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An ESR technique for measuring fluidity in semisolids

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Synopsis

An electron spin resonance (ESR) technique, basically devoid of external mechanical stress force was investigated as a possible method for measuring phase fluidity in semisolid systems. The stable free radical di-t-butylnitroxide, DBNO, was used as a probe for producing the ESR signals at temperatures between −50 and 100°C in petrolatum and selected polyethylene glycol (PEG) bases. The intensity ratio, I_1/I_3, derived from the ESR triplet spectrum of DBNO, reflecting the rotational diffusion of the probe, was shown to provide a highly sensitive measure for phase fluidity. Curves plotted for I_1/I_3 ratios against temperature indicate lower I_1/I_3 values corresponding to increased phase fluidity with elevated temperatures. Extrapolations of the linear portions of the steeply rising curves intersect the ordinate at $I_1/I_3 = 1$ to yield the intersection temperature, which theoretically represents the point of transition from rigid to fluid motion. A linear relationship was observed when the intersection temperatures were plotted as a function of the reported melting-point temperatures for homologous PEGs 1500, 1540, and 6000. Since the intersection temperatures, in turn, parallel in rank order reported Kinematic viscosities of PEGs, the I_1/I_3 plots can thus provide a relative measure of the zero-stress-obtained viscosities for a homologous series of semisolids.

INTRODUCTION

Fluidity or "viscosity" of semisolid systems determined by present methods is largely arbitrary, yielding variable results depending on the mode and level of applied stress during measurements. For practical applications, fluidity characteristics at zero-applied stress seem to provide the most desired data. This paper outlines a spectroscopic technique based on electron spin resonance, ESR, for these measurements, avoiding physical or mechanical disturbance of the sample under investigation. The ESR technique is shown to potentially provide a useful tool for characterizing fluidity or, inversely, "viscosity" in a rapid, sensitive, reproducible, and most importantly, a "non-destructive" manner, exemplified by preliminary tests with two commonly used pharmaceutical ointment bases. The term "viscosity" used in the context of this paper refers to the "rheological ground state" as discussed by Barry (1). In other words, it is that fundamental rheological parameter of semisolids which is independent of variables as a result of operating and continuous shear test methods. The ESR technique is, in fact, rheologically non-destructive and is presumed to test a semisolid without disrupting organized structures such as floccules in emulsions or networks in gels, and thus basically retains the original condition of the material. Results of such tests will provide analogous viscosity values as interpreted along the linear viscoelastic theory (2). Under such conditions where the ratio of stress to strain is a function of time, independent of stress magnitude, viscous effects will obey Newton's law and the elastic effects are described as being Hookean (3). Among known methods capable of examining viscoelastic behavior of semisolids under small strains are those of the creep test (4), oscillatory technique (5), and, more recently, of that using normal stress with parallel plates (6). The ESR technique is adapted from the spin probe procedure for characterizing the transport properties of biological samples, first introduced by McConnell and coworkers (7). It consists of dissolving a small concentration (approximately 10^{-4} M) of a stable free radical, di-t-butylnitroxide or DBNO, in about 0.5 mL of sample. Absorbance of microwave radiation by the DBNO probe is then obtained at its characteristic magnetic field in a suitable ESR spectrometer (7). The shape of the absorption band, the ESR signal usually recorded as a first derivative, provides a sensitive function of the rotational diffusion of the probe and, consequently, a measure for fluidity or viscosity of the medium. The signal is narrow with a Lorentzian line shape if the medium or phase is viscous but broadens and becomes non-Lorentzian with increased viscosity or rigidity. Thus, in principle, the width and shape of the ESR signal of the spin probe serves to measure fluidity or viscosity of liquid or semisolid systems without being subjected to external physical stress.

Figure 1 shows the DBNO probe and its typical ESR spectrum when dissolved in ethanol, a low viscosity medium. The three lines of the nitroxide triplet, labeled 1, 2 and 3, show the same intensity and shape, as the nitroxide molecules are essentially free to rotate and diffuse in the low viscosity medium. With increased viscosity of the medium, the Brownian motion of the probe molecule becomes more restricted, and thus each line, especially line 3, broadens by different amounts. Since the areas under all three lines remain unchanged, a broader signal indicates a lower peak-to-peak height ratio.

