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Riboflavin as a possible probe in studies of molecular aggregate microviscosity

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Abstract

Fluorescence polarization studies, using riboflavin as a probe, were conducted in a model fluid made up of 20:80 water–glycerin mixture. The viscosity of this medium and the fluorescence polarization of riboflavin solubilized therein were determined at temperatures varying from 10°C to 50°C ± 0.2°C. The data obtained were used to analyze and characterize the overall fluidity, microviscosity and flow activation energy of the glycerin–water medium. The fluidity and microviscosity of this medium, though temperature dependent, showed opposite tendencies. While the former showed an increase, the latter showed a decrease as the temperature is increased. The flow activation energy so obtained was 19.8 kJ mole^{-1}. This value is in good agreement with literature values obtained for some liposomes, vesicles and lipid bilayer aggregates of similar viscosity. © 1998 Elsevier Science S.A.

Keywords: Riboflavin; Water–glycerin mixture; Viscosity

1. Introduction

The microenvironment of molecular aggregates, cell membranes, lipid bilayers and vesicles control the mechanism of the activities of these systems in their respective functions. For these reasons research activities geared towards the understanding of these microenvironments have been and continues to engage the attention of many researchers [1–3]. In the probing of the interiors of these systems, fluorescence polarization is one of the most used techniques [3,4]. The depolarizers that are frequently used for this purpose include pyrene, perylene and 1,6-diphenyl, 1-3-5-hexatriene (DPH) [5–8]. The choice for these depolarizers stems from their high molar absorptivity and good quantum yield. In our laboratories we focus on the use of a naturally occurring fluorophore that is found in biological membranes as a depolarizer. Riboflavin is such molecule and it is ubiquitous in both plants and animal cell membranes. It has a good quantum yield, 0.26–0.40, [9,10] and high molar absorptivity, $1.22 \times 10^4\ M^{-1}\ cm^{-1}$ [10]. These last features have been taken advantage of to the extent that riboflavin has been analyzed in intact cell, with satisfactory result, without prior separation or purification [10]. It has also been used in various other chemical analyses [11–15]. In addition, riboflavin is soluble in both aqueous, vesicular and bilayer media. For these features, it is conceivable that riboflavin should be usable as an alternative probe in the investigation of the microviscosity of molecular aggregates and can be extended to the investigation of membrane dynamics. In this preliminary study, a medium consisting of glycerin and water whose viscosity is comparable to those of known aggregates such as micelles and cell membranes was used [16]. The water–glycerin composition was 20:80 v:v.

2. Experimental

2.1. Materials

Riboflavin (98%) was obtained from Aldrich Chemical. Reagent grade glycerin was obtained from Fisher Scientific. All reagents were used as received.

2.2. Method

2.2.1. Fluorescence measurements

All fluorescence measurements were made using a Perking Elmer luminescence spectrophotometer, model LS 50B. The excitation and emission wavelengths were 398 and 524 nm, respectively. All solutions were prepared using a triply-distilled deionized water of specific conductance of 7.0×10^{-8}

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Ω^{-1} obtained from the Reagent Grade Water System by Photronix. The riboflavin concentration in all experiments was kept constant at 2.0×10^{-4} M.

2.2.2. Temperature studies

The influence of temperature on the microviscosity of the system under study was also determined by fluorometry. The temperature range for this study was from 10 to 50°C ± 0.2°C and was controlled using a refrigerated bath and circulator, model KT-2, supplied by Haake Mess-Technik, Germany.

2.2.3. Viscosity measurements

The viscosity experiments were conducted using a Brookfield viscometer, model RDVD II+ with the spindle speed set at 50 rpm. In order to determine an appropriate viscosity for the experiment, several dilutions were made ranging from 20 to 80% v:v in glycerine–water mixture.

