

## ESR/Spin Probe Study of Ice Cream

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Spin probes based on the 1,1,3,3-tetramethylisindolin-2-yl structure have been used, in conjunction with electron spin resonance spectroscopy (ESR), to study the physical changes occurring in ice cream during freezing and melting. The ESR measurements allowed the rotational correlation times,  $\tau(B)$ , of the spin probes to be determined. Two probes were used together in a given sample of ice cream, namely, 1,1,3,3-tetramethylisindolin-2-yl (TMIO), which samples the fat phase, and the sodium salt of 1,1,3,3-tetramethylisindolin-2-yloxy-5-sulfonate (NaTMIOS), which samples the aqueous phase. Data from the TMIO probe showed that when ice cream is cooled, the fat phase is a mixture of solid and liquid fat until a temperature of  $\sim -60$  °C is reached. The water-soluble probe NaTMIOS showed that the aqueous phase changes completely from liquid to solid within 1 °C of  $-18$  °C. On cooling further to  $-24.7$  °C and then allowing it to warm to  $+25.0$  °C, the rotational correlation times of the NaTMIOS were slow to recover to their previous values. For the lipid phase,  $\tau(B)_{298}$  was found to be  $65.7 \pm 2.0$  ps and the corresponding activation enthalpy,  $\Delta H^*$ , was  $32.5 \pm 0.9$  kJ mol<sup>-1</sup>. These values are typical of those expected to be found in the type of fat used to make ice cream. The water phase gave corresponding values of  $32.2 \pm 0.5$  ps and  $24.5 \pm 0.4$  kJ mol<sup>-1</sup> values, which are those expected for a sucrose concentration of 24%.

**KEYWORDS:** ESR; spin probes; ice cream; rotational correlation times; enthalpies

### INTRODUCTION

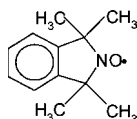
Ice cream is a complicated physiochemical system having air cells dispersed in a continuous liquid phase with embedded ice crystals (1, 2). Dispersed in the liquid phase are solidified fat globules, milk proteins, lactose (in some cases), stabilizers, sugars, and soluble and insoluble salts (2). "Ice cream mix" refers to the system before air is incorporated by the freezing process. The mix is comprised of (i) the aqueous phase containing sugar and salts; (ii) a colloidal suspension of milk proteins, stabilizer, insoluble sweetener solids, and possibly phosphates of milk minerals; and (iii) a coarse dispersion of fat globules. The proteins in the colloidal suspension help to make the ice cream more compact and smooth. Typically, dairy ice cream contains 65% water, 15% sugar, 10% fat, 10% milk-solids-not-fat, and small quantities of emulsifier and stabilizer. The viscosity of the ice cream mix is an important factor in producing a good ice cream: The freezing point is also important, with high-fat and low-sugar and milk-solids-not-fat solids yielding a higher freezing point. There are many formulations and methods for making ice cream (2). An

important part of the process is to incorporate air so that, by volume, the finished product typically contains 50% air, 30% ice, 15% sugar solution, and 5% fat: There are three states of matter present as follows: solid (ice and lipid), liquid (sugar solution), and gas. To develop new formulations and processes, it is important to understand the microstructure of ice cream. Most methods of characterization of the microstructure involve microscopy: There are no reports of magnetic resonance studies being made on the physical changes occurring in the water and fat phases of ice cream during the freezing process. Previously, we have employed successfully electron spin resonance (ESR) in conjunction with small spin probes to study palm oil/water emulsions (3) to investigate temperature and storage effects at low temperature. Because palm oil and butter fat contain similar amounts of C<sub>14</sub> to C<sub>18</sub> fatty acids, it is likely that a similar application to ice cream would be useful. Furthermore, the two types of fat have similar melting profiles. In order for the ice cream composition to be well-defined and for spin probes to be incorporated, ice cream had to be prepared in the laboratory. Also, adding spin probes to a commercial ice cream would have resulted in a loss of structure due to mixing and agitation. An emulsifier and a stabilizer (typically present to  $\sim 0.2\%$  each) affect some of the macroscopic properties of the ice cream, but

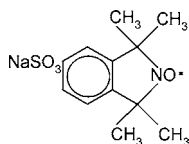
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the influence of temperature on the microviscosities of the water and the fat should be unaffected.