While computer simulation of ESR signals represents the most accurate procedure for

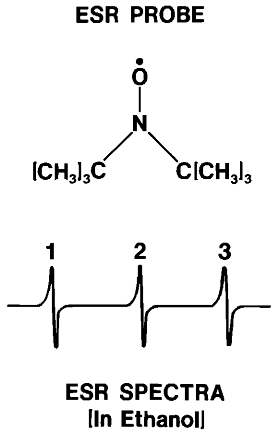

Figure 1. Structure of the paramagnetic (spin) probe, di-t-butylnitroxide, DBNO, and its typical spectrum in a highly fluid medium, ethanol at room temperature.

using the ESR technique in determining the correlation time, τ (and hence the viscosity, η) (8) as seen via the Debye-Stokes equation

$$\tau = \frac{4}{3} \frac{\pi r^3 \eta}{kT},$$

a preliminary estimate of η as presented in this paper may be made more simply by comparing the relative heights of peaks 1, 2 and 3. It had been noted previously that for biological samples the ratio of peak-to-peak heights of lines 1 and 3, I_1/I_3, at a given temperature could yield a fairly reliable measure for relative viscosities (9).

MATERIALS AND METHODS

Preliminary ESR measurements were made on two spectrometers, operating at X-band (9.5×10^9 Hz) and Q-band (33.8×10^9 Hz) microwave frequencies. Detailed measurements were subsequently made at the X-band frequency using a Brüker spectrometer (Model ER 200D) and a variable temperature accessory. Samples for ESR measurements were prepared by mixing 1 μL of DBNO with 1 mL of the ointment base and heating the mixture to slightly above its melting point before being drawn into standard melting point capillary tubes via capillary action. A standard microwave cavity (TE_{102}) was used. It may be noted, however, that in general the use of capillaries and heating of the sample are not required. The probe may be added during the ointment preparation, as discussed later. All spectrometer parameters, e.g., microwave power, microwave frequency, sample temperature, magnetic field scan, modulation amplitude and frequency, scan rate, etc., were optimized for best signal-to-noise ratio without signal distortion. The ESR spectra were recorded with a 100-kHz magnetic field modulation frequency unit and care was taken to employ low modulation amplitude to avoid line shape distortion. The sample temperature was controlled to within ±0.5°C and varied in 1° steps.

RESULTS AND DISCUSSION

Table I shows literature data for the molecular weights of PEG 1500 which is reported to be in the range of 550 ± 50 and PEG 6000 at around 6000. Thus, at a given temperature the viscosity of PEG 6000 was expected to be significantly higher than that of PEG 1500. Figure 2 represents the ESR spectra of DBNO in PEG 1500 and PEG

Table I
Physical Characteristics of Ointment Bases

Base	Melting Point T°C Range	Viscosity (Cks) Range 210°F	Average Molecular Weight
PEG 1500*	38–41	13–18	500–600
PEG 1540*	43–46	25–32	1300–1600
PEG 6000*	60–63	700–900	6000–7500
Petrolatum USP	38–60	–	

* Obtained from Table of Physical Properties, Carbowax Booklet, Union Carbide Chemicals Co., New York.

6000 measured at 25°C, with line 3 shown to be noticeably broader for PEG 6000 than for PEG 1500, in agreement with previously discussed theoretical considerations. Additional tests based on changing the sample temperature confirmed that broadening of peak 3 was related to phase fluidity. Figure 3 shows the typical X-band spectra for DBNO in PEG 6000 at 87, 37, and −3°C, taken after sample temperature equilibration for 30 min at each temperature. The expected increase in broadening of line 3 becomes evident with a decrease in temperature and resultant increased viscosity.

Again, theoretical considerations (8,9) indicate that if I_1/I_3 data for the X-band (Figure 2) and Q-band measurements are plotted on the same temperature scale, then the Q-band (higher frequency) curve would be expected to correspond to the higher I_1/I_3 values. This was indeed confirmed as may be noted from Figure 4.

Figure 5 shows a plot of the intensity ratio I_1/I_3 as a function of temperature for the four semisolid bases studied. It is observed that with the lower molecular weight PEG 1500 and petrolatum, the ratio I_1/I_3 approaches unity at their reported melting-point temperatures (Table I) since the probe molecule is entirely free to undergo Brownian diffusion in the then liquefied base. It should be noted that PEG 1500, which represents an equal blend of PEG 300 and PEG 1540 (Union Carbide), is said to demonstrate the "consistency of petrolatum." Our data in Figure 5 show the relatively close proximity between the curves of these two substances when their I_1/I_3 ratios were plotted against T°C in agreement with this descriptive statement.

