

CHAPTER III

METHODOLOGY

3.1 Materials

3.1.1. Ionic Solutions:

Lithium chloride (LiCl), Sodium chloride (NaCl), Potassium chloride (KCl), and Cesium chloride (CsCl) solutions were purchased from Kanto Chemical, Co. Ltd., Japan.

3.1.2. Non-ionic solutions:

D-(+)-ribose, D-(+)-glucose, D-(-)-fructose, lactose, and sucrose solutions were purchased from BDH Laboratory Supplies, England.

3.1.3. Colloidal solutions:

Blue dextran (M.W. 2,000,000) and dextran (M.W. 100,000 and 9,000) were purchased from Sigma Chemical Co. Ltd., USA. and Fluka Chemie, Switzerland, respectively.

3.1.4. Coolant:

Ethylene glycol was purchased from APS Ajax Finechem, Australia.

3.2 Equipment

3.2.1. Cylindrical Sample Vessel

- The vessel was 4.9 cm inner diameter and 19.75 cm high
- The wall of the vessel was made of acrylic, and the cooling plate, placed at the bottom of the vessel, was made of stainless steel



Fig. 3.1 The cylindrical sample vessel

3.2.2. Cooling Bath

The cooling bath (Neslab, USA) was able to maintain at the temperature of $-15\text{ }^{\circ}\text{C}$ or lower.

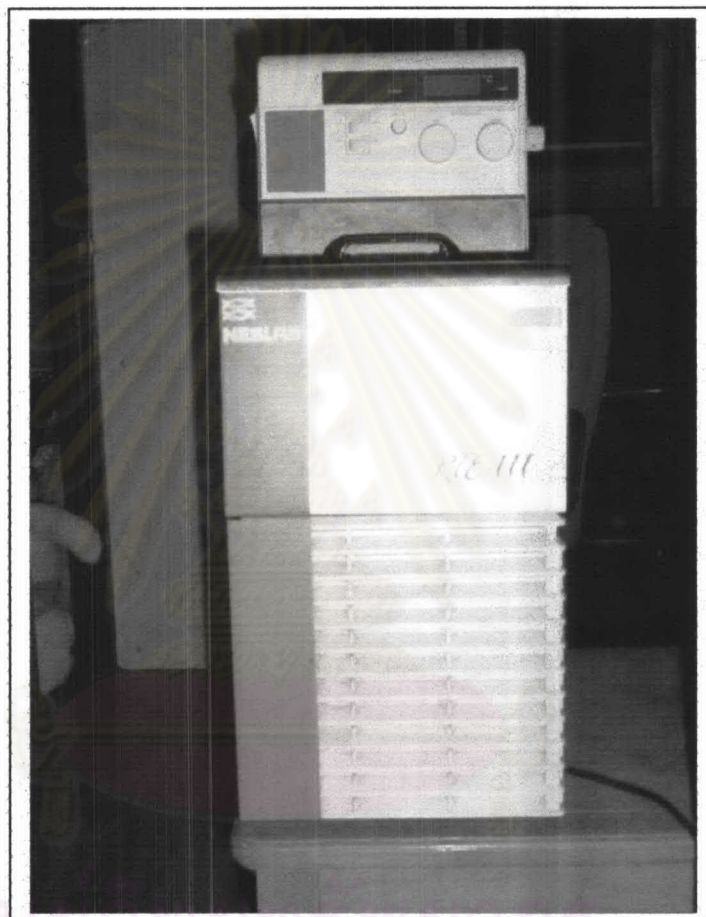


Fig. 3.2 Cooling Bath

3.2.3. Driving and Stirring Units

- A driving unit was used to lower the sample vessel into the cooling bath at a constant speed. The lowering rate, controlled by this driving unit, controlled the advance rate of ice front.
- A propeller-type stirring unit was used to enhance the mass transfer at the ice-liquid interface.



