McClung and Kivelson suggested,[2] its justification is

questionable for non-spherical particles undergoing anisotropic rotational diffusion. Long-chain spin-labelled nitroxide is a non-spherical particle, and the discussion on their rotational diffusion seems not to have been done satisfactorily.

In this study, spin-labelled compounds consisting of the nitroxide moiety attached at different positions along the hydrocarbon chain were used to examine the motions and ordering of a long chain and the viscosity dependence of rotational diffusion is discussed.

EXPERIMENTAL

Spin-labelled nitroxides (Scheme 1) were purchased from Aldrich Chemical and used without further purification. To avoid line broadening from intermolecular spin exchange, the concentrations of the sample solutions of spin-labelled nitroxides were chosen as low as 5×10^{-4} mol dm^{-3}. Sample solutions were deoxygenated by bubbling nitrogen.

ESR spectra were recorded on a JEOL FE–3XG X-band spectrometer with 100 kHz field modulation. Temperature was controlled by flowing nitrogen around the sample tube using a JEOL variable-temperature regulator. The high-pressure technique and procedures for ESR measurements were almost the same as those described elsewhere.[3]

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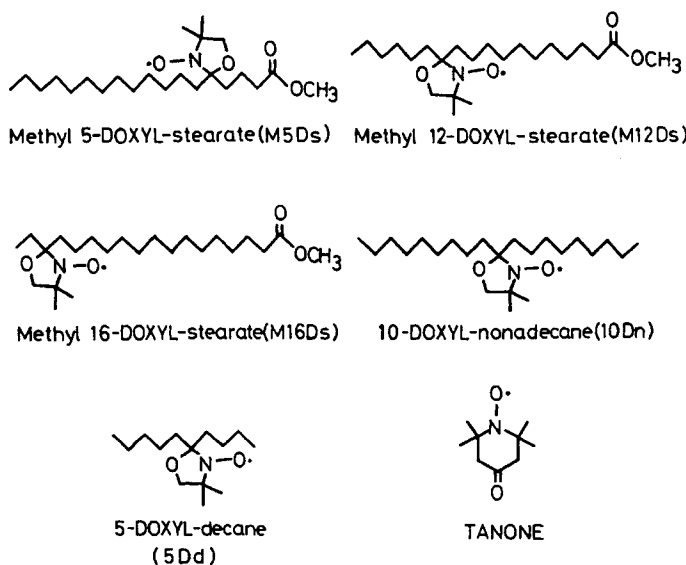
Scheme 1

RESULTS AND DISCUSSION

Estimations of τ_c and activation parameters

We observed ESR spectra which consist of three absorption lines for spin-labelled nitroxides. Such a shape of the ESR spectrum suggests that the rotational motion of radical species is rapid and the following simple analysis is allowable. From the line broadening of ESR signals, the rotational correlation time (τ_c) of the nitroxide radical can be estimated by using the following equation:

$$\tau_c = A\Delta H_{(m = +1)}[(I_{(m = +1)}/I_{(m = -1)})^{1/2} - 1] \qquad (1)$$

where $\Delta H_{(m = +1)}$ is the peak-to-peak linewidth (in G) of the low-field absorption line, $I_{(m = +1)}$ and $I_{(m = -1)}$ are the corresponding peak-to-peak heights for the low- and high-field lines, respectively, and the constant A has been given to be $6\cdot6 \times 10^{-10}$ s G^{-1}.[4] The τ_c-values for various spin-labelled nitroxides at 253–328 K are given in Table 1. Table 2 gives the activation parameters, estimated from the temperature dependence of τ_c.

The correlation time for the isotropic rotational diffusion is generally formulated by the Stokes–Einstein–Debye (SED) equation:

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

where a is the effective hydrodynamic radius of a radical and the other symbols have their usual meanings. As can be seen in Table 1, the observed τ_c-values are in the order of 10^{-10} s, and decrease with increasing temperature, in agreement with the prediction of

equation (2). When one considers the position of the nitroxide moiety along the hydrocarbon chain, the τ_c value of M5Ds is found to be comparable with those of M12Ds and 10Dn. However, the τ_c value of M16Ds, with the nitroxide group attached the end of the hydrocarbon chain, is about half that of M5Ds, and is comparable to that of short-chain 5Dd.