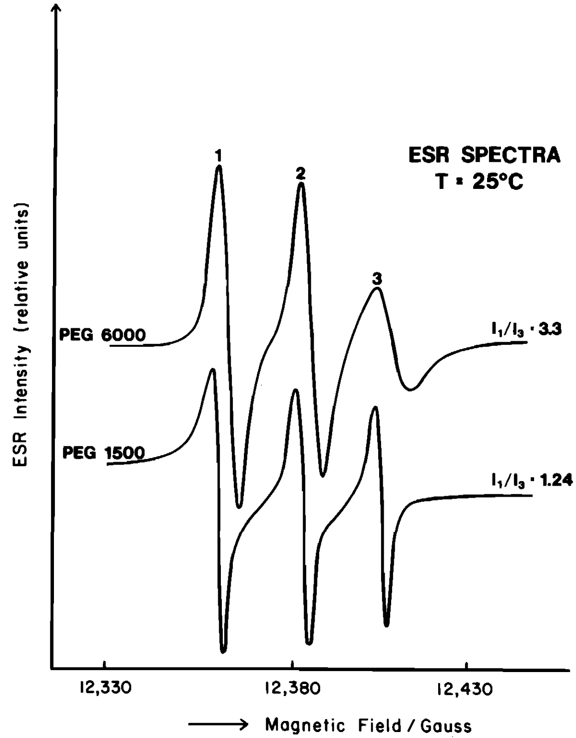

Figure 2. ESR spectra for DBNO in PEG 6000 and PEG 1500, illustrating the effect of sample fluidity on the relative heights of the components of the DBNO ESR triplet.

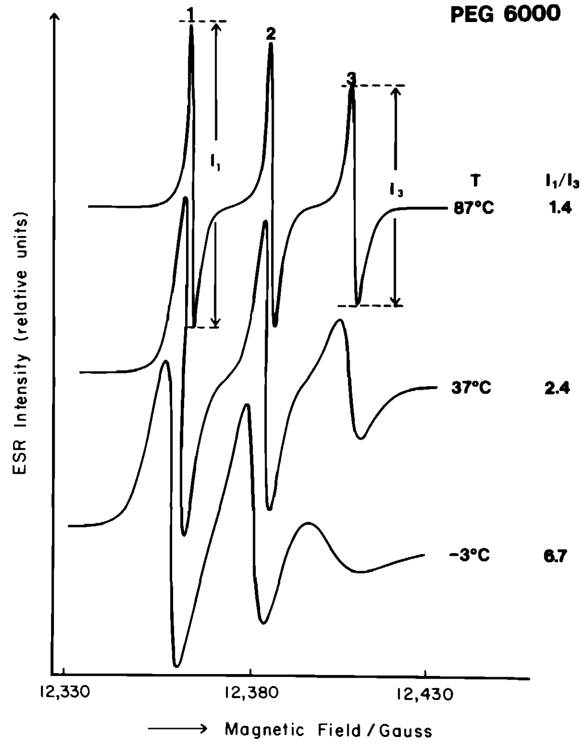

Figure 3. The ESR spectra for DBNO in PEG 6000 at various temperatures, exhibiting the relative broadening of the components of the DBNO triplet with decreasing base fluidity.

With a lowering of temperature, the I_1/I_3 ratio is seen to increase sharply for all the bases investigated because of decreased fluidity upon solidification. In fact, the intersection temperatures of the extrapolated linear portion of the steeply rising curves with a line perpendicular to the ordinate where the I_1/I_3 ratio $= 1$, as indicated in Figure 5 for PEGs 1500, 1540, and 6000, parallels at least in rank order their respective reported melting-point temperatures (Table I). These intersections may be considered to correspond to the "theoretical" melting-point temperatures as they represent the phase-commencing unrestricted random Brownian motion of *all* molecules, with physical changes of the material regardless of external appearance.