Fig. 3.3 Driving and stirring units

3.3 Sample Preparation

3.3.1. Ionic Solutions:

The ionic or salt solutions used in the experiments were prepared by dissolving each type of salt in a known amount of purified water in order to obtain the concentrations of 0.5, 2.5, and 10% (w/w). The solutions were then cooled to 0 °C.

3.3.2. Non-ionic Solutions:

The non-ionic or sugar solutions used in this study were prepared by dissolving each type of sugar in a known amount of purified water in order to obtain the concentrations of 2.5, 5, 10, and 20% (w/w) The solutions were then cooled to 0 °C.

3.3.3. Colloidal Solutions:

Colloidal solutions were prepared by dissolving blue dextran in known amounts of purified water in order to obtain the concentrations of 0.01, 0.05, and 0.25% (w/w). Dextran was dissolved in a known amount of purified water in order to obtain the concentration of 0.25% (w/w). All solutions were then cooled to 0 °C.

3.3.4. Coolant:

Ethylene glycol (25% (v/v)) was used as a coolant.

3.4 Method

Before initiating the freeze concentration, 3 ml of purified water was applied onto the bottom of the sample vessel to form an ice lining on the cooling surface as shown in Figure 3.4. This was done in order to prevent supercooling at the initial moment of the freeze concentration, as suggested by Lui *et al.* (1998). Adjusting a propeller's controller controlled the stirring speed rate at the ice-liquid interface. Adjusting the lowering speed of the plunger into the cooling bath controlled the advance rate of the ice front. At the beginning of each test run, approximately 120 ml of sample solution (at 0 °C) was poured into the sample vessel. The running equipment was shown in Figure 3.5. The test run was then stopped when the height of ice layer reached the level of approximately 1/3 of that of the whole sample. The operating time was then recorded. The solution and the ice phases were withdrawn from the sample vessel for further analysis.

The schematic in Figure 3.6 simulates the experiment and shows the ice lining at the beginning of test run. During the test, the ice crystal continuously grows as shown in Figure 3.7.

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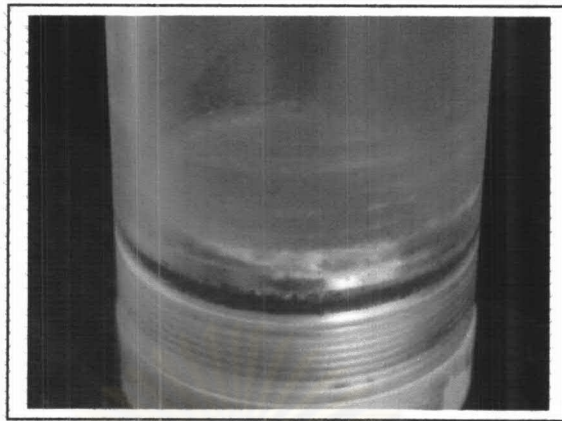