Combined with the selection of an appropriate spin probe, ESR can be invaluable for studying materials containing fat and water (3, 4). We have found that spin probes based on the 1,1,3,3-tetramethylisindolin-2-yl structure (TMIO) are superior to commercially available spin probes such as TEMPO (5): The main reasons are that there are fewer unresolved proton interactions and the molecules are rigid so that rapid interconversion does not increase the line width. Thus, (i) the TMIO-based probes have smaller ESR line widths and a higher sensitivity to molecular motion (4), and (ii) substitution in the aromatic ring allows the partition coefficient to be tailored over a much wider range than is possible with commercial probes. A further advantage is that they are chemically and thermally more stable. The probes used in this work are 1,1,3,3-tetramethylisindolin-2-yloxy (TMIO) (6, 7),



which has a partition coefficient ( $\log K_{OW}$ ) of 2.40 (8) and thus is only slightly soluble in water, and the sodium salt of 1,1,3,3-tetramethylisindolin-2-yloxy-5-sulfonate (NaTMIOS) (9)



This spin probe is soluble only in polar media.

TMIO and NaTMIOS can be incorporated together into a sample of ice cream because of the differences in their partition coefficients and their ESR spectra. They can report on the microviscosities of the fat and water, respectively: This is particularly important for the fat where fat can solidify within the liquid phase.

## MATERIALS AND METHODS

**Sample Preparation.** Ice cream was prepared using a Magimix Le Glacier ice cream maker model 1.1, which mimics the factory process by simultaneously aerating, freezing, and beating the ice cream mix to generate ice crystals, air bubbles, and the matrix (sugar solution). A 142 cm<sup>3</sup> amount of fresh double cream was mixed with 15 cm<sup>3</sup> of fresh full-fat milk, and to this was added, with gentle stirring (to avoid whipping), 22.5 g of icing sugar, a few grams at a time. The double cream consisted of 48 g of fat, 1.7 g of protein, and 2.6 g of carbohydrate per 100 cm<sup>3</sup>. The ice cream mix was chilled (aging process) for 1 h at ~5 °C. The mix was then added to the mixer bowl (previously stored at -18 °C for 24 h) and churned for 10 min in order to incorporate air and to freeze partly. The frozen ice cream was transferred to plastic tubs and stored at -18 °C (hardening process). Prior to mixing, typically 50 mg of NaTMIOS was added to the milk and 30 mg of TMIO was added to the milk and cream: These amounts produced spin probe concentrations each of ~1 mM. Experiment showed that a spectrally negligible amount of only about 0.2% of TMIO was dissolved in the aqueous phase, as compared with the spectrum from the NaTMIOS.

Because ice cream contains ~65% water, it is very "lossy" to microwave radiation; hence, it is necessary to introduce samples of small cross-sections into the spectrometer cavity. To achieve this, ice cream samples were drawn into thin-walled 0.8 mm i.d. borosilicate capillary tubes, which were then put into 5 mm NMR tubes. The

required spectrometer temperature was set before a sample was introduced, but no measurements were made until the sample had been allowed to thermally equilibrate for 20 min.

**ESR Measurements.** A JEOL JES-RE1X X-band spectrometer fitted with a JEOL DVT-2 sample temperature controller was used. A Hewlett-Packard 53181A counter was used to measure the microwave frequency, and a JEOL NMR field meter ES FC5 was used to measure the magnetic field. Spectra were recorded digitally with a personal computer fitted with a Real Time Devices ADA2000 digitizer and EPRW software supplied by Scientific Software Services (Bloomington, IL). The data were processed off-line. Because the ESR spectra comprised two overlapping three-line nitroxide spectra, deconvolution was necessary. This was accomplished using the EWVOIGT software supplied by Scientific Software Services. This iterative fitting program calculates (i) nitrogen-14 hyperfine coupling constants,  $a^N$ ; (ii) Gaussian line widths,  $W_G$ ; (iii) Lorentzian line widths,  $W_L$ ; (iv) line positions; and (v) fractions of the two components, together with error estimates on all parameters. The deconvolution process was assisted by the fact that  $a^N$  values were slightly different: NaTMIOS in a polar medium had  $a^N$  values of ~1.56 mT, and TMIO in a nonpolar medium had  $a^N$  values of ~1.40 mT. The  $a^N$  values had positive temperature coefficients. The rotational correlation times  $\tau(B)$  and  $\tau(C)$  were calculated from the  $W_L$  values (8):

$$\tau(B) = 5.75 (\pm 0.3) [W_L(-1) - W_L(+1)/B_0] \times 10^{-7} \text{ s} \quad (1)$$