When we examined the activation parameters shown in Table 2, a large difference among the $\Delta H^{\ddagger}$ values of various types of spin-labelled nitroxides was not observed. As Yoshioka suggested,[5] $\Delta H^{\ddagger}$ denotes the energy of formation of holes or free volume in a medium resisting intermolecular forces, and thus is a measure of intermolecular force. If equation (2) holds for the rotational diffusion of spin-labelled nitroxides, the observed $\Delta H^{\ddagger}$ should agree with the activation enthalpies ($\Delta H_\eta^{\ddagger}$) of the solvent viscosity. The $\Delta H_\eta^{\ddagger}$ values, calculated from the available viscosity data, were 35 kJ mol^{-1} for 5-methylheptan-3-ol, 16 kJ mol^{-1} for propan-1-ol, 11 kJ mol^{-1} for tetradecane, 13 kJ mol^{-1} for octylbenzene and 8 kJ mol^{-1} for butylbenzene. As can be seen in Table 2, the $\Delta H^{\ddagger}$ values of spin-labelled nitroxides in alcohols are smaller than the $\Delta H_\eta^{\ddagger}$ values. In contrast, the $\Delta H^{\ddagger}$ values in aprotic solvents are larger than the $\Delta H_\eta^{\ddagger}$ values. These results may be ascribed to the strength of solute–solvent and solvent–solvent interactions.

The $\Delta S^{\ddagger}$ value of M16Ds is clearly large compared with those of M5Ds and M12Ds. Yoshioka[5] suggested that the rotation of the N—O group in a long-chain nitroxide is the sum of that around the centre of gravity and that caused by internal rotation. The rate constant

Table 1. Rotational correlation times of various types of spin-labelled nitroxides

Solvent	Compound	$10^{10}\tau_c(S)$ (at 1 bar)												
		−25	−15	−5	0	5	10	15	20	25	30	35	45	55 °C
5-methyl heptan-3-ol	M5Ds				17·5	13·1		8·73		5·94		3·74		
	M12Ds				17·8	14·6		9·12		5·86		3·80		
	M16Ds			11·5	9·08	7·06		4·54		2·76		1·80		
	10Dn					16·4		10·0		6·42		4·35		
	5Dd			1·06	7·42	5·78		3·64		2·22				
	TANONE		8·28	4·65	3·55	2·51		1·43		0·85				
Propan-1-ol	M5Ds	7·90	5·87	4·31	3·73	3·30		2·47		1·97				
	M12Ds	8·38	6·16	4·62	4·03	3·50		2·69		2·05				
	M16Ds	4·07	2·92	2·23	1·99	1·68		1·28		1·01				
	TANONE	1·15	0·81	0·65	0·54	0·49		0·40		0·33				
Tetradecane	M5Ds					3·50		2·52		1·85			1·28	1·02
	M12Ds					3·76		2·81		2·10		1·63	1·29	1·05
	M16Ds					1·35		0·97		0·73		0·56	0·47	0·36
	TANONE					0·15		0·13		0·10		0·089	0·075	
Nitrobenzene	M5Ds					4·46	3·70	3·16	2·88	2·60	2·28			
	M16Ds					2·04	1·66	1·44	1·26	1·11	0·99			
	TANONE					0·40	0·35	0·32	0·30	0·28	0·23			
Octylbenzene	M5Ds	15·0	9·17	6·36	5·37	4·56		3·35		2·43				
	M12Ds	14·6	9·93	6·85	5·65	4·83		3·55		2·55				
	M16Ds	6·41	4·13	2·70	2·19	1·88		1·31		0·99				
	TANONE	0·79	0·53	0·38	0·32	0·30		0·24		0·19				
Butylbenzene	M12Ds	5·28	3·87	2·89	2·50	2·16		1·66		1·32				
	TANONE	0·42	0·32	0·27	0·24	0·22		0·19		0·16				

Table 2. Activation parameters for rotational motion, with probable errors in parentheses

