The Application of Malononitriles as Microviscosity Probes in Pharmaceutical Systems

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Three molecules were investigated for their ability to distinguish variations in the microviscosity of the surrounding medium. Julolidinemalononitrile (JMN), p-(N-dimethylaminobenzylidene) malononitrile (BMN), and p-(N-dimethylaminocinnamylidene) malononitrile (CMN) were dissolved in media of various micro- and bulk viscosities. The fluorescence intensity of each dissolved probe and the bulk viscosity of each medium were measured. In solutions of low molecular weight substances, where the micro- and bulk viscosities are expected to correspond, the fluorescence behavior of each probe was a function of bulk viscosity and was independent of solution composition. In contrast, in aqueous solutions of methylcellulose, the fluorescence behavior of the probes corresponds to microviscosities significantly lower than the measured bulk viscosities. Thus, the probes are useful in resolving the microviscosity from bulk viscosity of neat liquid and solution systems. The sensitivity of the probes to viscosity is in the order JMN > BMN > CMN. Due to its limited water solubility, JMN is not particularly useful for pharmaceutical systems. CMN is the preferred probe for these applications due to its high fluorescence intensity over a large viscosity range.

KEY WORDS: microviscosity; viscosity; malononitrile; diffusion; fluorescence; p-(N-dimethylaminocinnamylidene) malononitrile.

INTRODUCTION

Viscosity has long been recognized as an important determinant of drug dissolution and diffusion (1,2). However, diffusion of drugs through viscous media rarely correlates to bulk viscosity (3–6). In addition, other processes such as hydrolysis have a similar lack of correlation with bulk viscosity (7). It is now recognized that there are two viscosities that describe a system (4–11). Macroviscosity is a measure of the convective or bulk flowability of a material. The resistance to bulk flow is due to the slippage of molecular surfaces over one another as well as the entanglements of long chain molecules. Macroviscosity is dependent on shear rate, particularly in the case of solutions of long chain molecules. Microviscosity, on the other hand, is a measure of only the slippage between molecular surfaces. Since microviscosity rather than macroviscosity affects the movement of small molecules within a system, it is the more appropriate measure to be included in the Stokes-Einstein equation:

$$D = \frac{kT}{6\pi\eta r} \quad (1)$$

where D denotes the diffusion coefficient, k is Boltzmann's constant, T is temperature (Kelvin), η is viscosity and r is the radius of the diffusing molecule. Hence, the diffusion and eventual release of drugs is significantly and correctly described using accurate measurements of microviscosity rather than macroviscosity.

A number of rheologically nondestructive methods have been used to measure microviscosity. In an early study, Ibrahim and Rippie monitored the rate of a cyclization reaction in anisotropic systems (12). Florence et al. identified an "effective" viscosity which is related to microviscosity (8). Dynamic light scattering (DLS) of polystyrene latex spheres has recently been used to measure microviscosity (13,14). However, the DLS results do not always correlate with diffusion of small molecules in polymers due to the obstruction effect of the polymer on the polystyrene spheres. In addition, the spheres aggregate under some conditions. Another widely used method for measuring microviscosity is electron spin resonance of TEMPOL and similar probes (5–7,15). Unfortunately, a number of problems with this method have been identified (15).

Viscosity-sensitive fluorescence of malononitriles represents an elegant and useful method to measure microviscosity. While this method has not been used for pharmaceutical systems, it has been used to monitor the rates of polymerization reactions (16–18). In such systems, the fluorescence intensity of the dyes increases 20–40 fold during polymerization (16). The malononitrile dyes consist of dialkylaminophenyl and malononitrile moieties (Figure 1). These dyes absorb light in the visible region at wavelengths which depend on the polarity of the solvent; however, the shapes of the absorption and emission bands are reported to be the same in all solvents at all temperatures (19). Unlike most fluorescent compounds, the emission intensities of the malononitriles are uniquely sensitive to local viscosity. The mechanism of this behavior (Fig. 1) has been reported to be nonradiative transmission of energy through torsional relaxation about bonds a and b as well as photoisomerization about bond c (20–22). Since internal rotation about bonds a and b is intrinsically faster than solvent relaxation, the internal rotation is thought to be governed by solvent relaxation (19). The same may be said for isomerization of bond c. Solvent relaxation is a direct measure of viscosity over molecular distances—microviscosity. With no other reported deactivation pathways, malononitrile fluorescent emission is a useful tool for measuring the microviscosity in pharmaceutical systems.