3. Results and discussion

Neat glycerin has a viscosity of 954 cP at 25°C [17]. This very high viscosity presents difficulties in using it directly for dissolution and analysis of riboflavin. It was therefore decided to moderate it by dilution with water. Three compositions, 20:80, 40:60 and 80:20 water–glycerin mixtures were tried. The observed viscosities of these mixtures plotted against temperature are shown in Fig. 1. As can be seen from these figures, the viscosity resulting from the 20 and 60% glycerin were too low to be of practical use in the contemplated work. Therefore, all subsequent experiments were conducted using 80% glycerine. In both cases, however, the observed viscosities exhibited asymptotic decay as the temperature is increased indicating a thinning tendency of the bulk viscosity of the fluid with a rise in temperature.

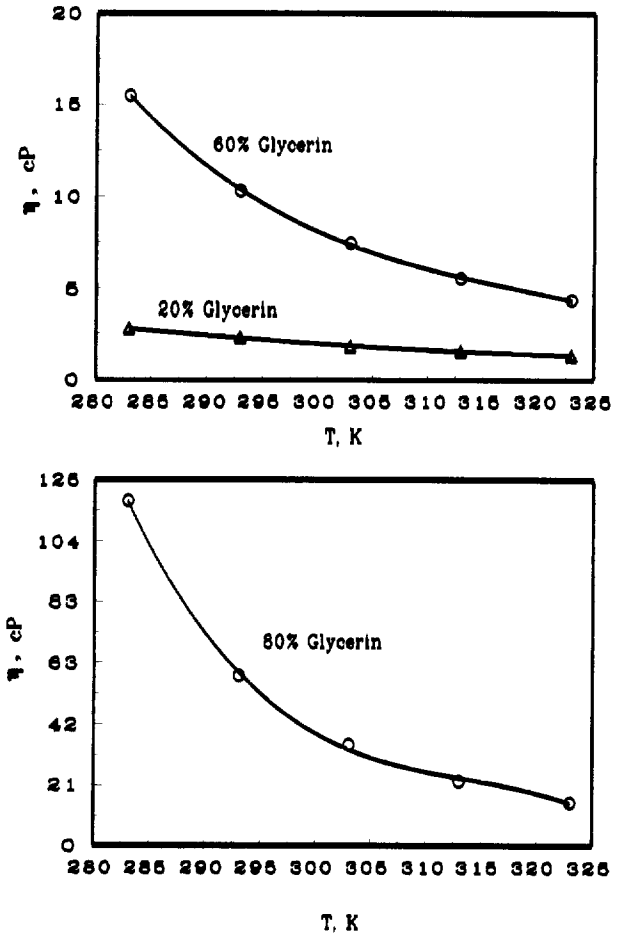

Fig. 1. Plot of the observed viscosity as a function of temperature at different glycerin compositions.

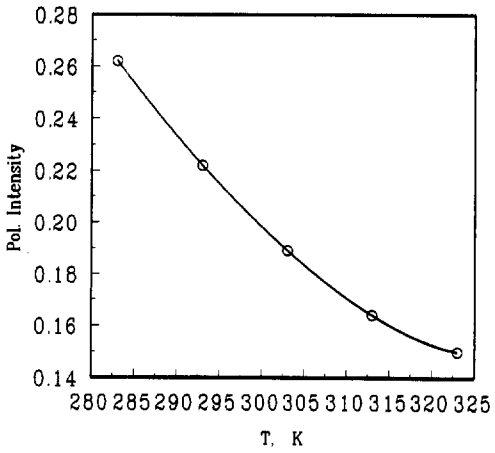

Fig. 2. Plot of the observed fluorescence polarization intensity vs. temperature.

Table 1
List of observed fluidity and microviscosity at different temperatures

Temperature (K)	Microviscosity (cP)	Fluidity (cP^{-1})
283	2.65	0.008
293	1.86	0.017
303	1.39	0.029
313	1.11	0.045
323	0.97	0.067

A similar temperature-dependent decrease in fluorescence polarization intensity of riboflavin solubilized in 80% glycerin was also observed as can be seen in Fig. 2. Polarization intensity is enhanced in or near vitrified medium. Therefore, the observed decrease in polarization intensity as the temperature increases is not unexpected and is attributable to an increase in the thermal motion of riboflavin in a thinning solution.