$$\tau(C) = 1.72 (\pm 0.7) [W_L(+1) + W_L(-1) - 2W_L(0)] \times 10^{-9} \text{ s} \quad (2)$$

where  $B_0$  is the magnetic field at the center of the spectrum and the values +1, 0, and -1 refer to the low-, center-, and high-field lines. The above equations apply to systems in which the rotation is isotropic and for values of  $\tau(B)$  and  $\tau(C)$  less than ~500 ps. Comparison of the two rotational correlation times gave an indication of the isotropy of the probe motion: When  $\tau(B)/\tau(C) \sim 0.95$ , the motion was isotropic, as was found in this study. Only  $\tau(B)$  values are reported here since they can be calculated somewhat more accurately than those of  $\tau(C)$ , because they represent averaging of both the hyperfine and the  $g$  factor anisotropies (5, 8).

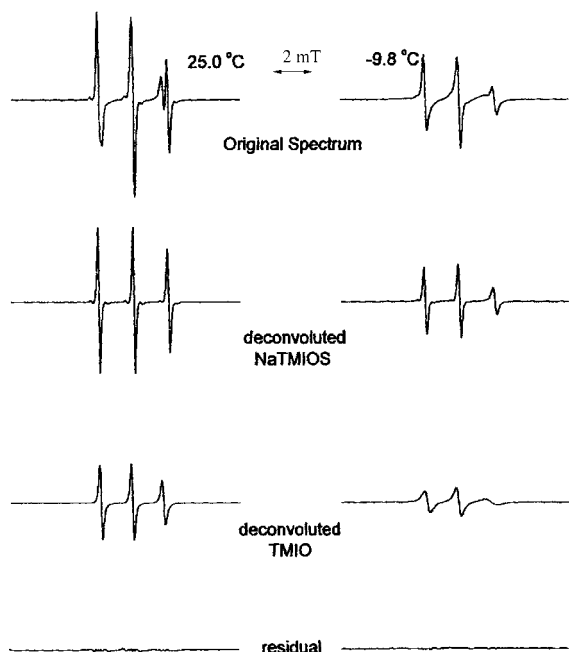
At low temperatures, the spin probes can no longer be regarded as tumbling rapidly; hence, the above equations are not then appropriate. For this low-temperature region, the approach used by Goldman et al. (10) was adopted. Slow motional rotational correlation times were estimated by measuring the separation,  $2A_z'$ , of the outer lines as seen in the low-temperature spectra shown in **Figure 3**. This separation was compared with that,  $2A_z$ , observed for the rigid limit spectrum. The rotational correlation time,  $\tau$ , is given by

$$\tau = a(1 - S)^b \quad (3)$$

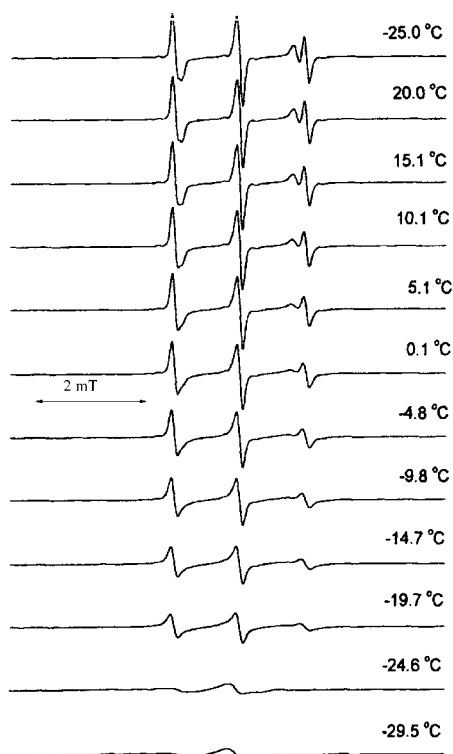
where  $S = A_z'/A_z$  and the parameters  $a$  and  $b$  were determined by Goldman et al. (10) for different models. Here, the Brownian diffusion model was adopted and for this  $a$  and  $b$  have values  $2.57 \times 10^{-10}$  s and -1.78, respectively, for a Lorentzian line width of 0.03 mT (to be referred to as set 1) and values of  $5.4 \times 10^{-10}$  s and -1.36 for a Lorentzian line width of 0.3 mT (to be referred to as set 2). Values of rotational correlation times calculated from eq 3 can only be semi-quantitative.