Solvent	Compound	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}_{298\,K}$ (J K^{-1} mol^{-1})	α	a/a_{TANONE}
5-Methylheptan-3-ol	MSDs	28 (1)	26 (2)	0·68	1·8
	M12Ds	28 (1)	26 (1)	0·71	1·8
	M16Ds	29 (1)	34 (1)	0·69	1·4
	10Dn	29 (1)	29 (1)	0·66	1·9
	5Dd	31 (1)	45 (2)	0·73	1·3
	TANONE	34 (1)	63 (2)	0·72	1·0
Propan-1-ol	M5Ds	15 (1)	−10 (1)	0·84	1·9
	M12Ds	15 (1)	−10 (1)	0·84	1·9
	M16Ds	15 (1)	−3(1)	0·90	1·4
	TANONE	13 (1)	−2 (1)	0·82	1·0
Tetradecane	M5Ds	16 (1)	−6 (2)	1·2	2·3
	M12Ds	17 (1)	−3 (1)	1·2	2·4
	M16Ds	17 (1)	7 (1)	1·3	1·6
	TANONE	10 (1)	−1 (1)	0·72	1·0
Nitrobenzene	M5Ds	16 (1)	−9 (2)	1·2	2·0
	M16Ds	18 (1)	5 (2)	1·3	1·5
	TANONE	11 (1)	−4 (3)	0·92	1·0
Octylbenzene	M5Ds	19 (1)	3 (1)	1·4	2·2
	M12Ds	19 (1)	1 (1)	1·3	2·4
	M16Ds	20 (1)	14 (1)	1·5	1·6
	TANONE	15 (1)	10 (2)	1·0	1·0
Butylbenzene	M12Ds	15 (1)	−7 (1)	1·5	2·0
	TANONE	9 (1)	−7 (1)	0·98	1·0

for the rotation of spin-labelled nitroxides can be expressed as follows:[5]

$$k = (kT/h)[\exp(\Delta S_g^{\ddagger}/R) + \exp(\Delta S_{int}^{\ddagger}/R)]\exp(-\Delta H^{\ddagger}/RT) \quad (3)$$

where $\Delta S_{int}^{\ddagger}$ and $\Delta S_g^{\ddagger}$ denote the activation entropies for internal rotation and the rotation around the center of gravity, respectively. Since M16Ds, M12Ds and M5Ds have the same molecular weight and similar structures, the difference in their $\Delta S_g^{\ddagger}$ values must be small. The nitroxide moiety of M5Ds and M12Ds is located in the vicinity of the centre of gravity. In fact, the $\Delta S^{\ddagger}$ values obtained for M5Ds are comparable to those of M12Ds, and hence, it could reasonably be assumed that $\Delta S^{\ddagger}$ of M5Ds corresponds to $\Delta S_g^{\ddagger}$ for the methyl doxylstearates used here. M16Ds has the several single bonds between the nitroxide moiety and the centre of gravity, which contribute to the internal rotation. Therefore, the large $\Delta S^{\ddagger}$ values of M16Ds may be attributable to the increase in motional freedom involving the internal rotation ($\Delta S_{int}^{\ddagger}$). Using equation (3) and $\Delta S^{\ddagger}$ values ($\Delta S_g^{\ddagger}$) of M5Ds, the $\Delta S_{int}^{\ddagger}$ values of M16Ds in various solvents can be calculated and are given in Table 3. It is difficult to give a clear explanation for the negative $\Delta S^{\ddagger}$ values for the rotational diffusion. Although the kind of interaction that is operative has not yet been clarified,

Table 3. Entropies of activation for rotational motions in various solvents

Solvents	$\Delta S_g^{\ddagger}$ (J K^{-1} mol^{-1})	$\Delta S_{int}^{\ddagger}$ (J K^{-1} mol^{-1})
Propan-1-ol	−10	−8
Nitrobenzene	−9	4
Tetradecane	−6	5
Octylbenzene	3	12
5-Methylheptan-3-ol	26	30

the compensated relationship between $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ roughly holds for the solvent effect (Table 2), and the $\Delta S_{int}^{\ddagger}$ value increases with increasing $\Delta S_g^{\ddagger}$ value (Table 3).