Fig. 3.4 Ice lining at the bottom of cylindrical vessel

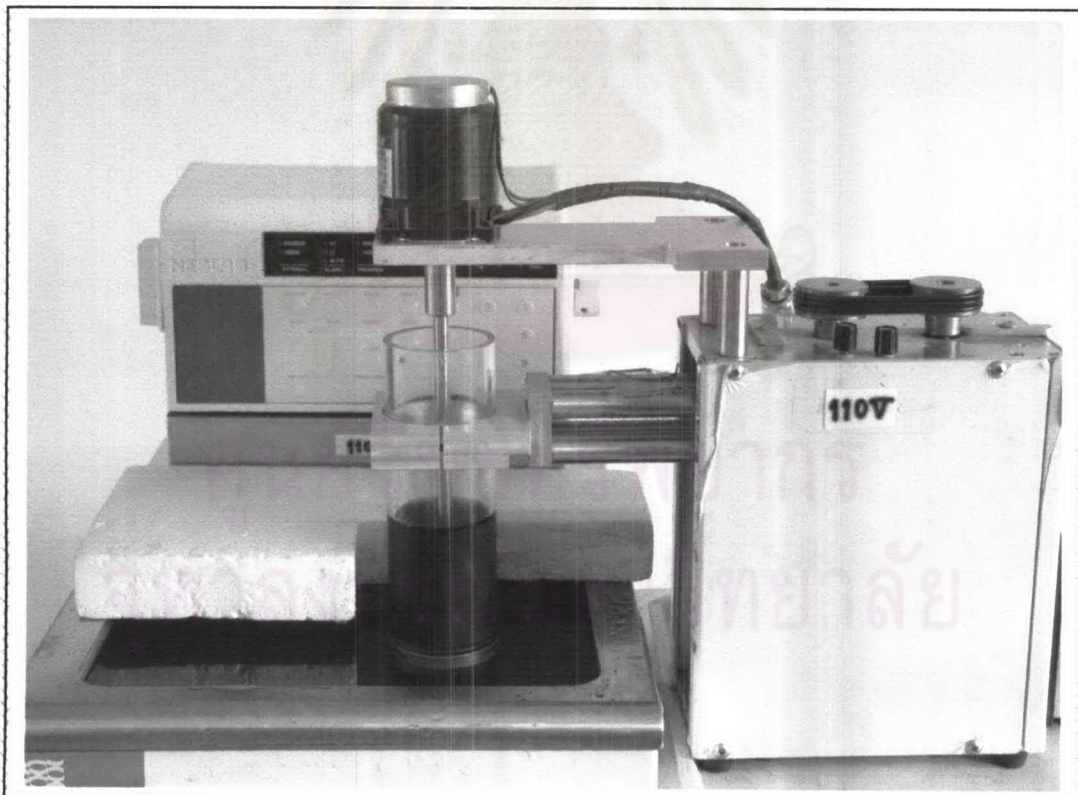


Fig. 3.5 The operation of equipment.

