DIFFERENTIAL SCANNING CALORIMETRIC STUDY ON ICE NUCLEATION IN WATER AND IN AQUEOUS SOLUTIONS OF HYDROXYETHYL STARCH

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ABSTRACT

   An experimental investigation has been performed on ice nucleation rates over a range of degrees of undercooling, in water and in 15%, 25% and 35% w/w aqueous solutions of hydroxyethyl starch. An emulsion of droplets of the aqueous phase to be studied was cooled in a differential scanning calorimeter. The heat of crystallization was measured from which the nucleation rate was deduced after the droplet size (assumed uniform) had been determined. All the results obtained are in the main consistent with classical nucleation theory. The presence of hydroxyethyl starch is found to lead to pronounced increases in the exponential factor; this effect may be explained by changes in the interfacial free energies between ice and the aqueous phases. Discrepancies seen at both the high and the low temperature ends of the scans can be attributed to contributions to the exotherms by droplets of volumes larger and smaller, respectively, than the average.

INTRODUCTION

   Knowledge about nucleation mechanisms of ice in condensed phases is important to the studies of cloud physics and cryobiology. Much work has been done (for a summary see, for example, ref. 1 or 2) but is in the main restricted to the case of nucleation in pure water. Critical data relating to the effect of solutes on undercooling and freezing mechanisms may be provided by investigating the process in aqueous solutions of different substances. Thus the interfacial free energies between ice and undercooled solutions have been deduced from measurements of so-called homogeneous nucleation temperatures [3]. Nucleation is a time-dependent process; a more general

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method is therefore the direct determination of nucleation rates as a function of temperature.

This paper reports the results of such an attempt using the technique of differential scanning calorimetry (DSC). The solute chosen is hydroxyethyl starch (HES) which is increasingly used as a cryoprotectant, e.g., of blood components. An emulsion of droplets of water or an aqueous solution is subjected to decreasing temperature. Since ice crystal growth is rapid compared to nucleation, the rate of the latter phenomenon may be assumed equal to that of droplet freezing and calculated from the heat of crystallization that is liberated.

EXPERIMENTAL

Materials

Doubly distilled water was used throughout. HES (ex McGaw Laboratories, California) was purified by dialysis against distilled water and freeze drying. It had a degree of substitution of 0.75 and a nominal molecular weight of 450 000. Aqueous solutions were made up by weight.

Sorbitan tristearate (SPAN 65, ex Fluka) was employed as emulsifier. It was recrystallized from ethanol and dried under vacuum. A 5% w/w solution was prepared in silicone oil of viscosity 1 cP (constant over 200–300 K range) and this solution was employed as the continuous phase.

Preparation

The nucleation of ice was studied in pure water and aqueous solutions of 15%, 25% and 35% w/w HES. The aqueous phase to be investigated was added slowly from a pipette into the oil phase, which was being continuously homogenized by a Polytron ultrasonic blender operating at 10 000 rpm. The weight added was always a third of that of the oil phase. Droplet size distributions were obtained from micrographs of sample volumes of the emulsion after suitable dilution with 1 cP silicone oil.

Method

In each experiment between 2 and 4 mg of the mixture, equivalent to 0.5 to 1 mg of aqueous phase, were transferred in a sealed aluminium sample pan to a Perkin-Elmer DSC-2 instrument. An empty sealed pan served as the reference. The calorimeter was fitted with the Intracooler II subambient accessory and a dry box. The DSC head and the dry box were purged continuously with dried nitrogen.

The same cooling rate of 2.5 K min^{-1} was used in all experiments.

Indium, supplied by Perkin-Elmer (melting point specified as 429.78 K) and ice were utilized for temperature calibration. For greater reliability, comparison was made between the indicated temperature (where the line of greatest positive slope on the melting endotherm intersected the baseline) and the true value at heating rates of 0.313, 0.625, 1.25 and 5.0, as well as 2.5 K min^{-1} [4]. No calibration of the energy scale was undertaken as only relative quantities were of significance [see eqn. (2)].