## RESULTS AND DISCUSSION

**High-Temperature Measurements.** **Figure 1** shows typical ESR spectra of ice cream containing both NaTMIOS and TMIO at 25.0 and -9.8 °C. Also shown are deconvoluted (simulated) spectra obtained by the EWVOIGT program. Note that the small peaks arise from carbon 13 satellites and that all of the lines of the TMIO spectra are broader than those of NaTMIOS due to the higher concentration of paramagnetic oxygen dissolved in the lipid. **Figure 2** shows the effect of temperature on the mobility of both probes in the temperature range of -29.5 to +25.0 °C. From the spectra in the temperature range of -14.7



**Figure 1.** Deconvoluted X-band ESR spectra of TMIO and NaTMIOS in a single sample of ice cream at 25 and  $-9.8$  °C.



**Figure 2.** X-band ESR spectra of TMIO and TMIO in a single sample of ice cream at various temperatures.

to  $+25.0$  °C were derived the Lorentzian line widths, and the rotational correlation times are shown in **Table 1**. It may be seen from the latter, despite the small difference in size of the two probes, that  $\tau(B)$  for TMIO is larger than  $\tau(B)$  for NaTMIOS due to the fact that the lipid has a greater viscosity than water. As a result, the low-temperature values of  $\tau(B)$  for TMIO are becoming too large for accurate measurement. It may also be seen that the water phase is still liquid and no ice crystals have formed. From plots of  $\ln\tau(B)$  vs absolute temperature ( $R$  values were in the range of 0.997–0.999), the thermodynamic parameters could be calculated as follows: NaTMIOS in the water

phase  $\tau(B)_{298} = 32.2 \pm 0.5$  ps and  $\Delta H^* = 24.5 \pm 0.4$  kJ mol $^{-1}$ ; TMIO in the lipid phase  $\tau(B)_{298} = 65.7 \pm 2.0$  ps and  $\Delta H^* = 32.5 \pm 0.9$  kJ mol $^{-1}$ .

Experiments were also carried out with NaTMIOS and TMIO used separately in order to verify the procedure; similar data were obtained. In the case of NaTMIOS, spectra were obtained at 1 °C intervals lowering the temperature from  $-15$  °C when it was found that there was a sharp decrease in probe mobility at  $-18$  °C. Data were also obtained for NaTMIOS in pure water when values of  $\tau(B)_{298}$  and  $\Delta H^*$  were found to be 10.1 ps and 17.5 kJ mol $^{-1}$ , respectively—in good agreement with earlier studies (8). The enthalpies of activation reported here refer strictly to microviscosity, but the enthalpy of activation derived from the macroviscosity of water has been found to be  $17.23 \pm 0.05$  kJ mol $^{-1}$ . As expected in this case, micro- and macroviscosities and their enthalpies of activation should be the same for a pure liquid. From our previous work on the water-soluble probe in sucrose solutions (8), the present NaTMIOS results correspond to a sucrose concentration in the region of 24%, which produced  $\tau(B)$  and  $\Delta H^*$  values of 32.8 ps and 23.9 kJ mol $^{-1}$ , respectively. This concentration is close to that expected, based on the composition of the ice cream mix used in the experiments, and thus, the aqueous phase can be expected to behave like a normal liquid. Hence, for the temperature range 25.0 to  $-14.7$  °C, it can be concluded that both the lipid and the water phases behave like normal liquids. Once the solid state had been reached, ice crystals may form but these could not be detected in these studies: The NaTMIOS would become immobile in both the crystalline and the glassy state and the resulting solid state (“polycrystalline”) ESR spectra would be indistinguishable. However, NMR deuterium spin–lattice measurements have shown that solid solutions of glucose in water contain both a crystalline ice phase and an amorphous glass phase (12). The probe changes its mobility from relatively mobile to relatively immobile in the very narrow temperature range of  $-18$  to  $-19$  °C.

