

## COMMUNICATIONS

### The use of electron spin resonance to measure microviscosity

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**Abstract**—The viscosities of a series of mixtures of glycerol and water were measured by electron spin resonance (ESR), photon correlation spectroscopy and Ostwald viscometry. Close agreement was obtained between the last two methods but viscosity as measured by ESR was always significantly lower. The difference, the magnitude of which depended on the spin probe used, was attributed to interaction between water and glycerol. Similar results were obtained using water-sorbitol and water-sucrose mixtures.

The technique of electron spin resonance (ESR) has been used to measure the so-called microviscosity or internal viscosity of a variety of systems. Thus, the internal viscosity of erythrocytes has been studied by Daveloose et al (1983) and Morse (1985), and of gelatin gels by Charkoudian (1983). ESR is potentially useful in the measurement of the microviscosity of a medium through which diffusion occurs. The medium resists the spinning of the charged probe, and so gives a measure of the resistance which is encountered by the diffusing substance. The principal advantage claimed for the method is that it is rheologically non-destructive. In the case of gel and mucus-like structures, the microviscosity can be very different from the bulk viscosity. Thus Armstrong et al (1987) and Gebre-Mariam et al (1989) studied the diffusion of molecules through glycerogelatin matrices representing the shells of soft gelatin capsules, and found that the rate of diffusion was linearly related to microviscosity, but wholly unrelated to bulk viscosity.

It is known that in a glycerogelatin shell, some of the water is associated with the gelatin, and hence does not form part of the diffusion medium, which is mainly a mixture of glycerol and water. It was thought that by measuring the microviscosities of the gels and of mixtures of glycerol and water, the composition of the interstitial fluid in the gel could be established, and hence an estimate made of the amount of water taken up by the gelatin. Because of the relative complexity of determining viscosity by ESR, it was hoped to measure viscosities of the glycerol-water mixtures by a simpler technique, such as Ostwald viscometry or photon correlation spectroscopy (PCS). The last two methods gave almost identical results, but these were significantly different from those measured by ESR. This communication investigates these differences.

#### Materials and methods

**Materials.** Glycerol, sorbitol and sucrose were obtained from BDH Ltd (Poole, UK) and used without further purification. The ESR probes used were 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy (TEMPOL), 4-oxo-2,2,6,6-tetramethylpiperidinyloxy (OTEMPO), di-tert-butyl nitroxide (DTBN) and 1,2-doxyl

stearic acid (DSA). All were obtained from Aldrich Chemical Company Ltd, Gillingham, UK.

**Measurement of viscosity by ESR.** A solution of the probe in water was added to the mixture of glycerol and water to give a final probe concentration of  $10^{-4}$  M. The samples were degassed and transferred to capillary tubes. ESR spectra were recorded on a Varian E3 X-band spectrometer at a power level of 5 mW, using 100 kHz field modulation. The temperature was kept constant at 25°C.

The mobility of the probe was assessed in terms of the rotational correlation time ( $\tau$ ), which is determined from the relative peak heights of the ESR triplet and the line width of the control line. The rotational correlation time, which can be thought of as the time taken by the probe to rotate through an angle of 1 radian around its axis, is given by equation 1 (Kivelson 1960).

$$\tau = 6.5 \times 10^{-10} \Delta H_0 \left[ \sqrt{\frac{h_0}{h_{-1}}} - 1 \right] \quad (1)$$

where  $h_0$  and  $h_{-1}$  are the amplitudes of the central and high fields, respectively, and  $H_0$  is the line width in gauss. The viscosity of water ( $\eta$ ) is known, and the rotational correlation times in water and glycerol-water mixtures are measured. Hence the viscosities of the mixtures can be calculated from equation 2.

$$\frac{\tau_{\text{mixture}}}{\tau_{\text{water}}} = \frac{\eta_{\text{mixture}}}{\eta_{\text{water}}} \quad (2)$$

**Measurement of viscosity by Ostwald viscometry.** Viscosities were determined from flow times ( $t$ ) of the solutions in calibrated U-tubes and their densities, using equation 3. Densities were measured in a DM602 densitometer (Anton Parr).

$$\frac{t_{\text{mixture}}}{t_{\text{water}}} = \frac{\eta_{\text{mixture}}}{\eta_{\text{water}}} \times \frac{\text{density of mixture}}{\text{density of water}} \quad (3)$$

**Measurement of viscosity by dynamic light scattering.** This was determined using a Malvern Instruments photon correlation spectrophotometer, type 144, with a 64 channel multibit correlator K7025 interfaced to a Commodore 32 PET computer, a 15 mW helium-neon laser (SpectraPhysics) and a dual trace oscilloscope. Each solution was probed with monodisperse polystyrene spheres, diameter 91 nm. Scattered light was collected at an angle of 90° and the four signals evaluated from the Stokes-Einstein equation (eqn 4), using a Malvern Applications Program.

$$D = \frac{kT}{6\pi\eta r} \quad (4)$$

where  $k$  is the Boltzmann constant,  $T$  absolute temperature,  $D$  the diffusion coefficient and  $r$  the particle radius. Hence the viscosity  $\eta$  can be calculated.