The equilibrium freezing temperatures (i.e. melting temperatures) of 15%, 25% and 35% starch solutions were measured from their respective melting endotherms.

RESULTS

The droplet size distribution obtained for 25% HES solution is compared in Fig. 1 with that reported by Michelmore and Franks [5] for water. The distributions for 25%, 15% and 35% solutions are almost identical. Their displacements from that for pure water are probably due to the higher viscosities. They are sufficiently narrow so that, in the data analysis to be considered below, the droplets may be treated as having a uniform volume in
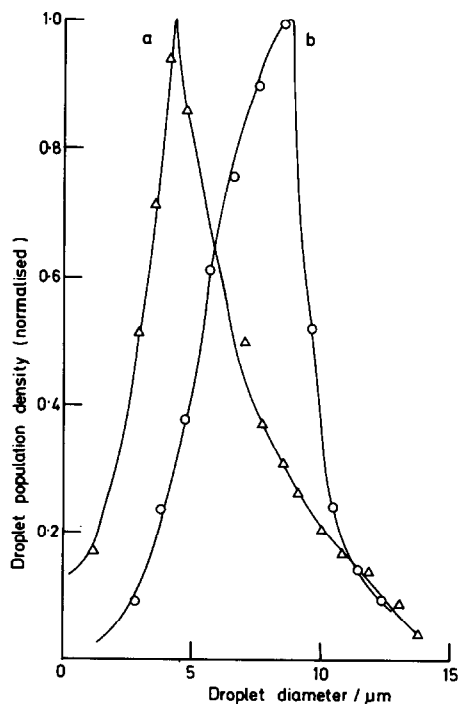

Fig. 1. Normalized droplet diameter distributions for (a) water (b) 25% HES solution.

each case. These volumes, calculated from the most probable values of droplet diameter, are 0.34, 3.6, 3.6 and 3.7 (10^{-16} m^3) for emulsions of water and 15%, 25% and 35% solutions, respectively. These results are in good agreement with drop size distributions previously reported for water and aqueous solutions of polyethylene glycol [5].

The number of droplets in each experiment was therefore in excess of 10^7, and should be large enough for the ice nucleation rate to be measured reliably as a function of temperature. On the other hand, the DSC sample weights were judged to be sufficiently small for self-heating due to freezing to cause insignificant (time-dependent) errors in temperature readings. The difference between indicated and true temperature was found to be a virtually linear function of the heating rate (slope = 0.107 min) and thus attributable to thermal lag between the pan holder and the sample; being systematic, it is assumed to be eliminated by the calibration. Temperature data are regarded as accurate to ± 0.1 K.

A typical DSC power–time trace obtained on cooling an emulsion is shown in Fig. 2. The absence of a long 'tail' on the high temperature side of the scan confirms that the emulsion was of a sufficiently homodisperse nature and did not contain appreciable concentrations of large droplets that would freeze at higher temperatures. Indeed, the quality of the emulsion is sensitively reflected in the shape of the power–time curve [6].

The ice nucleation rate J at temperature T, corresponding to time t, may be calculated as follows. Let the emulsion be characterized by the most probable droplet volume v and $N(t) \equiv N$ represent the number of droplets that have frozen at time t. Consider a time interval Δt and equivalently ΔT so short that $J(T + \Delta T) \approx J(T) \equiv J$. If the assumption is valid that the time scale for ice crystal growth in volume v is shorter still than Δt (and not more than one ice nucleus has time to form within v) then, from the definition of J
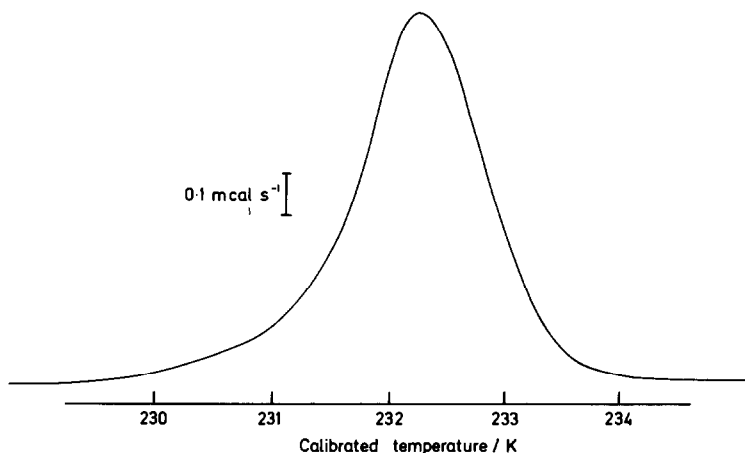