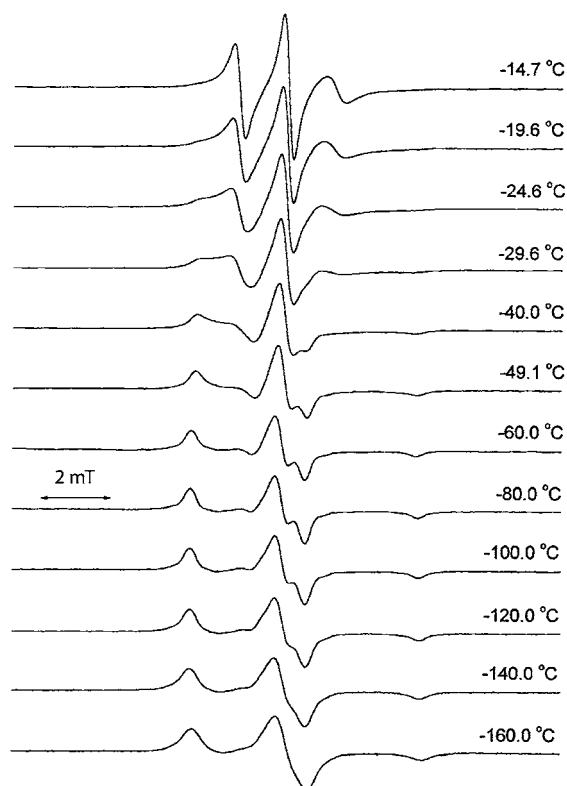
To check whether the physical changes are reversible, the temperature was decreased incrementally in  $\sim 10$  °C intervals from 25 to  $-25$  °C and then similarly increased from  $-25$  to 25 °C: The sample was maintained at each temperature for 20 min. The TMIO data showed no difference in rotational correlation times on cooling and on heating. However, the NaTMIOS showed that the probe became immobile on cooling when  $-24.7$  °C was reached and then, on warming, did not become mobile again until  $-4.9$  °C was reached. This effect is probably caused by the fact that the ice content would not be significantly reduced until the sample had warmed to  $\sim 5$  °C (1). It was found that NaTMIOS and TMIO gave values of  $\tau(B)_{298}$  and  $\Delta H^*$  within experimental error of those reported above, showing that the overall changes are reversible.

**Low-Temperature Measurements.** From the spectra at  $-24.6$  and  $-29.5$  °C shown in **Figure 2**, it may be seen that the spin probes are beginning to approach the rigid limit. Because the deconvolution program EWVOIGT could not be applied to data obtained below  $\sim -20$  °C, experiments were carried out with the probes used separately. A series of ESR spectra of NaTMIOS were obtained in the temperature range of  $-24.6$  to  $-104$  °C, which all showed that the rigid limit had been reached; that is, the value of  $\tau(B)$  was greater than about 1  $\mu$ s for all of the temperatures in this range.

ESR spectra of the spin probe TMIO in ice cream were recorded in the temperature range of  $-14.7$  to  $-160$  °C when it was found that the rigid limit spectrum gradually evolved (see **Figure 3**). From a series of spectra similar to these, values

**Table 1.** Lorentzian Linewidths and Rotational Correlation Times of NaTMIOS and TMIO in Ice Cream

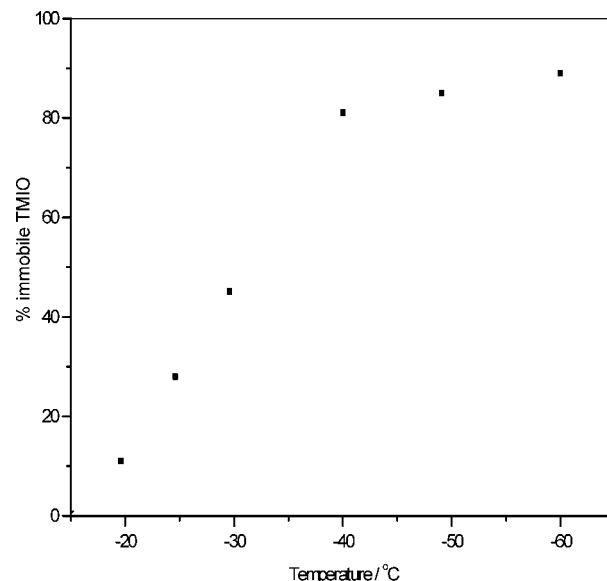
temp (°C)	NaTMIOS				TMIO			
	$W_L(+1)$ (mT)	$W_L(0)$ (mT)	$W_L(-1)$ (mT)	$\tau(B)$ (ps)	$W_L(+1)$ (mT)	$W_L(0)$ (mT)	$W_L(-1)$ (mT)	$\tau(B)$ (ps)
25.0	0.0245 ± 0.0006	0.0240 ± 0.0006	0.0424 ± 0.0006	31.7 ± 0.1	0.0613 ± 0.0013	0.0597 ± 0.0012	0.0977 ± 0.0014	64.4 ± 1.7
20.0	0.0268 ± 0.0006	0.0257 ± 0.0006	0.0479 ± 0.0007	37.3 ± 1.0	0.0671 ± 0.0013	0.0665 ± 0.0013	0.1124 ± 0.0015	80.2 ± 1.9
15.1	0.0300 ± 0.0006	0.0281 ± 0.0006	0.0555 ± 0.0007	45.1 ± 1.1	0.0763 ± 0.0014	0.0763 ± 0.0013	0.1349 ± 0.0016	104 ± 2
10.1	0.0338 ± 0.0006	0.0316 ± 0.0006	0.0645 ± 0.0007	54.3 ± 1.1	0.0957 ± 0.0015	0.0943 ± 0.0014	0.1705 ± 0.0018	132 ± 3
5.1	0.0406 ± 0.0006	0.0374 ± 0.0006	0.0782 ± 0.0007	66.5 ± 1.2	0.1234 ± 0.0015	0.1187 ± 0.0015	0.2179 ± 0.0021	167 ± 3
0.1	0.0495 ± 0.0007	0.0451 ± 0.0007	0.0960 ± 0.0008	82.3 ± 1.4	0.1592 ± 0.0019	0.1504 ± 0.0018	0.2776 ± 0.0028	209 ± 3
-4.8	0.0546 ± 0.0013	0.0502 ± 0.0012	0.1119 ± 0.0016	101 ± 3	0.2029 ± 0.0024	0.1832 ± 0.0024	0.3732 ± 0.0041	301 ± 5
-9.8	0.0483 ± 0.0015	0.0429 ± 0.0015	0.1142 ± 0.0018	116 ± 5	0.2538 ± 0.0024	0.2188 ± 0.0024	0.4903 ± 0.0039	418 ± 5
-14.7	0.0563 ± 0.0018	0.0488 ± 0.0019	0.1383 ± 0.0023	145.1 ± 5.2	0.3089 ± 0.0030	0.2511 ± 0.0027	0.5919 ± 0.0052	501 ± 7