The present work describes the fluorescence behavior of three malononitrile dyes (Fig. 1): p-(N-dimethylaminobenzylidene) malononitrile (BMN), julolidinemalononitrile (JMN) and p-(N-dimethylamino) cinnamylidenemalononitrile (CMN) in various viscosity environments. One of these dyes, CMN, has not previously been discussed in the malononitrile literature. The three dyes were evaluated for their

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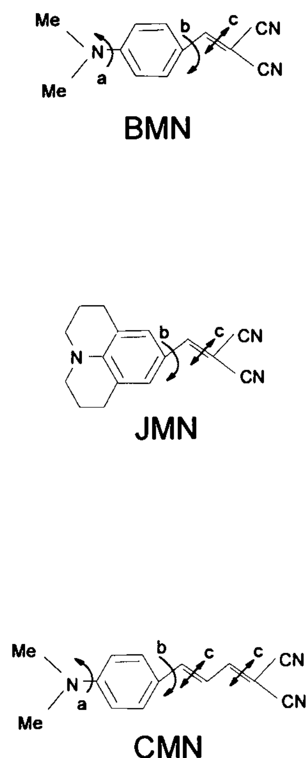

Fig. 1. Chemical structure of julolidine malononitrile (JMN), p-(N-dimethylamino benzylidene) malononitrile (BMN) and p-(N-dimethylamino cinnamylidene) malononitrile (CMN) with proposed modes of nonradiative transmission of absorbed energy—rotation about bonds (a) and (b) and isomerization of bond (c).

sensitivity to changes in microviscosity and ability to distinguish between microviscosity and bulk viscosity. In addition, shifts in the fluorescence spectrum due to changes in the polarity of the media are reported. The media studied include a) a neat solvent at various temperatures, b) binary solvents at room temperature, c) various concentrations of a low molecular weight polymer in aqueous solutions at room temperature, and d) various concentrations of a high molecular weight polymer in aqueous solutions at room temperature. Lastly, the ability of the malononitriles to predict microviscosity is also compared with estimates from electron spin resonance and diffusion experiments.

METHODOLOGY

The three malononitrile dyes used in this study, julolidine malononitrile (JMN), p-(N-dimethylamino benzylidene) malononitrile (BMN) and p-(N-dimethylamino cinnamylidene) malononitrile (CMN), were used as received from Molecular Probes, Eugene, OR.

Four solvent systems were used to create viscosity environments. Glycerol (Fisher, Springfield, NJ) was used at various temperatures to study a wide range of viscosities in a low molecular weight liquid. Various compositions of glycerol and ethanol were used at room temperature to resolve out any temperature effects seen in the neat glycerol system. Various concentrations of PEG 1450 (Carbowax 1450, Union Carbide, Danbury, CT) were prepared in 20% v/v ethanol in water to study the effect of this small polymer on bulk and microviscosity. Finally, methylcellulose (Methocel A4C, Dow Chemical, Midland, MI) solutions were prepared in 20% v/v ethanol in water to study the effect of a larger hydrophilic polymer. The final two sets of solutions were prepared in 20% v/v ethanol due to the poor water solubility of one dye, JMN.

The absorption maxima for the dyes were determined in aqueous solutions for CMN and BMN; that for JMN was determined in 20% v/v aqueous ethanol. The dyes were incorporated into each of the four series of solvent systems to achieve concentrations of 1.6×10^{-5}M BMN, 1.8×10^{-5}M CMN, and 2.0×10^{-5}M JMN. The concentrations of BMN and CMN were chosen so as to maintain solubility of the dyes in aqueous solutions. Solutions were prepared in triplicate. Each solution was subjected to triplicate analysis of both fluorescence and bulk viscosity.

Fluorescence spectra of the solutions were recorded using a luminescence spectrometer (Model LS-50, Perkin-Elmer, Norwalk, CT) with excitation and emission slit widths set to 5nm. The solutions were excited at the absorption maximum determined for each dye as described above. With a scan speed of 150 nm/min., the emission maximum was recorded. The fluorescence intensity was measured by integration at the emission maximum for 10 seconds.

Bulk viscosities were determined using a series of Cannon-Fenske viscometers (#150, #200 and #350, Fisher Scientific, Springfield, NJ) in a constant temperature bath.

