Freeze Concentration of Dyes

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ABSTRACT

Concentration of water soluble direct, acid, basic, and reactive dyes occurs when dilute solutions are frozen at a temperature below the melting point and above the eutectic point of the solution. When freezing is done in still solution, a concentration of up to 500% is achieved in one step, with the dye solution collecting in an oblate spheroidal liquid pocket surrounded by clear ice containing voids, formed from air pockets, radiating outward. Three repetitive freezing cycles concentrate the dye 13,000%. The higher the water solubility of the dye, the lower the ability to concentrate during freezing. The freezing rate of four acid and direct dyes had no close relationship with the size of the dyes studied. Over a larger molecular weight range, an effect was observed for other kinds of molecules. Freeze concentration of salt solution (MW = 58.5) gives almost a 70% concentration, detergent solution (sodium dodecyl sulfate, MW = 298.4) gives 400%, and bovine serum albumin, a large water soluble macro-molecule (MW = 66,200), gives 160%. A theory is presented suggesting that the concentration effect and the formation of the central sphere are consistent with minimizing of the free energy of the overall system. This simple technique may find application in the concentration of heat sensitive, labile dyes for analytical purposes, as well as in the recovery of dyes and other chemicals on a bench scale.

The recovery and concentration of dyes is an important technique in the textile, food, pharmaceutical, and cosmetic industries. It is used in dye and forensic chemistry where the dyes are first extracted from dyed materials, concentrated by evaporation, and then analyzed [20]. Since dyes are often unstable during evaporation under heating, a vacuum at a lower temperature may be used, e.g., freeze drying. Freeze drying cools a solution to a temperature below its melting point and removes the solvent via vacuum suction of vapor sublimated from the frozen solution [4, 7, 12]. Workers have reported that freeze-dried dyes can be finely dispersed so that the photosensitivity and charge stability of photosensitive dyes are improved and aggregation of the dye particles is prevented [8, 16]. Drying of mordanted wool with freeze-dried cochineal produced a high color yield [9].

We report an alternate approach in which we used a freezing process to obtain concentrated dye solutions at conditions where the dyes remain stable. This process has been called freeze concentration. Using this method, a concentrated solution can be obtained without evaporating or sublimating the solvent out of the solution. Thus freeze concentration consumes less energy than freeze drying, and it does not need the relatively expensive vacuum system. Freeze concentration requires only a beaker (or a polyolefin microfuge tube) and a freezer. It can be done at volumes as small as 1 ml, and one freeze cycle will concentrate samples by 500%.

During a still freezing process, solute dissolved in a solvent concentrates in the remaining liquid when the temperature of the solution is decreased below its melting point but above its eutectic point, which is the lowest temperature at which a mixture of two or more substances melts [19]. An alternate approach uses agitation to achieve super cooling and to promote formation of pure ice crystals. The ice is then separated from the concentrated solution by centrifugation, filtration, and decantation [2, 17]. Freeze concentration is common in the food industry, since the low temperature preserves labile and volatile components in food products while giving a significant increase in the solids content of the remaining liquid [5, 17, 18].

This technique has also been used to concentrate acrylamide [15] and to treat bleach effluents [21] on an industrial scale. Such a technique is particularly useful for reactive dyes, since these dyes (e.g., chlorotriazines) will react with water to produce a hydrolyzed dye incapable of fixation with fibers. Increased temperatures will increase the rate of hydrolysis [13]. Freeze concentration could prove to be an appropriate method to concentrate such dyes.

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This report describes the freeze concentration of direct, reactive, acid, and basic dyes. Thermodynamic analysis of the process is consistent with a homogeneous/heterogeneous nucleation mechanism. The results suggest that glass and polyolefin surfaces are sufficient to initiate the process, and that a concentration of up to 500% is achievable in one step starting from a solute concentration of 0.1 g/l.

Experimental

**DYES AND OTHER SOLUTES**

Characteristics of the commercial dyes examined in this study are listed in Table I. These dyes were selected to include each of the major water soluable dye classes (i.e., reactive, direct, acid, and basic) and were used directly without purification. Dyes from each class were examined to determine whether freeze concentration was applicable to water soluable dyes. Standard dye solutions to be frozen were prepared by adding 100 mg dye to a 1-liter volumetric flask, which was then brought to volume with deionized water. Standard solutions, and therefore standard curves for dye concentrations, were based on the total weight of commercial dye, which contain significant amounts of salt and other nondye components.