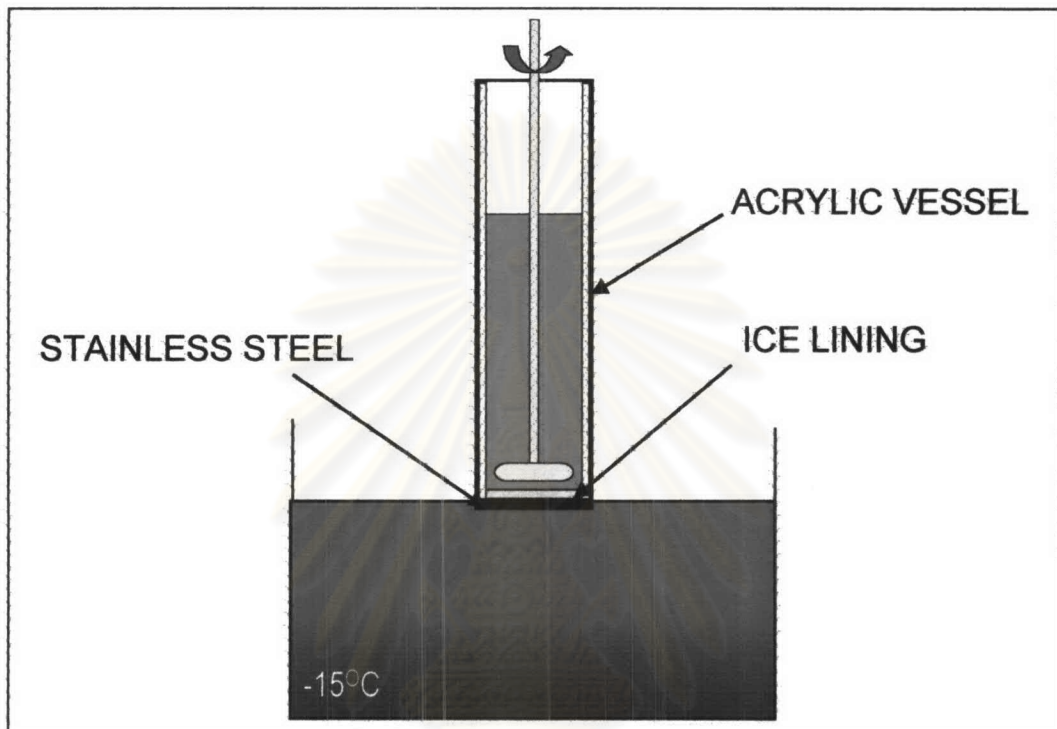


Fig.3.6 The beginning of progressive freeze-concentration experiment

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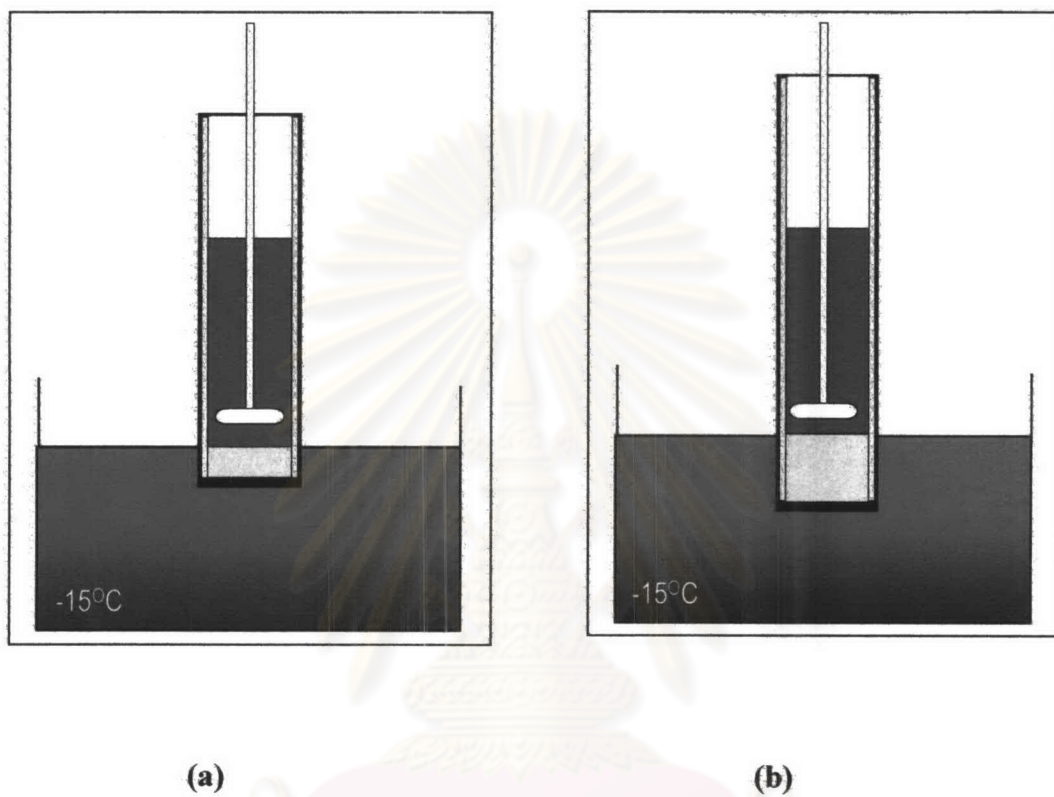


Fig. 3.7 Ice crystal growth at the bottom of vessel from (a) to (b)

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3.4.1 An Investigation of Factors Affecting the Efficiency of the Progressive Freeze-concentration in the Ionic Solutions

3.4.1.1 *Parameters*

Factors studied in this research included:

- Type of solute: four types of solutes comprising: LiCl, NaCl, KCl, and CsCl were used.
- The initial concentration of the solution: the initial concentrations of 0.5, 2.5, and 10% (w/w) were used.
- The stirring speed at the ice front (N_r): the speeds ranging from 200-1000 rpm were used.
- The advance rate of the ice front (u); the advance rate of the ice front was controlled within the range of 0.12-2.67 cm/h using the following relationship:

$$u = (V_{sa} - V_{il}) \times 1.085 / (\pi r^2 \times t), \quad (3.1)$$

where V_{sa} and V_{il} are volumes (cm^3) of ice phase and ice lining after melting, respectively; r is the inner radius of a sample vessel (cm); and t is the operating time (h). The calculation example is shown in Appendix C.