The τ_c values given in Table 4 increase as the external pressure increases. The activation volume ($\Delta V^{\ddagger}$) for the rotational motion of nitroxide was estimated from the ln$k$ vs pressure plot according to the following equation:

$$\Delta V^{\ddagger} = -RT(\partial \ln k/\partial P)_T = RT(\partial \ln \tau_c/\partial P)_T \quad (4)$$

The apparent activation volume ($\Delta V_\eta^{\ddagger}$) for the rotational diffusive process can be calculated from equations (2) and (4):

$$\Delta V_\eta^{\ddagger} = RT(\partial \ln \eta/\partial P)_T \quad (5)$$

The $\Delta V_\eta^{\ddagger}$ values, determined from the available $\eta - P$ data,[6] are 41 cm^3 mol^{-1} for 5-methylheptan-3-ol and 18 cm^3 mol^{-1} for propan-1-ol. The observed $\Delta V^{\ddagger}$ values are about 35 cm^3 mol^{-1} for 5-methylheptan-3-ol and 15 cm^3 mol^{-1} for propan-1-ol, independent of the position of the nitroxide moiety along the hydrocarbon chain.

Viscosity dependence of τ_c

Figure 1 shows the representative plots of τ_c against η/T. It should be noted that the plots in 5-methylheptan-3-ol and propan-1-ol fall on the same curve irrespective of temperature and pressure effects. The variation of viscosity with temperature inevitably involves unfavourable energetic problems. Jonas[7] and Hwang et al.[8] have pointed out the importance of experiments in which the solvent viscosity is varied through pressure at a fixed temperature. Recently, Wakai and Nakahara[9] have reported differences in the pressure- and temperature-variable viscosity dependences of the rotational correlation times for D$_2$O in CH$_3$CN and CHCl$_3$. However, in the present system, we unfor-

Table 4. Rotational correlation times at high pressure in 5-Methylheptan-3-ol and propan-1-ol

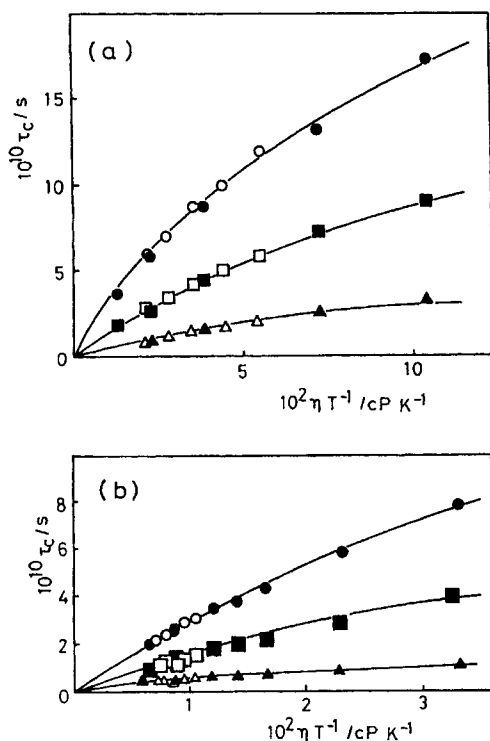|		$10^{10}\tau_c$(S) (at 298 K)					$\Delta V^{\ddagger}$ (cm^3 mol^{-1})	$\alpha \Delta V_\eta^{\ddagger}$ (cm^3 mol^{-1})
		1	150	300	450	600 bar		
5-Methylheptan-3-ol	M5Ds	5·94	7·09	8·90	10·0	12·1	34	28
	M12Ds	5·86	6·42	9·09	10·1	12·0	35	29
	M16Ds	2·76	3·43	4·18	5·06	5·91	37	28
	10Dn	6·42	7·95	9·46	11·1	13·3	34	27
	5Dd	2·22	2·86	3·48	4·32	5·40	38	30
	TANONE	0·85	1·08	1·24	1·60	1·84	36	30
Propan-1-ol	M5Ds	1·97	2·13	2·39	2·77	2·95	18	15
	M12Ds	2·05	2·26	2·38	2·84	3·07	13	15
	M16Ds	1·01	1·11	1·26	1·36	1·53	17	16
	TANONE	0·33	0·36	0·39	0·43	0·46	15	15

Figure 1. Relationship between τ_c and η/T in (a) 5-methylheptan-3-ol and (b) propan-1-ol. (●,○) M5Ds; (■,□) M16Ds; (▲,△) TANONE. Solid and open symbols are for temperature- and pressure-variable viscosity variations, respectively

tunately could not observe differences in properties which are changed by applying temperature or pressure.