<table>
<thead>
<tr>
<th>CI dye</th>
<th>Chemical class</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct red 81</td>
<td>diazo</td>
<td>505</td>
<td>675</td>
</tr>
<tr>
<td>Direct blue 80</td>
<td>phthalocyanine</td>
<td>620</td>
<td>780</td>
</tr>
<tr>
<td>Direct green 26</td>
<td>triazo</td>
<td>610</td>
<td>1332</td>
</tr>
<tr>
<td>Direct red 80</td>
<td>polyazo</td>
<td>530</td>
<td>1372</td>
</tr>
<tr>
<td>Direct brown 74</td>
<td>polyazo</td>
<td>380</td>
<td>1816</td>
</tr>
<tr>
<td>Reactive blue 4</td>
<td>anilinophthaleine</td>
<td>500</td>
<td>637</td>
</tr>
<tr>
<td>Reactive orange 13</td>
<td>monoazo</td>
<td>485</td>
<td>762</td>
</tr>
<tr>
<td>Basic red 29</td>
<td>monoazo</td>
<td>505</td>
<td>357</td>
</tr>
<tr>
<td>Basic blue 54</td>
<td>monoazo</td>
<td>590</td>
<td>426</td>
</tr>
<tr>
<td>Acid orange 52</td>
<td>azo</td>
<td>465</td>
<td>327</td>
</tr>
<tr>
<td>Acid yellow 36</td>
<td>monoazo</td>
<td>435</td>
<td>375</td>
</tr>
<tr>
<td>Acid red 151</td>
<td>diazo</td>
<td>510</td>
<td>454</td>
</tr>
<tr>
<td>Acid yellow 151</td>
<td>azo (metal complex)</td>
<td>430</td>
<td>811</td>
</tr>
<tr>
<td>Acid red 111</td>
<td>diazo</td>
<td>500</td>
<td>830</td>
</tr>
</tbody>
</table>

Chemicals used in other freeze concentration experiments (100 mg/l) were sodium chloride, a sodium chloride/dye mixture (100 mg/l each), D-glucose, sodium dodecyl sulfate (SDS), and bovine serum albumin (BSA) [3]. Our objective was to examine whether freeze concentration occurs for other water-soluble substances that may be found in a dye bath. We examined BSA (MW = 66,200) [22] in order to observe the effects of the presence of water-soluble macromolecules in solution on freeze concentration.

**Concentration Determination**

Dye concentrations in solution were determined using a liquid chromatographic (LC) instrument, where a zero dead volume fitting replaced the column, as shown in Figure 1. A Consta-Metric I liquid chromatography pump (LDC, Milton Roy, Riviera Beach, FL), a Varichrom VUV detector (Varian, Palo Alto, CA), and a model 7125 syringe loading sample injector (10 µl) (Rheodyne, Berkeley, CA) were used. The volume between the injector and detector was 0.1 ml and the flow rate was 1.0 ml/min, so that the peak recorded by the detector was very sharp. In essence, the LC was modified to inject samples directly into the VUV detector. Injection of standard solutions showed that the height of the peak was directly proportional to solute concentration (Figure 2).

**Figure 1.** Schematic diagram of a zero dead volume fitting used in a liquid chromatography system for determining dye concentration. Detector set at 0.1 V to 2X absorbance range, and 2X to 8X attenuation. Assay carried out at ambient temperature.

There are two ways of using a VUV detector to determine dye concentration. One method is to find λ<sub>max</sub> in the visible light region of a dye solution and then determine peak height. The other method is to use the UV region (254 nm) to detect the conjugated systems of dyes. The first method is generally more sensitive, while the second is easier to do. Both methods were evaluated and gave R<sup>2</sup> = 0.990 or higher for detector response as a function of concentration in the range of 1 to 100 mg/l of dissolved solids. Visible wavelength measurements made with the VUV detector and a Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, NY) were the same within experimental error (±5%). The correlation between calibration curves obtained from LC and conventional spectrophotometric absorbance measurements is given in Figure 2 (inset).
son that $\Delta H_f^\circ$ transferred to the inner solution more rapidly than through the glass wall to the surrounding environment is that the thermal conductivity of water is much larger (about 0.08 cal/(s·cm$^2$·°C/cm)) than that of glass (about 0.002 cal/(s·cm$^2$·°C/cm)) [19], while the thermal conductivity of ice is 30 cal/(s·cm$^2$·°C/cm) [11]. Therefore, the glass of the beaker wall is the limiting resistance to heat transfer. Further crystallization is thus facilitated in the ice because the ice serves as a nucleation center with a favorable temperature gradient extending from the glass wall through the ice to the liquid solution held in the center of the beaker.