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## Results and discussion

The viscosities of a series of glycerol-water mixtures, measured using an Ostwald viscometer, PCS and ESR are shown in Table 1. The Ostwald and PCS results are virtually identical, but are both considerably greater than the ESR viscosities. The ESR results agree closely with those of Charkoudian (1983). The divergence of the ESR results increases with the proportion of glycerol in the mixture. Viscosity measurements using spin labels require that the solvent does not interact with the probe (Morse et al 1979). Hwang et al (1975) studied the motion of TEMPONE (2,2,6,6-tetramethyl-4-piperidone *N*-oxide) in 85% glycerol, and found that motion of this radical deviated significantly from that predicted by simple Brownian motion. This they attributed to hydrogen-bond formation between the probe and the polyol. Similarly, Schreier et al (1978) suggested that hydrogen-bonding occurs between TEMPOL and glycerol. This was considered unlikely in the present investigation, since complexation between glycerol and probe would increase the recorded viscosity, rather than cause the observed decrease. The suggestion was nevertheless investigated. UV spectra of the probe were recorded in solvent mixtures, and were identical in all combinations of glycerol and water, indicating that the probe either does not interact, or interacts identically in all mixtures.

The viscosity of a liquid varies with temperature according to equation 5 (Eyring 1936).

$$\eta = Ae^{\Delta G/RT} \quad (5)$$

Where  $A$  is a constant, and  $\Delta G$  the free energy required to initiate flow between molecules. If there were preferential interaction between the probe and either liquid component, the ESR method would yield a different free energy from the other two. The viscosities of a 0.226 mol fraction solution of glycerol in water were measured by ESR, using TEMPOL, Ostwald viscometry and PCS, over a range of temperatures from 25 to 70°C. Fig. 1 shows the plots of  $\ln \eta$  against  $1/T$  for the three methods. The lines are parallel, indicating that  $\Delta G$  does not vary. Complexation between probe and components of the mixtures is therefore unlikely.

By analogy with vapour pressures and other colligative properties, one would expect viscosities of ideal binary liquid systems to vary rectilinearly from one pure component to the other. In our systems, the plot of viscosity against composition is curved, suggesting interaction between water and glycerol occurs, the first part of the plot representing increasing concentrations of a complex in aqueous solution, and the second representing decreasing quantities of a complex in glycerol solution. Scrutiny of the plot of viscosity, using TEMPOL as a probe, against mol fraction concentration of glycerol (Fig. 2) suggests that the first four points follow a straight line. Regression analysis gave a line which crosses the extrapolated line joining the last two points at  $X_2$  (mol fraction concentration

Table 1. Viscosities ( $\text{Pas} \times 10^3$ ) of glycerol-water mixtures.

Mole fraction glycerol	Method					
	Ostwald	PCS	ESR			
			TEMPO	OTEMPO	DSA	DTBN
0.000	0.89	0.89	0.89	0.89	0.89	0.89
0.046	1.49	1.56	1.37	1.23	1.33	1.12
0.077	2.00	2.17	1.57	1.76	1.92	1.41
0.115	2.97	3.07	1.87	2.04	2.91	1.82
0.163	4.63	4.64	2.53	2.47	4.11	1.94
0.226	8.00	8.13	4.36	3.63	6.32	2.48
0.313	14.20	14.34	6.42	5.59	—	4.84
0.439	32.32	33.87	13.70	10.32	—	11.45

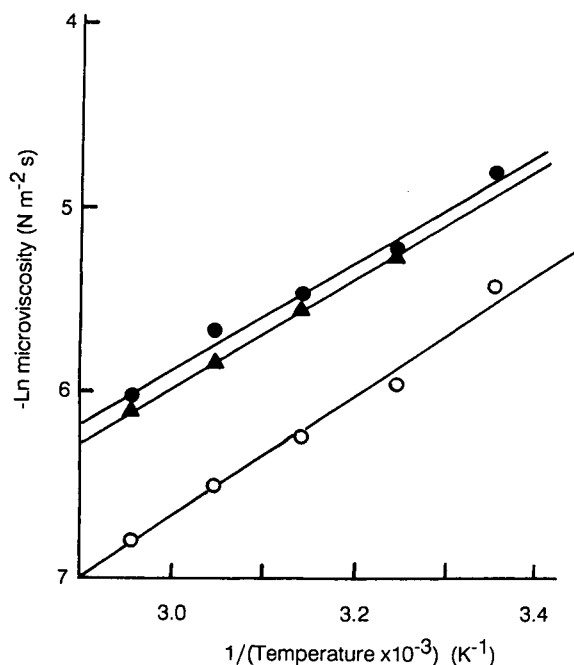


Fig. 1. Arrhenius type plots of viscosities of 60% glycerol in water, measured by different methods.  $\blacktriangle$ , Ostwald viscometry;  $\bullet$ , photon correlation spectroscopy;  $\circ$ , electron spin resonance.

of glycerol) = 0.25, indicating 1:3 complexation. In the absence of contrary experimental evidence, this is the anticipated stoichiometric ratio of glycerol to water, since the glycerol molecule has 3 hydroxyl groups, each theoretically capable of complexing with water. A similar treatment of the Ostwald viscometer results produced the same molecular ratio.