We note, however, an apparent reversal of phase fluidity between PEG 1540 and PEG 6000 which occurs below approximately 20°C. While this observation was unexpected, on further examination the I_1/I_3 ratio data of PEG 6000 for the lower temperatures (Table II) show a maximum or spur at around -10°C, indicating a possible cooperative molecular ordering such as a coiling-uncoiling phase transition. Thus PEG 6000 may show deviations from the general behavior for fluidity in the lower temperature range. Further, it may be pointed out here that the I_1/I_3 ratios for the higher molecular weight PEGs, i.e., PEG 1540 and PEG 6000, do not reach 1, within our measurement accuracy as indicated by the error bars on the graphs, even at the 70–80°C range. This is quite possible since the probe molecule is envisioned microscopically to be still incompletely free to rotate in the medium at a rate sufficient for rendering $I_1/I_3 = 1$. It is

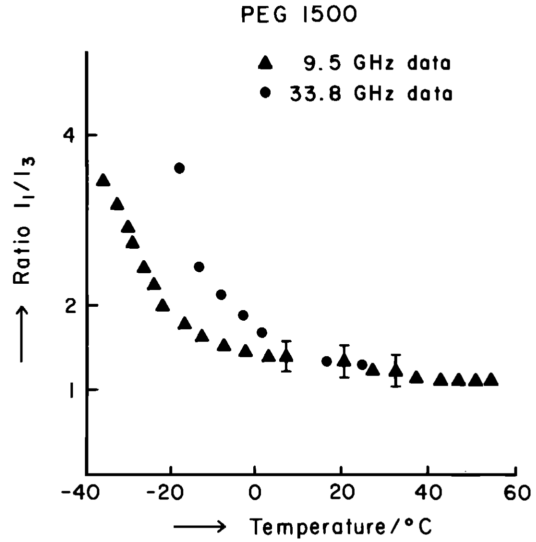

Figure 4. The intensity ratio I_1/I_3 for DBNO in PEG 1500 at 9.5 and 33.8 GHz frequencies.

projected, however, that this ratio will eventually reach unity with much higher temperatures.

Figure 6 presents the reported average melting-point temperatures for PEGs 1500, 1540, and 6000 plotted against their intersection temperatures. As anticipated, the melting-point temperatures demonstrate, within our measurement accuracy, a linear relationship with the intersection temperatures or "theoretical" melting-point temperatures as previously discussed. In turn, this relationship is seen to parallel in favorable fashion the Kinematic viscosities reported for the three PEGs obtained at 210°F (Table I), with the lowest point associated with PEG 1500 and the highest point with PEG 6000.

It should be pointed out that since these bases themselves do not exhibit sharp melting-point temperatures, deviations may occur within the experimental data. Nevertheless, the approximate linearity of the curve is encouraging in that such a plot can serve as a calibration curve for characterizing a family of bases in terms of their relative viscosities. Furthermore, the scale can be made quantitative if standard samples with established viscosity values are used in relation to the curve for measuring viscosities.

The ESR technique may be extended to temperature-related studies based on the Arrhenius relationship:

$$\eta = \text{constant } e^{\Delta E/kT} \qquad (1)$$

where ΔE is the activation energy for the molecular reorientation processes, representing in essence the barrier to the rotational diffusion of the molecules. Since theoretical estimates of ΔE would be sensitively dependent on intermolecular forces, a comparison of theoretical and experimental values of ΔE could serve as a basis for learning the microscopic details of the structure and bonding of the bases investigated. It is worthwhile noting here that variable temperature measurements for η are not easily attainable by other methods.