As the outer ice shell grew, the inner liquid assumed an oblate spheroidal shape, which minimized the interfacial area between ice and liquid. This could be quantitatively expressed as follows [1]:

$$dG = -SDT + \sum_i \mu_i d_n + V^e dP^e + \gamma dA.$$  \hspace{1cm} (9)

The last term of Equation 9 represents the interfacial energy change. Since $\gamma > 0$ (ice-melt interfacial energy $\gamma = 22$ ergs/cm$^2$) [19], a smaller interface area $A$ gives a more stable system. A perfectly spherical shape has the minimum interfacial area, but the shape of the liquid core is also determined by other factors. These include the extent of deformation of the liquid from a sphere, which decreases with decreasing liquid volume, with decreasing magnitude of the density gradient, and with increasing interfacial tension [10]. Ice has a lower density than water (water = 0.9999, ice = 0.9168 g/ml at 0°C [19]), so that the shapes of the inner liquid of water and solutions would be oblate spheroidal. Lucassen's theory describes the shape of a droplet in a medium with higher density [10], which applies to this case. When freezing continued, the volume of the inner liquid decreased, so that the shape of the liquid tended to be even more spherical. Finally, when the remaining amount of the inner liquid became solid with many voids, the density differences between outer shell and inner solid and the volume of the inner phase were both at a minimum, and the shape of the inner solid was spherical, as predicted by Lucassen's theory.

Conclusions

Concentration of water-soluble direct, acid, basic, and reactive dyes occurs when dilute solutions are frozen at a temperature below the melting point and above the eutectic point of the solution. The freeze concentration of dye solutions is consistent with thermodynamic theory, which predicts the formation of a spherical upon freezing of a solution. The freezing rate has no direct relationship with the size of the dyes studied. The process presented here is a useful method for concentration of water soluble dyes, particularly heat labile dyes. More than 500% concentration is attainable in one freezing cycle. If liquid from one freezing cycle is recovered and taken through a second or third cycle, even higher concentration is possible. Since volumes as small as 1 ml can be conveniently processed through this technique, freeze concentration could be useful in the analysis and characterization of very dilute solutions, including dye identification on an analytical scale for textile, food, pharmaceutical, cosmetic, and forensic applications.

ACKNOWLEDGMENTS

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Literature Cited

9. Kazuhiko, E., Endo, Y., and Hasumi, S., Studies on the
The concentration of these substances in solution was determined by LC as described previously. For BSA, the VUV detector with $\lambda = 280$ nm was used. For NaCl, SDS, and glucose, the VUV detector in Figure 1 was changed to a refractive index detector (Refracto Monitor, model 1170, Milton Roy).

**FREEZING PROCEDURE**

A 100 ml glass beaker containing 60 ml of solution was covered with plastic film and stored in a freezer at $-10^\circ$C $\pm 1^\circ$C for 0.5 to 15 hours. The results were recorded using a Nikon F3 camera with a 55 mm lens and T-Max black and white film. Solution volumes as small as 1 ml were frozen in polystyrene microfuge tubes. Concentrations were determined by removing liquid samples from partially frozen solution using a syringe inserted through a hole in the ice, which had previously been made with a fine sewing needle. Since the center of the liquid was surrounded by solid ice, a liquid sample was readily obtained. The sample could also be removed from the frozen shell by hanging a syringe needle with its tip in the middle of the dye solution before freezing. Samples taken in this manner gave the same result. Consequently, the second technique was preferable, since the concentrated solution could be moved out easily through the pre-set needle. The sample thus obtained was then appropriately diluted and its absorbance measured using the VUV spectrophotometer.

One dye solution, CI reactive blue 4, was frozen three times. The first freezing cycle began with a dye concentration of 100 mg/L, the second cycle used only the concentrated dye solution from the first cycle, and the third cycle used the concentrated dye solution produced by the second cycle.

**Freezing Rate Determination**

Freezing rate was determined by measuring the volume of the concentrated solution at different freezing times. Two liters of 100 mg/L dye solution in a 190 mm diameter $\times$ 100 mm deep glass crystallizing dish was covered with Parafilm (American Can Company, Greenwich, CT) and then stored at $-28.5 \pm 1.5^\circ$C. The liquid volume was calculated from the measurement of $oa$, $ob$, and $oc$ (Figure 3). As shown in Figure 4, the shape of the liquid was approximately an oblate spheroid, which is similar to the volume formed by rotating the shorter axis of an ellipse $180^\circ$ (Figure 3), but during the initial freezing period, the shape of the liquid was not a completely formed oblate spheroid. The top of the liquid was flat as shown by the straight line that crossed $c$ and was parallel to the x-axis. Thus, the liquid volume could be calculated as follows:
where $\gamma_{i,j}$ are the three interfacial free energies that define the system at equilibrium. The function $f(m, R)$ is defined so that $0 < f < 1$ and $\frac{df}{dm} < 0$, $\frac{df}{dR} < 0$.