The nitroxide spin probe, 4-hydroxy-TEMPO (Sigma Chemicals, St. Louis, MO) was added to various solutions to achieve a final concentration of 1×10^{-4} M. An electron spin resonance spectrum of each solution was obtained at 25°C using a microwave of 20.3 mW and frequency of 9.34 GHz. The rotational correlation time was calculated as previously described (23). The microviscosity of each solution was determined from the rotational correlation time by assuming the microviscosity of purified water to be 1.0 cps.

The microviscosities influencing the diffusion of a probe molecule through various media were also determined using the method of Stout, et al. (24). Briefly, siliconized glass tubes of 60 mm in length and 2 mm inner diameter were filled with various media containing 4.10×10^{-3} M niacinamide. The tubes were sealed at one end with silicone putty and positioned vertically with the open end in a 50 ml beaker containing 30 ml of the same medium without the niacinamide. The external medium was well mixed by a magnetic stir bar positioned close to the tube opening. The apparatus was maintained at 20°C and covered to prevent evaporation of the external medium. At a time when $Dt/L^2<0.2$ (where D is the diffusion coefficient of niacinamide, t is time and L is the length of the tube), the average concentration of niacinamide, C_{AVE} was assayed spectrophotometrically. The value of the diffusion coefficient of niacinamide in each medium was determined using Equation 2.

$$D = \frac{\pi L^2}{4t}\left(1 - \frac{C_{AVE}}{C_0}\right)^2 \quad (2)$$

The microviscosity of each medium was calculated from Equation 3

$$\eta_i = \frac{D_{H_2O}\eta_{H_2O}}{D_i} \quad (3)$$

Table I. Emission Maxima and Intensities of the Malononitrile Dyes in Glycerol at Various Temperatures and Viscosities

Dyes		JMN		BMN		CMN	
Excitation wavelength		468 nm		444 nm		500 nm	
Temperature of neat glycerol	Viscosity, cps (s.d.)[a]	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}
80°C	32 (1)	37 (1)	511	36 (5)	489	50 (10)	583
70°C	48 (2)	52 (4)	511	52 (3)	489	78 (4)	582
65°C	66 (2)	62 (2)	510	57 (5)	489	89 (5)	582
50°C	150 (0)	120 (20)	509	100 (0)	488	160 (10)	582
37°C	350 (20)	250 (20)	508	190 (10)	488	270 (0)	582
30°C	550 (20)	370 (10)	508	260 (10)	487	350 (10)	581
25°C	840 (20)	410 (20)	508	320 (50)	486	410 (40)	581

[a] Sample standard deviation.

where η is the microviscosity of the medium, D_i is the diffusion coefficient of niacinamide in that medium as determined in Eqn. 2, η_{H_2O} is the microviscosity of water (assumed to be 1 cps) and D_{H_2O} is the diffusion coefficient of niacinamide in water as determined from Eqn. 2. All studies were performed in triplicate.

RESULTS AND DISCUSSION

Diffusion is governed by the movement of solvent molecules out of the path of a permeant, the strength of solute-solvent and solvent-solvent interactions and the size of the gap necessary for the permeant to move forward. These are

Table II. Emission Maxima and Intensities of the Malononitrile Dyes in Ethanol/Glycerol Solutions of Various Bulk Viscosities

Dyes		JMN		BMN		CMN	
Excitation wavelength		468 nm		444 nm		500 nm	
Solvent Composition (% w/v in ethanol)	Viscosity, cps (s.d.)[a]	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}
30% glycerol	3.2 (0.1)	22 (1)	499	22 (2)	479	45 (2)	568
50% glycerol	12 (1)	31 (2)	502	30 (1)	480	56 (2)	574
60% glycerol	20 (1)	38 (1)	502	34 (2)	481	68 (1)	575
70% glycerol	46 (1)	53 (1)	504	47 (2)	482	84 (12)	575
80% glycerol	98 (2)	88 (4)	505	72 (3)	483	110 (4)	578
85% glycerol	160 (0)	130 (0)	506	100 (10)	485	140 (20)	579
90% glycerol	270 (20)	150 (10)	507	120 (10)	486	210 (0)	580
100% glycerol	840 (20)	410 (10)	508	320 (40)	486	410 (30)	581

[a] Sample standard deviation.