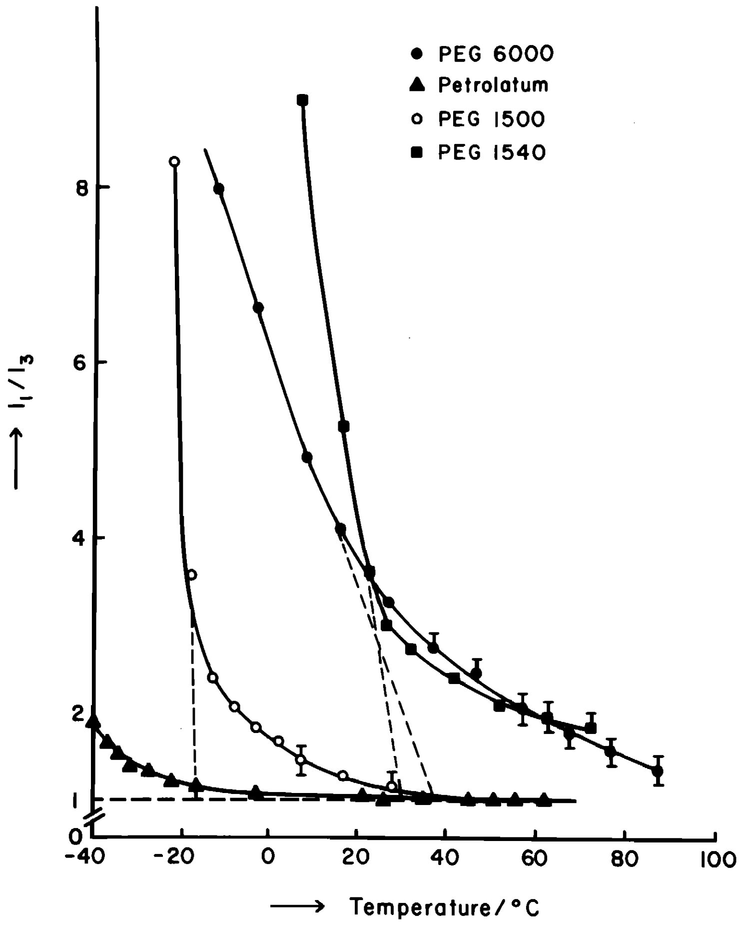

Figure 5. Plots of the intensity ratio, I_1/I_3, for DBNO in PEGs 1500, 1540, 6000, and Petrolatum USP as a function of temperature. The estimated measurement error in I_1/I_3 is ±5%.

Although this paper describes ESR measurements made by dispersing the DBNO probe via melting of the semisolids, this procedure of applying heat to the test samples is not an essential part of the methodology itself; the probe may be mixed in during the preparation of the ointment or emulsion prior to the analysis. Approximately 10 milli-

Table II

I_1/I_3 Ratio as a Function of Temperature for PEG 6000

Temperature (°C)	I_1/I_3 Ratio
−20	6.9
−10	8.0
0	6.7
+10	4.9
+20	4.1
+31	3.2

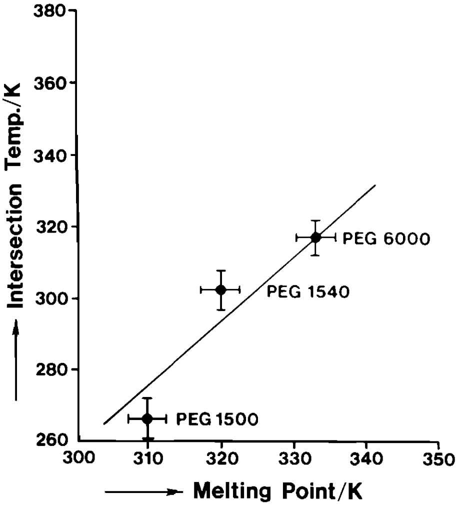

Figure 6. A plot of the average melting-point temperature of PEG bases 1500, 1540, and 6000 as a function of the intersection temperatures.

grams of the product can then be placed in a glass or quartz tube (5 mm o.d.) at any desired temperature without further subjecting the sample to any mechanical disturbance. The application of heat is necessary only if the fluidity data are to be obtained as a function of temperature. It should be noted that only trace amounts (ppm level) of the probe are required, and hence the addition of the probe during the emulsion/ointment preparation is not expected to alter the sample's structural characteristics.

Potential interferences with ESR measurements may be related to the type of the spin label probe added since it can modify fluidity of the system under test. This is not considered a serious shortcoming since the required concentration of the probe is usually quite low ($\cong 10^{-4}$ M). However, insolubility or other chemical interactions may occur necessitating replacement of the DBNO probe by other nitroxide molecules (7). In cases where the ESR spectra are different from that of DBNO, Freed's procedure (9) may be employed to take into account the shape and size of a given nitroxide probe for studying molecular motions. Studies are planned in our laboratories for obviating these possible complications.

CONCLUSION

In conclusion, preliminary results presented in this paper strongly suggest that ESR spectroscopy can be successfully developed into a rapid, accurate, and sensitive method for characterizing the fluidity or ground state viscosity of pharmaceutical liquids or semisolid systems. The proposed method requires only 10^{-4} to 10^{-5} molar amounts of probe, and these sensitive measurements may easily be conducted, without external stress to the sample, and are thus regarded as "non-destructive." Moreover, this method allows an easy measurement of barriers (i.e., ΔE in Equation 1) to the activation energy for molecular reorientation in a system as a basis for improving understanding of material structures. A direct application of the ESR method could be its further development towards diffusivity studies, with the ultimate aim of optimizing vehicle selection in controlling drug release processes.

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