**Figure 3.** X-band ESR spectra of TMIO in ice cream at low temperatures.**Table 2.** Estimated Rotational Correlation Times for TMIO in Ice Cream at Low Temperatures

temp (°C)	$2A_z'$ (mT) <sup>a</sup>	$S^b$	$\tau(1)$ (ns)	$\tau(2)$ (ns)
-30.0	6.090	0.933	31.6	21.3
-35.0	6.165	0.944	43.5	27.2
-40.0	6.180	0.947	47.9	29.3
-45.0	6.285	0.963	90.9	47.8
-50.0	6.285	0.963	90.9	47.8
-60.0	6.345	0.972	149	69.9
-80.0	6.420	0.984	404	150
-100.0	6.450	0.989	788	248
-120.0	6.510	0.998		
-140.0	6.525	1.000		
-160.0	6.525	1.000		
-170.0	6.525	1.000		

<sup>a</sup> The errors are ±0.015 mT. <sup>b</sup> The errors are ±0.002.

of  $2A_z'$  were measured and from these values the  $S$  parameters of eq 3 were measured, as shown in **Table 2**. Also in the table are values of  $\tau(1)$  and  $\tau(2)$  for sets 1 and 2 as defined above: Set 2 is more likely to be appropriate since the line width is ~0.2 mT. Arrhenius plots for  $\tau(1)$  and  $\tau(2)$  give enthalpies of

**Figure 4.** Estimation of the proportion of TMIO in an immobile phase in ice cream at low temperatures. Estimations made from the spectra shown in **Figure 3**.

activation of  $19.4 \pm 1.4 \text{ kJ mol}^{-1}$  ( $R = 0.984$ ) and  $14.9 \pm 1.0 \text{ kJ mol}^{-1}$  ( $R = 0.984$ ), respectively. These do not agree well with the enthalpy of activation found for TMIO for temperatures above  $\sim -15 \text{ }^\circ\text{C}$ . However, the Arrhenius plots are linear suggesting that no major phase change occurs in cooling the lipid phase down to  $-170 \text{ }^\circ\text{C}$ . In **Figure 3**, it can be seen that at a temperature of  $-29.6 \text{ }^\circ\text{C}$  there is still a fraction of TMIO in a mobile environment. Using the Freed/Schneider computer simulation program (13), it is possible to obtain semiquantitative data on the effect of temperature on the relative amounts of mobile and immobile phases present. The immobile phase is likely to be solidified fat globules in the liquid matrix. The results are shown in **Figure 4** where the plot, essentially a curve within experimental error, shows that there is no sharp phase change as the fat solidifies.

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