Available online at www.sciencedirect.com
SCIENCE DIRECT
Thermochimica Acta 425 (2005) 149–163
thermochimica acta
www.elsevier.com/locate/tca

# Reliable determination of freeze-concentration using DSC

Bakul S. Bhatnagar[a], Stephane Cardon[a], Michael J. Pikal[a,b], Robin H. Bogner[a,b,*]

[a] School of Pharmacy, University of Connecticut, U2092, Storrs, CT 06269, USA
[b] Institute of Materials Science, University of Connecticut, Storrs, CT 06269, USA

Received 3 March 2004; received in revised form 22 June 2004; accepted 28 June 2004
Available online 14 August 2004

## Abstract

The objective of this study was to determine the feature of a DSC endotherm that can be most reliably used to determine the composition of a freeze-concentrate. Samples (3–10 mg) of sucrose in water (0–60%, w/v) were frozen and then heated (at 0.2–2.0 °C/min) on a DSC. The peak ($T_{peak}$) and offset ($T_{offset}$) temperatures were obtained from the melt endotherms. A freezing point osmometer was also used to determine the freezing point depression of sucrose solutions. Two theoretical models were developed, one that describes melting in a one-component system (ice) with a heterogeneous temperature distribution, and a second model, which describes melting in a binary system (sucrose and ice) with a homogeneous temperature distribution. Modeling Laboratory (MLAB) was used to simulate melt endotherms using the two models. In Gray's theory for analysis of dynamic thermal measurement (1968), the end of the melting occurs at the peak of the endotherm followed by a much sharper recovery to the baseline than that observed experimentally. Our theoretical model for one- and two-component systems predicts that the melting continues beyond the peak resulting in a delayed recovery to the baseline as experimentally observed. Both experimental and simulated $T_{peak}$ and $T_{offset}$ (also defined as the return to the baseline after completion of the peak) increased with an increasing scan rate. Extrapolation of the experimental $T_{offset}$ to a zero scan rate yielded a value of the equilibrium melting temperature, $T_m$, which is closer to accepted literature values and osmometry data than did the extrapolation of $T_{peak}$. Neither $T_{peak}$ nor $T_{offset}$ reliably (at a finite scan rate) define the multicomponent freezing point. Rather, an extrapolation of $T_{offset}$ to a zero scan rate gives the most reliable measure of freezing point using DSC. Simulation of DSC endotherms supports this conclusion and also exhibits the same trends as observed from experiment.

*Keywords:* Sucrose; DSC; Freeze-concentration; $T_{offset}$; Ice melting

## 1. Introduction

Freeze-drying is the process of choice to stabilize many parenteral antibiotics, peptides and proteins [1]. Ice formation during the freezing step in a freeze-drying process results in the increase in concentration of the dissolved solutes. This process, known as freeze-concentration, may lead to changes in pH, aggregation, phase separation, and other destabilizing processes [2]. To better understand the destabilizing mechanisms that occur during freeze-concentration, the composition of the freeze-concentrate must be well-defined at all relevant temperatures. The composition of the freeze-concentrate for a formulation can be calculated from the equilibrium liquidus curve of the phase diagram if known. To resolve the effect of pure freeze-concentration on stability of frozen protein or antibiotic solutions, one can study the isothermal degradation kinetics of unfrozen formulations of a concentration same as that of the freeze-concentrate as defined by the liquidus curve. Thus, an accurate determination of the freeze-concentrate has significant implications in the study of the stability of frozen proteins and antibiotics during freeze-drying.

Glucose, sucrose, trehalose, and other sugars are widely used as bulking agents and stabilizers in lyophilized formulations. The universal quasi-chemical (UNIQUAC) [3] and universal functional activity coefficient (UNIFAC) [4] models have been utilized to predict freezing points of binary

---

[*] Corresponding author. Tel.: +1 860 486 2136; fax: +1 860 486 4998.
*E-mail address:* robin.bogner@uconn.edu (R.H. Bogner).