Table III. Emission Maxima and Intensities of the Malononitrile Dyes in PEG 1450 in 20% (v/v) Ethanol in Aqueous Solutions of Various Bulk Viscosities

Dyes		JMN		BMN		CMN	
Excitation wavelength		468 nm		444 nm		500 nm	
Concentration of PEG 1450 % (w/v)	Viscosity, cps (s.d.)[a]	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}
10	2.8 (0.2)	10 (1)	511	12 (0)	488	23 (1.0)	581
20	5.1 (0.1)	13 (1)	511	16 (1)	488	31 (1)	581
30	9.2 (0.3)	16 (1)	511	20 (1)	488	41 (2)	581
40	17 (1)	23 (1)	511	28 (1)	488	49 (2)	581
50	30 (0)	32 (1)	511	37 (1)	488	71 (2)	581
60	55 (1)	47 (1)	511	47 (1)	488	79 (2)	581

[a] Sample standard deviation.

the same factors which govern the rotation of the malononitrile moieties and the fluorescence intensity of the molecules in Figure 1. It seemed reasonable, therefore, to use these malononitriles to differentiate between the microviscosity that controls diffusion and the macroviscosity that regulates bulk flowability.

The three malononitrile dyes shown in Figure 1 are structurally similar; an electron donor (i.e., the lone pair of electrons) and an electron acceptor (i.e., the nitrile groups) are separated by a conjugated structure. The methyl groups on JMN are rotationally constrained, while those on the other two molecules are freely rotating. In addition, CMN has an additional double bond in its structure. These differences in structure were expected to lead to differences in fluorescence behavior as a function of microviscosity.

U-tube viscometry measures bulk or macro-viscosity. However, for small molecules, macro- and microviscosity values are expected to be equal. In low molecular weight liquids both of these viscosities are simply due to the resistance of one molecule moving past another; entanglements do not occur in these solutions such as those seen in solutions of high molecular weight polymers. Thus, glycerol, a low molecular weight liquid which exhibits a relatively high viscosity, was used to study the ability of each of the three dyes to detect changes in microviscosity as measured by U-tube viscometry. The results are shown in Table I. From

Table IV. Emission Maxima and Intensities of the Malononitrile Dyes in Methylcellulose Solutions of Various Viscosities

Dyes		JMN		BMN		CMN	
Excitation wavelength		468 nm		444 nm		500 nm	
Concentration of methylcellulose % (w/v)	Viscosity, cps (s.d.)[a]	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}	Intensity (s.d.)[a]	λ_{max}
1.00	27 (4)	11 (1)	509	8.7 (0.6)	489	14 (1)	582
1.25	55 (5)	12 (1)	509	9.7 (0.6)	489	16 (1)	582
1.50	118 (5)	13 (1)	510	11 (1)	490	17 (1)	582
1.75	180 (0)	14 (1)	510	11 (1)	490	18 (1)	582
2.00	400 (10)	14 (1)	510	12 (1)	490	21 (1)	582
2.25	640 (10)	15 (1)	510	12 (0)	490	22 (1)	582

[a] Sample standard deviation.

80°C where the viscosity is 32 cps to 25°C where the viscosity is 840 cps, the fluorescence intensities increase 11, 8.9 and 8.2 fold for JMN, BMN, and CMN, respectively. Over the broad range, JMN is the most sensitive to changes in viscosity, followed by BMN and CMN.

The fluorescence of the dyes in solutions of glycerol in ethanol was studied to determine whether fluorescence changes seen in the neat glycerol systems were due primarily to microviscosity. Table II shows that the fluorescent behavior of the three dyes is similar to that in the neat glycerol system indicating that the fluorescence behavior in neat glycerol was free from temperature effects. The neat and the binary systems yielded equivalent results with the differences in sensitivity of the three dyes to the broader viscosity range becoming more striking. In addition, the change in solvent polarity, from the more polar 100% glycerol to the less polar 30% glycerol, results in a characteristic blue shift in the fluorescence maximum for each of the dyes. This shift did not effect the measurement of viscosity by fluorescence in these experiments, since the intensity was always measured at the emission maximum. However, a shift in the fluorescence spectrum would make it impossible to monitor microviscosity at a single emission wavelength.