Equation (2), given by the SED law, can be applied to the rotational diffusion of a spherical solute with stick boundary conditions. Ben–Amotz and Drake[10] suggested that molecular diffusion in liquids gradually approaches the stick hydrodynamic condition as the solute size increases. As the solute size becomes comparable to or smaller than the solvent size, the solute experiences a reduced frictional force. Therefore, the SED law is usually modified for rotational dynamics:[11]

$$\tau_c = (4\pi a^3 \eta/3kT)\theta + \tau_0 \qquad (6)$$

where θ is the rotational friction coefficient and depends on the boundary conditions and the shape of the rotating molecule, and τ_0 is the rotational correlation time at zero viscosity. Equation (6) predicts a linear relationship between τ_c and η/T, whereas the plots in alcohols deviate downwards with an increase in η/T value and show curvilinearity (Figure 1). There have been several measurements of diffusion coefficients (D) in different systems for which an expression of the form $D \propto 1/\eta^a$

was sought for microscopic diffusion.[12] Thus, the experimental results for τ_c fit well the following empirical expression:

$$\tau_c = (4\pi a^3 \eta^a)/(3kT) \qquad (7)$$

The value of a can be taken as a measure of the deviation of the experimental results from the SED law, and corresponds to solute–solvent frictional coupling. The fitting parameters a were obtained from the experimental results by non-linear regression (OPTIM), and are given in Table 2. The a values observed in alcohols are obviously small compared with those in aprotic solvents. In the case of alcohols, a values of less than unity indicate that coupling between the solvent and solvent is stronger than that between the solute and solvent.

For the rotational diffusion of long flexible molecules, such as methyl doxylstearates, the predictions of the slip and stick limits seem to be a future problem.[10,11] At least, it could safely be said that a series of methyl doxylstearates have similar rotational motions and the rotational diffusion is close to a sticking boundary condition, judging from the size of the solute and solvent molecules.[10] As shown in Figure 1, however, there are large differences in the viscosity dependences of the τ_c values of M5Ds and M16Ds. A measure of non-hydrodynamic to hydrodynamic behaviour can be derived from the slope of the τ_c vs η/T plots [equation (6)]. Although the non-linear form is better than the linear form in the present data fit, we tentatively estimated slopes at $\eta/T = 0$ for M5Ds and M16Ds using the data in Table 1. The slope ratio of M16Ds to M5Ds in 5-methylheptan-3-ol was estimated to be 0·48. If the slope of M5Ds were to mean perfect hydrodynamic behaviour, the slope ratio indicates a 52% deviation from hydrodynamic behaviour in M16Ds. This is not the case. Rather, we consider that the flexibility at the end of the long chain allows for more rapid reorientation than that at the centre, resulting in a small τ_c value which is taken as an indication of segmental motions. The observations on 10Dn and 5Dd also support the idea presented above.

According to equations (4) and (7), the apparent activation volume ($\Delta V^{\ddagger}_{\eta,a}$) for the rotational motion can be expressed as follows:

$$\Delta V^{\ddagger}_{\eta,a} = RTa(\partial\ln\eta/\partial P)_T = a\Delta V^{\ddagger}_{\eta} \qquad (8)$$

The $\Delta V^{\ddagger}_{\eta,a}$ values in 5-methylheptan-3-ol and propan-1-ol were recalculated, and are given in Table 4. Owing to the approximate nature of the above treatment, the agreement between $\Delta V^{\ddagger}_{\eta,a}$ and $\Delta V^{\ddagger}$ could be said to be fair.