The microviscosity, $\bar{\eta}$, of riboflavin in the water–glycerin mixture was determined using the equation developed by Shinitzky and Barenholz [4].

$$\bar{\eta}=2p/(0.46-p)$$

In the above equation p refers to the observed polarization intensity of the probe. The calculated values are shown on Table 1. Also shown on this table is the fluidity, the reciprocal of viscosity, which may be defined as the tendency of a fluid

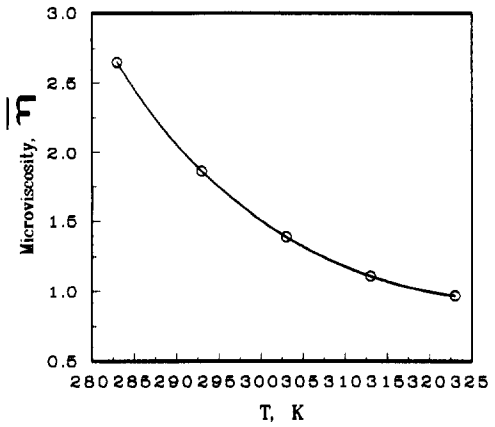

Fig. 3. Plot of the calculated microviscosity vs. temperature.

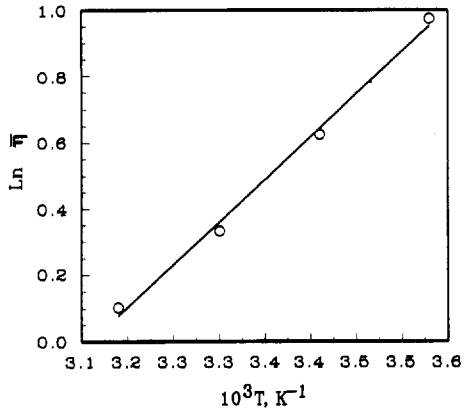

Fig. 4. Plot of ln of microviscosity vs. the reciprocal of temperature.

to flow [3]. From this table it is seen that the microviscosity varies inversely with fluidity as it should be. The easier the tendency for a fluid to flow the lower the microviscosity of the medium. In other words, where there is less mechanical barrier for flow the lower the viscosity and the greater the flow. The microviscosity is plotted against temperature as can be seen on Fig. 3. Again, it can be seen that as the temperature is increased, the microviscosity decreases in a way consistent with what was observed for the bulk viscosity of the medium.

The flow activation energy, E_a, of this system was also determined by the slope of the plot of the natural logarithm of microviscosity vs. the inverse of temperature, in absolute scale, in accordance with the Newtonian flow parameter:

$$\ln \bar{\eta} = Ae^{\Delta E_a/RT}$$

where R and T are the universal gas constant and absolute temperature, respectively. A is a characteristic constant for the system. The plot, as can be seen in Fig. 4, is linear and consistent with the Arrhenius plot for energy of activation determination. From the slope of this equation the flow activation energy was determined as 19.80 kJ mole^{-1}. This value together with the microviscosity values shown on Table 1 are in good agreement with the literature values of some vesicles, proteins and synthetic liposomes whose viscosities are similar to our model fluid.

4. Conclusion

It has been shown in this preliminary work that riboflavin is useable as a fluorescent probe compound in the study of the fluidity of molecular aggregates and clusters. The microviscosity of the model medium (20:80 v:v water–glycerin mixture) probed by the use of riboflavin, and the calculated flow activation energy obtained from the polarization data were comparable with those obtained using the conventional probes in the media of similar viscosity. It is envisaged that from the result of this work, riboflavin could find application as a probe in the study of the dynamics and rigidity of biological liposomes and vesicles.

Acknowledgements

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