Solutions of the dyes in aqueous polyethylene glycol 1450 (which consists of polymer chains averaging 32.5 oxyethylene units) show the same fluorescence behavior as those of the smaller molecules (Table III). PEG 1450 solutions are similar to lower molecular weight solutions in that they have no significant entanglements which would make the bulk viscosity greater than the microviscosity. In contrast, in solutions of methylcellulose, the dyes have fluorescence behaviors much different from those of the previous three solvent systems (Table IV). The fluorescence intensity of the probes in solutions of high bulk viscosity is much lower than would be expected if the micro- and bulk viscosities were assumed equal. If, on the other hand, we assume that the microviscosity of the methylcellulose solutions can be determined from the fluorescence intensity of the probes, then we conclude that the microviscosity of the methylcellulose solutions is far less than its corresponding bulk viscosity. In fact, the probes measure a highly localized resistance to molecular motion (see Figure 1) while the U-tube viscometer measures resistance to bulk flow. The viscosity measured by the U-tube viscometer gives an indication of a solution's resistance to bulk or convective flow (e.g., a resistance to fluid motion during shearing); the fluorescence intensity of the probes gives a measure of the molecular mobility or diffusivity in solution. Thus, while a solution of methylcellulose with its high bulk viscosity would resist convective flow, its resistance to the diffusion of small molecules would be considerably less. This general phenomenon has been previously discussed for other polymer solutions (11). The malononitrile probes, therefore, appear to be a useful tool for resolving the microviscosity from the bulk viscosity in order to predict diffusion coefficients.

Table VI. Model Parameters for Fluorescence Versus Viscosity Data for BMN

Solvent system	I_{max}	η_{50}	I_0
1) Glycerol	656	955	16.3
2) Ethanol/glycerol	794	1551	22.2
3) PEG 1450/water	561	727	11.8
1, 2 and 3 pooled data	713	1152	16.1
(95% confidence interval)	(460–966)	(559–1745)	(12.1–20.1)
4) Methylcellulose/water	5.92	45.8	6.45

In order to use the data for predictive purposes, a model is fit to the data. This model relates intensity to viscosity. The three model parameters used in Eq. 4 are defined as: baseline intensity in the absence of measurable viscosity (I_o), an intensity at infinite viscosity (I_{max}), and the viscosity at half the maximal intensity (η_{50}).

$$I = I_{max} \frac{\eta}{\eta + \eta_{50}} + I_0 \quad (4)$$

These parameters are fit to the observed mean data for each malononitrile probe in each solvent system by a least-squares algorithm, and 95% confidence intervals were constructed about the parameter estimates. In addition, the observed data from glycerol, ethanol/glycerol solutions and PEG 1450 solutions are superimposable, so the model is fit to a composite set of these data. It can be seen from the data in Tables V–VII that for each probe the fluorescence behavior in glycerol, ethanol/glycerol solutions and PEG 1450 solutions falls within the 95% confidence interval for the composite data with few exceptions. In contrast, the fluorescence behavior of the probes in methylcellulose solutions is well outside the 95% confidence interval for the pooled data. The difference between the pooled intensity data and the intensities of the probes in the methylcellulose solutions is shown in Figure 2. The data clearly produce two separate

Table V. Model Parameters for Fluorescence Versus Viscosity Data for JMN

Solvent system	I_{max}	η_{50}	I_0
1) Glycerol	889	925	5.65
2) Ethanol/glycerol	1062	1611	22.4
3) PEG 1450/water	500	634	8.82
1, 2 and 3 pooled data	985	1210	11.5
(95% confidence interval)	(578–1391)	(501–1919)	(6.3–16.7)
4) Methylcellulose/water	7.31	48.6	7.95

Table VII. Model Parameters for Fluorescence Versus Viscosity Data for CMN

Solvent system	I_{max}	η_{50}	I_0
1) Glycerol	627	492	15.1
2) Ethanol/glycerol	900	1273	47.8
3) PEG 1450/water	753	591	24.6
1, 2 and 3 pooled data	724	761	32.2
(95% confidence interval)	(523–924)	(413–1109)	(25.5–38.8)
4) Methylcellulose/water	12.2	219	12.8

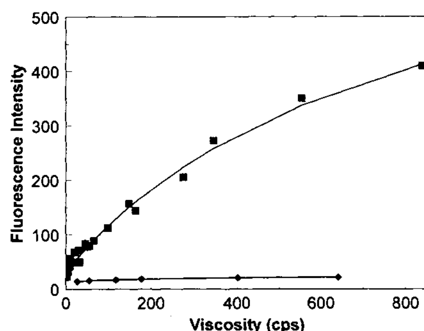

Fig. 2. Mean fluorescence intensities of CMN in glycerol, ethanol/glycerol solutions and PEG 1450 solutions (■) and in methylcellulose solutions (♦) as a function of the corresponding bulk viscosity of the medium. Solid line represents the model fitted to the pooled data in Table 7. Dotted line represents the model fitted to the methylcellulose data in Table 7.

functions. This indicates that the microviscosity as measured by fluorescence intensity of the malononitriles is a unique function of the measured macroviscosity in the small molecular solutions; however, the microviscosity of the methylcellulose solutions is much less sensitive to changes in its macroviscosity. These results clearly show a difference in the factors affecting the viscosities of these two classes of solutions.