3.4.1.2 *Analytical Procedure*

- The amounts of the solution and ice phase (after melting) were volumetrically measured.
- The salt concentrations in both ice and liquid phases were measured using a conductivity meter (SC-12A, Suntex, USA), calibrated with a standard KCl solution at the temperature of 20 °C.

3.4.1.3 *Evaluation of the effects of the parameters as above on the K value*

3.4.1.4 *Determination of the limiting partition coefficient of solute based on the concentration polarization model.*

3.4.2 An Investigation of Factors Affecting the Efficiency of the Progressive Freeze-concentration on the Non-ionic Solutions

3.4.2.1 *Parameters*

Factors studied in this research included:

- Type of solute: five types of solutes comprising: D- (-)-ribose, D- (+)-glucose, D-(-)-fructose, lactose, and sucrose were used.
- The initial concentration of the solution: the initial concentrations of 2.5, 5, 10 and 20% (w/w) were used.
- The stirring speed at the ice front (N_r): the speeds ranging from 200-1000 rpm were used.
- The advance rate of the ice front (u): the advance rate of the ice front was controlled within the range of 0.12-2.67 cm/h, as calculated in Eq. (3.2)

3.4.2.2 *Analytical Procedure*

- The amounts of the solution and ice phase (after melting) were volumetrically measured.
- The sugar concentrations in both ice and liquid phases were measured using a refractometer (N-1E, Atago, Tokyo). The measurement was carried out at the temperature of 20 °C.

3.4.2.3 *Evaluation of the effects of the parameters as above on the K value*

3.4.2.4 *Determination of the limiting partition coefficient of solute based on the concentration polarization model.*

3.4.3 An Investigation of Factors Affecting the Efficiency of the Progressive Freeze-concentration on the Colloidal Solutions

3.4.3.1 *Parameters*

Factors studied in this research included:

- Type of solute: blue dextran (M.W. 2,000,000), and dextran (M.W. 100,000 and 9,000) were used.
- The initial concentration of the solution: the initial concentrations of 0.01, 0.05, and 0.25% (w/w) were used.
- The stirring speed at the ice front (N_r): the speeds ranging from 200-1000 rpm were used.
- The advance rate of the ice front (u): the advance rate of the ice front was controlled within the range of 0.12-2.67 cm/h, as calculated in Eq. (3.2)

3.4.3.2 *Analytical Procedure*

- The amounts of the solution and ice phase (after melting) were volumetrically measured.
- Blue dextran concentrations in both ice and liquid phases were analyzed using a spectrophotometer at the wavelength of 256 nm, as suggested by Lui *et al.* (1997). The standard curve of blue dextran concentration is presented in Appendix A.

– Dextran concentration in both parts were measured using Robert's copper method (AOAC, 1995). A dextran solution in the amount of 2 ml was placed in a 20 mm × 150 mm test tube. Water, which was used as a blank, in the amount of 2 ml was also placed in the same size of test tube. The 5 % aqueous phenol solution in the amount of 10 ml was added into those two test tubes. Concentrated sulfuric acid in the amount of 10 ml was then added rapidly into those two test tubes to ensure that the solutions were thoroughly mixed. The test tubes were placed in a water bath maintained at 100 °C for 2 min. After being allowed to cool to a room temperature, the color of the treated solutions was measured using a spectrophotometer at the wavelength of 485 nm. The standard curves of dextran concentration are presented in Appendix A.

3.4.3.3 Evaluation of the effect of the parameters as above on the K value

3.4.3.4 Determination of the limiting partition coefficient of solute based on the concentration polarization model.

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