0040-6031/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2004.06.017

## Nomenclature

| | |
|---|---|
| $\mathrm{d}h/\mathrm{d}t$ | heat generated by the sample during melting |
| $\Delta h_0$ | heat of fusion of ice present in the DSC sample |
| $\Delta H_\mathrm{f}$ | heat of fusion of ice per gram |
| $(\mathrm{d}Q/\mathrm{d}t)_\mathrm{r}$ | heat flow to the reference during melting |
| $(\mathrm{d}Q/\mathrm{d}t)_\mathrm{s}$ | heat flow to the sample during melting |
| $\mathrm{d}T_\mathrm{r}/\mathrm{d}t$ | scan rate or heating rate, $S$ |
| $\mathrm{d}T_\mathrm{s}/\mathrm{d}t$ | rate of change of sample temperature with time |
| $\Delta T_\mathrm{f}$ | freezing point depression |
| $\Delta T_\mathrm{melt}$ | difference between $T_\mathrm{offset}$ and $T_\mathrm{onset}$ for pure water |
| $a_1$ | coefficient 1 for the binary system in Eq. (17) and is used in fitting of the model to describe DSC data |
| $a_2$ | coefficient 2 for the binary system in Eq. (17) and is used in fitting of the model to describe DSC data |
| $A_{00}$ | parameter in evaluation of area, $A(z)$ |
| $A_{01}$ | parameter in evaluation of area, $A(z)$ |
| $A(z)$ | area of the ice:water interface in the HTD model |
| $C$ | constant in $z(m)$ empirical equation |
| $C_2$ | constant in $z(m)$ empirical equation |
| $C_\mathrm{ice}$ | heat capacity of ice |
| $\bar{C}_p^\mathrm{l}$ | specific heat of water |
| $C_\mathrm{r}$ | heat capacity of the reference pan |
| $C_\mathrm{s}$ | sample heat capacity (includes heat capacity of the sample pan) |
| $C_\mathrm{w}(m)$ | effective heat capacity of water |
| $C_\mathrm{w}^0$ | initial value of $C_\mathrm{w}$ |
| $h$ | height of ice sample in sample pan assuming a cylindrical geometry in the HTD model |
| $\bar{H}_1^0$ | partial molal enthalpy of water before dilution of the freeze-concentrate |
| $\bar{H}_1(m')$ | partial molal enthalpy of water after dilution of the freeze-concentrate where the new composition of the freeze-concentrate is $m'$ |
| $k$ | thermal conductivity of water |
| $K_\mathrm{f}$ | cryoscopic constant |
| $m_0$ | mass of the ice sample in the HTD model |
| $m$ | mass of water |
| $m_\mathrm{onset}$ | molality of the maximal freeze-concentrate |
| $m_\mathrm{solution}$ | molality of a solution in Eq. (2) |
| $n_1$ | number of moles of solvent (water) |
| $n_2$ | number of moles of solute |
| $n_\mathrm{m}$ | number of moles of water in the freeze-concentrate following dilution |
| $r$ | radius of ice sample in sample pan assuming a cylindrical geometry in the HTD model |
| $R$ | thermal resistance and is same as total thermal resistance, $R_\mathrm{T}$ which is a sum of $R_0$ and $R_\mathrm{s}(m)$ used in the one-component model |
| $R_0$ | thermal resistance across the DSC:aluminum pan interface |
| $R_\mathrm{s}(m)$ | sample thermal resistance |
| $S$ | scan rate (heating rate) |
| $T_\mathrm{f}$ | furnace temperature |
| $T_\mathrm{l}$ | mean temperature of water in Eq. (24) |
| $T_\mathrm{melt}$ | temperature of melt end = $T_\mathrm{onset} + St$ |
| $T_\mathrm{r}$ | reference pan temperature |
| $T_{\mathrm{r}_0}$ | reference pan temperature at time zero, i.e., start of the scan |
| $T_\mathrm{s}$ | sample pan temperature |
| $T_0$ | freezing point of pure water |
| $T_\mathrm{m}$ | freezing point of a solution of molality (m) |
| $T_\mathrm{onset}$ | onset melting temperature in a two- (or a multi-) component system |
| $T_z$ | ice temperature |
| $\bar{X}_2^0$ | mole fraction in the freeze-concentrate before the onset of melting in the incorporation of the heat of dilution effect in Eq. (14) |
| $\bar{X}_2^\mathrm{F}$ | mole fraction in the freeze-concentrate after all ice is melted in the incorporation of the heat of dilution effect in Eq. (14) |
| $z$ | thickness of the water layer surrounding ice in Fig. 1 |
| $Z_\mathrm{s}(m)$ | derivative of $R_\mathrm{s}$ with respect to $m$, $\mathrm{d}R_\mathrm{s}/\mathrm{d}m$ |

sugar–water systems. Another model based on solute/solvent interaction corrections was used to account for non-ideal freezing point depression and extend freezing point depression theory to the high concentration range [5]. Even though such models are useful for defining the liquidus curve of simple carbohydrate–water systems, they are less useful for studying pharmaceutical systems, which are compositionally more complex.

Prior to the development of calorimetric techniques, the liquidus curve was constructed by careful visual observation of the end of melting [6]. Such measurements were often very slow and tedious. The freezing point depression for aqueous solutions of carbohydrates and/or other solutes reported in the widely used International Critical Tables are based on such traditional measurements performed in the late 19th and early 20th centuries [7–9], and are cited routinely in the thermal literature even today [10]. With the development of other methods such as osmometry, refractometry [11], DTA [12–14], and DSC [15,16], freezing point determination has become easier and faster.

Today DSC has, in fact, become the method of choice for the determination of freezing point depression and for the construction of the liquidus curve of the phase diagram