Fig. 2. DSC trace of 15% HES emulsion cooled at 2.5 K min^{-1}.

$$\mathrm{d}N = Jv[\,N(0) - N\,]\;\mathrm{d}t \tag{1}$$

or, in terms of $\alpha \equiv N/N(0)$

$$\int_t^{t+\Delta t} \frac{\mathrm{d}\alpha}{1-\alpha} \approx Jv \int_t^{t+\Delta t} \mathrm{d}t$$

so that, since $1 - \alpha(t) \propto A(t) \equiv$ the area under the DSC trace from t to the completion of freezing

$$J = \frac{1}{v\,\Delta t}\;\ln\frac{A(t)}{A(t+\Delta t)} \tag{2}$$

In the present work $\Delta t = 3.75$ s and correspondingly $\Delta T = 0.156$ K have been chosen and the areas measured with a planimeter: relative errors in A are taken as $\pm 2\%$. The values of $J(T)$ thus calculated are plotted in Fig. 3 to illustrate the functional dependence predicted by classical nucleation theory (discussed below).

The equilibrium freezing temperatures of 15% and 25% solutions were determined as $T_m = 272.6$ and 272.4 K, respectively; that for water is of course 273.15 K. The value for the 35% solution was estimated by extrapolation to be 272.2 K; the melting endotherm in this case is reproduced in Fig. 4 which shows two features which have been discussed previously [7]. A is the glass transition temperature T'_g of an HES solution of the limiting composition ($\sim 70\%$) obtained after all the freezable water has frozen; it is therefore independent of the initial solution concentration. The exotherm B is due to an instrumental artefact related to a temperature gradient across the sample pan which causes distillation of water with its subsequent
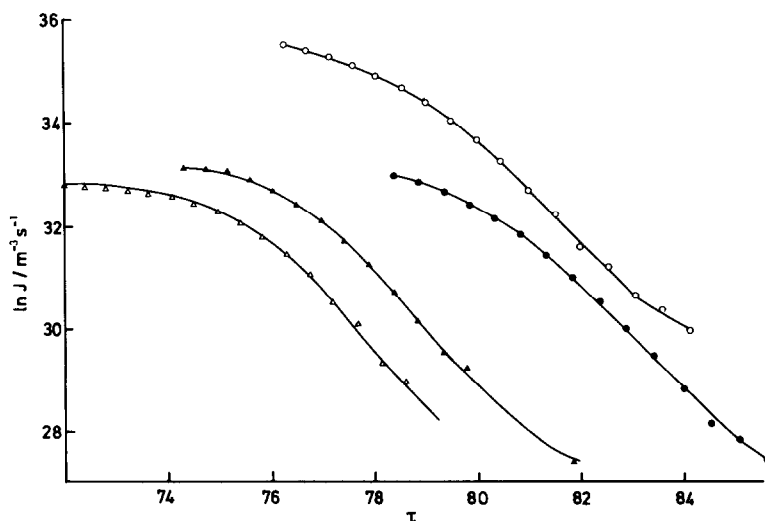