Keith et al (1970) pointed out that equations which depended on line broadening, such as equation 1, assume isotropic motion. It is possible that the difference between Ostwald viscometer and

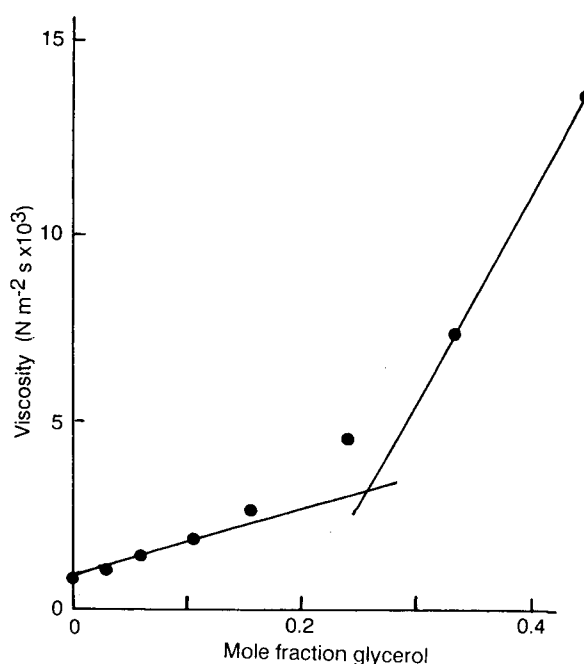


Fig. 2. ESR viscosities of glycerol-water mixtures, using TEMPOL as probe.

PCS results, on the one hand, and ESR results on the other, are a consequence of anisotropy in the glycerol-water system, arising from the complexation suggested above. One could speculate further, and suggest water bridges joining one glycerol hydroxyl to a hydroxyl group in another glycerol molecule, giving a polymolecular network, distributed throughout the liquid in excess. It follows that the Ostwald and PCS methods are measuring bulk viscosity, and the ESR method is measuring the viscosity of the interstitial fluid, and that this is the cause of the viscosity differences under investigation.

On the basis of this argument, one would anticipate similar behaviour with the other polyols, sorbitol and sucrose. Similar plots to Fig. 2 were obtained, suggesting complexation, but the stoichiometric ratios were different from those obtained with glycerol. Plots of ESR viscosities against Ostwald viscosities gave straight lines, and regression data are given in Table 2. The correlation coefficients indicate good relationships within systems, but the wide variation between slopes indicates that, while the systems behave similarly, the precise ways in which they behave are different. The most important conclusion is that the observed deviations of the ESR viscosities from the other two methods can be attributed to the same mechanism as that suggested for the glycerol-water mixtures.

Table 1 also reveals that viscosities obtained by ESR are probe dependent. This behaviour could be explained on the basis that different probes must experience different resistances to rotation, probably due to their molecular shapes, and this, coupled with the fact that magnetic parameters will vary from one nitroxide to another, would lead to the differences in viscosity. TEMPOL, OTEMPO and DTBN have similar geo-

Table 2. Linear correlation data of viscosity measured by ESR ( $\eta_{\text{ESR}}$ ) against Ostwald viscometer results ( $\eta_{\text{OS}}$ ).

Probe	$\eta_{\text{ESR}} = a + b\eta_{\text{OS}}$			
	a	b	n	r
Glycerol-water				
TEMPOL	0.740	0.403	8	0.999
OTEMPO	1.062	0.295	8	0.995
DSA	0.360	0.767	6	0.995
DTBN	0.497	0.330	8	0.995
Sorbitol-water				
TEMPOL	1.161	0.212	7	0.998
DSA	0.182	0.805	4	0.997
DTBN	1.499	0.195	7	0.988
Sucrose-water				
TEMPOL	1.174	0.315	7	0.992
DSA	0.019	0.963	5	1.000
DTBN	1.059	0.332	7	0.993

metry, whereas DSA is less symmetrical, and would be expected to encounter greater resistance to rotation. Indeed, with the more concentrated glycerol solutions, resistance was so great that viscosities could not be determined with this probe. This is confirmed in Table 2, in which the slopes for DSA are always significantly greater than the others.

It must be concluded that viscosity data obtained by ESR cannot be compared with those obtained with the other two techniques, except possibly when they are applied to ideal binary liquid systems. The observed differences are therefore due to the choice of systems, rather than to the technique.

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