Finally, the microviscosity as measured by malononitrile fluorescence intensity was compared with the microviscosity measured by electron spin resonance (ESR) and that determined by diffusion experiments. To use the malononitrile fluorescence intensity predictively, equation 4 was rearranged to yield:

$$\eta = \frac{\eta_{50}(I - I_o)}{(I_o + I_{max}) - I} \quad (5)$$

Table VIII shows the values of microviscosity of a variety of solutions determined by the three methods—ESR, the tube method and fluorescence intensity. The microviscosity in the last column of Table VIII was calculated using Eqn. 5 and the model parameters. It compares favorably with that measured by ESR, except in the very low viscosity ranges where Eqn. 5 predicts a negative viscosity. The inability of the model to predict low microviscosities may be due to the use of the broad range of microviscosities used to determine the model parameters. At the high viscosity exhibited by glycerol, the microviscosity determined by ESR and by the fluorescent probe differ by an order of magnitude. This is probably due to the reduced precision of the ESR value which results from the large degree of distortion of the ESR spectrum at high viscosities. ESR appears to be a useful measure of microviscosity in a limited range (ca. 1–10 cps).

The tube method (24) predicts microviscosity only in fluids with low *macroviscosities* (ca. 1–5 cps). In more viscous media, the receiver fluid cannot be adequately stirred and a hydrodynamically static layer exists at the opening of the tube. This creates non-sink conditions and results in an erroneously low value for diffusivity and a correspondingly elevated value for microviscosity. Thus, a determination of microviscosity from diffusion experiments may yield spurious values. In Table VIII, the methylcellulose solution data have high macroviscosities as measured by U-tube viscometry. This high macroviscosity leads to an elevated microviscosity determined by the diffusion of nicotinamide—a value that is inconsistent with the other measures of microviscosity. Thus, a determination of microviscosity from diffusion experiments may result in spurious values, whereas JMN fluorescence and ESR yield similar low microviscosity results for hydrophilic polymer solutions that corroborate the observations of other investigators (4,5,7,9).

CONCLUSIONS

The fluorescence of the three malononitriles investigated is sensitive to microviscosity independent of solution composition. The sensitivity of the probes follows the rank order JMN>BMN>CMN. Although JMN is the most sensitive of the three probes, its low water solubility limits its use. While CMN has the lowest ability to distinguish between differences in microviscosity, its fluorescence intensity is significantly higher than that of the other two probes. This high intensity allows CMN to be used in smaller concentrations which reduces the perturbation to the system it is measuring, while maintaining an adequate signal-to-noise ra-

Table VIII. A Comparison of the Viscosities of a Variety of Solutions Determined by Three Independent Methods

	Viscosity (cps) as measured by:			
Solution	U-tube viscometry	Electron spin resonance[1]	Diffusion of niacinamide[2]	Fluorescence intensity of JMN[3]
10% PEG in 20:80 ethanol:water	2.8	1.6	4.8	—
30% PEG in 20:80 ethanol:water	9.2	1.8	17	5.5
50% PEG in 20:80 ethanol:water	30	4.3	50	26
30% glycerol in ethanol	3.2	4.3	3.9	13
70% glycerol in ethanol	46	16	5.9	53
100% glycerol	840	72	1300	830
1.0% methylcellulose in 20:80 ethanol:water	27	1.3	4.5	—
1.25% methylcellulose in 20:80 ethanol:water	55	1.3	19	0.2
2.0% methylcellulose in 20:80 ethanol:water	400	2.3	130	3.5

[1] Calculated as in reference 23.
[2] Determined using Eqns. 2 and 3.
[3] Determined using Eqn 5 with model parameters 985, 1210 and 11.5 for I_{max}, η_{50}, and I_0, respectively for JMN from Table V.

tio. Thus, CMN is the preferred microviscosity probe based on its water solubility, adequate sensitivity to microviscosity and high fluorescence intensity.

Malononitrile fluorescence represents a useful alternative method to determine microviscosity. The relatively low cost of the spectrofluorimeter and the ease in sample preparation make this method accessible and elegant. Furthermore, it will be shown in future work that malononitrile fluorescence excited by a laser is uniquely suited to determine the microviscosity of various positions within a heterogeneous system.

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