Fig. 3. Ice nucleation rate as a function of 'reduced temperature' as defined by eqns. (4) and (5).
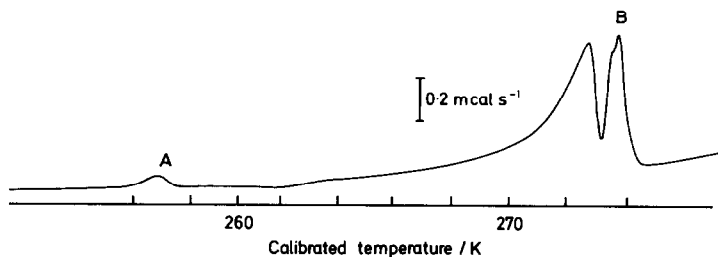
Fig. 4. DSC trace of 35% HES emulsion heated at 2.5 K min^{-1}.

condensation on the lid and/or the surface of the viscous HES solution with which it does not mix spontaneously. Endotherm B thus always occurs at 273.15 K. This separation of pure water means, of course, that the actual concentration of HES is higher than the nominal concentration. Any evaluation of the melting endotherm must be corrected for this artefact.

DISCUSSION

Classical nucleation theory, the relevant details of which may be found in ref. 8, predicts that

$$J = A \exp\left(B \big/ \left[T^3 (\Delta T)^2 \right] \right) \tag{3}$$

where A and B are constants characteristic of the aqueous phase and ΔT is the degree of undercooling. In this work, in order that results for different types of aqueous phase can be compared on an equal basis, T is replaced by the reduced temperature

$$\theta \equiv T / T_m \tag{4}$$

In Fig. 3 ln J is plotted against

$$\tau \equiv 1 \big/ \left[\theta^3 (1 - \theta)^2 \right] \tag{5}$$

It can be seen that the plots give straight lines over at least two orders of magnitude in J for each of the four aqueous phases, supporting the classical relation (3). In each case, nevertheless, the experimentally derived J levels off at small τ. This may be explained by the increasing contribution from smaller droplets [5], in which ice nucleations occur with much lower probabilities per unit time, as t increases (and correspondingly T decreases). The derived J thus becomes an underestimate, since the real effective value of v has not been used in eqn. (2). It must also be borne in mind that the basic assumption of rapid freezing of the droplets may no longer apply at the lowest temperatures, because the viscosities of HES solutions increase steeply with decreasing temperature and increasing concentration, as takes place during freezing.

TABLE 1

Kinetic constants of ice nucleation

% HES	$B(K^5)$	$A(10^{50} \text{ m}^{-3} \text{ s}^{-1})$
0	1.029 ± 0.01	3.0 ± 0.5
15	1.044 ± 0.01	4.0 ± 0.8
25	1.080 ± 0.01	10.0 ± 2.0
35	1.138 ± 0.01	30.0 ± 5.0

Consider now the curvature of the plots at high τ. In a previous investigation on nucleation in pure water by means of microscopic observation of 300–500 droplets [8], the same discrepancy was noted and interpreted as a consequence of heterogeneous nucleation. However, the possibility that the emulsifier used becomes active as nucleators at some temperature can be discounted under the experimental conditions employed [5]. It should be noted, nevertheless, that prolonged storage of water emulsions at subzero temperatures substantially higher than those associated with rapid nucleation does lead to eventual freezing [9,10], presumably by some heterogeneous mechanism involving the emulsifier. In the present case, the discrepancy may be more plausibly explained as due to ice nucleation in droplets larger than the mean volume.

The values of A and B as defined by eqn. (3) have been obtained from the linear sections of the plots and are given in Table 1. Note that the error in determining v, estimated as $3 \times 5\%$, affects the accuracy of A but not B.

It is obvious from Table 1 that the presence of HES leads to an increase in B, the effect being more marked the higher the concentration (up to 35%). This increase may most naturally be interpreted as an increase in the interfacial free energy between ice and the aqueous phase: the other quantities contained in B, namely the heat and the entropy of crystallization and the partial molar volume of water, are less likely to be significantly affected by the presence of the polymer in the aqueous phase. The effects observed are qualitatively consistent with the results obtained with solutions of polyethylene glycol, but the increase in B for HES solutions is rather more pronounced.

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