By fitting the experimental results to equation (7), the effective hydrodynamic radii (a) for rotation in solution can be obtained. In Table 2, the estimated radius ratios of the spin-labelled nitroxide to TANONE (a/a_{TANONE}) are listed. The a/a_{TANONE} values are about 2·0 for M5Ds

and M12Ds, and are comparable to that for 10Dn irrespective the nature of the solvent. It is interesting that the apparent molecular size of M16Ds is only 1·5 times that of TANONE, and is comparable to that of 5Dd. In the SED treatment, a hydrodynamically equivalent shape of rigid TANONE molecule is regarded as a sphere. Plachy and Kivelson[13] measured the translational diffusion constant of di-tert-butyl nitroxide (DTBN) and found that it obeys the Stokes–Einstein relationship with a radius $a = 3·2$ Å. We can estimate the hydrodynamic radius of a molecule with the help of Corey–Pauling–Koltum models (CPK) using the average of the three axial radii of DTBN as a standard. Assuming that the long-chain molecule is linear and rigidly extended, the a/a_{TANONE} value, estimated from CPK models, is 3·4 for M5Ds, M12Ds and M16Ds. When the SED equation is applied to the rotational motion of non-spherical long-chain compound, the segmental motion of the chain may be reflected in the effective hydrodynamic radius. Therefore, the above small a/a_{TANONE} values suggest an increase in the rotational motion at the end of the chain.

Rotational diffusion of TANONE

Figure 2 shows the plots of τ_c against η/T for the rotational diffusion of TANONE in nitrobenzene, butylbenzene and octylbenzene. The a values of the plots in the three solvents are almost unity, and the intercepts are negligibly small. According to equation (2), we can easily carry out the prediction of the stick limit for the rigid TANONE molecule. The solid line in Figure 2 represents the plots under the sticking boundary condition [$\theta = 1$ in equation (6)] using the mean radius $a = 3·9$ Å for TANONE.[14] From the slopes of the

plots in Figure 2, the θ values were estimated to be 0·12 in octylbenzene, 0·23 in nitrobenzene and 0·25 in butylbenzene. These are acceptable values.[11,15]

To improve the agreement between the hydrodynamic theory and experiment, Hu and Zwanzig[16] used a slipping boundary condition. Further, Fury and Jonas[15] improved the slip limit predictions by introducing a weighting factor which emphasizes molecular size. Applying the slip/stick ratio of Hu and Zwanzig to the rotational diffusion, they gave the following equations:[15]

$$\tau_c = (4\pi a^3 \eta \theta_{eff})/(3kT) \qquad (9)$$

$$\theta_{eff} = \Sigma[\rho_i^2 \theta(\rho_i)/\Sigma\rho_i^2] \qquad (10)$$

where θ_{eff} is the effective slip/stick ratio for the motion giving rise to relaxation. Axial ratios ρ_i for rotation about the ith axis are estimated with CPK atomic models.[16] The size of spheroid TANONE was estimated to be 2·9, 4·0, and 4·7 Å. The value of θ_{eff} can be calculated to be 0·047 according to equation (10). The dashed line in Figure 2 shows the prediction of the slip limit. The observed τ_c values fall between those predicted by the slip and stick models, and suggest that the rotational diffusion of TANONE is closer to the slip boundary condition than the stick boundary condition. The θ value decreases as the solvent size increases from butylbenzene to octylbenzene, clearly indicating the transition towards slip boundary conditions. The qualitative explanation of these results is that the rotational motion of TANONE can be understood by envisaging a small particle rotating among large solvent molecules with large interstitial gaps.

Figure 2. Relationship between τ_c and η/T for rotational diffusion of TANONE in (◑) octylbenzene, (○) nitrobenzene and (●) butylbenzene

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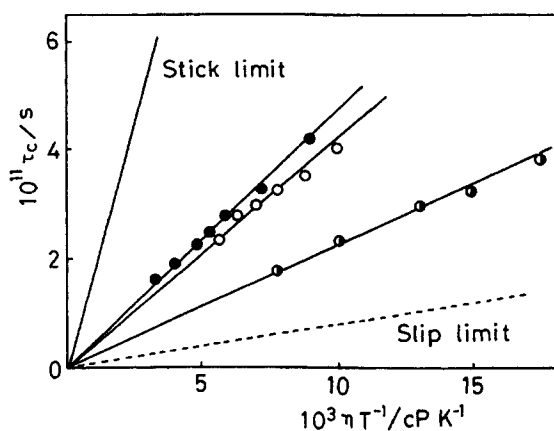13. W. Plachy and D. Kivelson, *J. Chem. Phys.*, **47**, 3312 (1967).
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