The relation between contact angle and $\gamma_{i,j}$ is illustrated in Figure 11. From this figure, we see that

$$\gamma_{\text{solid/liquid}} - \gamma_{\text{solid/ice}} = \gamma_{\text{liquid/ice}}(\cos \theta),$$

where $\theta$ is the contact angle. Comparing Equations 6 and 7,

$$m = \cos \theta.$$

![Figure 11. The relationship between interfacial free energies ($\gamma_{i,j}$) and contact angle ($\theta$).]

For a surface that is totally unwettable by water, $\theta = 180^\circ$. From Equation 8, $m = -1$, the minimum value, and the surface is totally incompatible with ice (nonnucleating). If a solid is totally wetted by water, then $\theta = 0^\circ$ and $m = 1$, which is the maximum value and indicates that the solid is efficient for heterogeneous nucleation. This latter condition is true for water as long as the radius $R \geq 10$ nm [6].

The container used in the experiments was a glass beaker. The ice that first formed at the glass wall was produced by heterogeneous nucleation. The glass served as a heterogeneous site, much like a glass rod inserted into a solution helps in recrystallization. As expressed in Equations 5 to 8, the hydrophilic wall gave a small contact angle between the wall and water, and heterogeneous nucleation conditions were easily satisfied. We observed similar results for small volumes (e.g., 1 ml) frozen in polyethylene microfuge tubes.

When water freezes, dies and other solid solutes move to the liquid phase, leaving the pure ice and giving the concentrated solution. The reason for this is that in the liquid phase, these solutes can have much higher entropies than in the solid phase. Although water also has a higher entropy in the liquid than in the solid phase, the latent heat released due to freezing is greater than the entropy lost. Thus it is thermodynamically favorable to form the pure ice and leave the solid dyes in the solution. If the solution is liquid and the temperature used is below the freezing point of that solution, the solute could be frozen due to the latent heat released. In other words, the freeze concentration is not always true if the solute is not solid.

The dyes used in this work have structures similar to that of a surfactant, i.e., a hydrophilic head (e.g., -SO$_3$Na) and a long hydrophobic tail (e.g., conjugated hydrocarbon chains). They tend to form a micelle with their hydrophilic heads facing outward. As the concentration of the solution increases due to the formation of ice, the number and size of these micelles increase. When the radius of a micelle is larger than 10 nm, it is possible that the micelle can become a heterogeneous nucleation site of ice. Since the outside layer of the micelle is hydrophilic, Equations 5 to 8 show that ice formed from these seeds is favored. The ice near or adjacent to the concentrated liquid (Figure 4) and the final solid ice-solute mixture (Figure 5) are not as pure as those in the outer layer. This arises from the heterogeneous nucleation of ice with dye micelles as seeds. Since larger micelles are heavier, a high proportion of nucleation occurs below the liquid phase. For this reason, ice below the liquid and below the final ice-solute mixture has more dye than any other area surrounding the inner liquid and middle ice-solute mixture. We also observed the micelle properties of dyes at the ice/air interface (Figures 4 and 5). Before the solution was frozen, some of the dye molecules at this interface were positioned with their hydrophilic heads toward the solution and their hydrophobic tails in the air, which decreased the energy of the system. The interfacial energy increase from moving these dyes to the concentrated solution during freezing is more than the free energy decrease due to the entropy increase. Hence these dyes stay at the surface of the ice.

From Table II, we see that BSA has been concentrated the least. In solution, proteins such as BSA also form micelles. Since BSA is physically larger than dyes, the extent of heterogeneous nucleation with BSA as seeds is greater than that in the dye solution. As a result, the concentration of BSA in the inner liquid is lower than that of the dye solution.

**Oblately Spheroidal Liquid Phase in the Ice Shell Center**

When ice formed, the latent heat $\Delta H_{f}^o$ (about 6.02 kJ/mole at 0°C) [6] released was transferred to the solution. This prohibited further decrease of the temperature of the system and slowed down the freezing rate, which facilitated the growth of pure ice. The rea-
\[ V_{os} = 2\pi \int_0^b x^2 dy = 2\pi a^2 \int_0^b \left(1 - \frac{y^2}{b^2}\right) dy , \]
\[ = \frac{4}{3} \pi a^2 b \]
\[ V_{sk} = \pi \int_0^a x^2 dy = \pi a^2 \int_0^a \left(1 - \frac{y^2}{b^2}\right) dy , \]
\[ = \pi a^2 \left[\frac{2b}{3} - c + \frac{c^3}{3b^2}\right] \]
\[ V_L = V_{os} - V_{sk} = \pi a^2 \left[\frac{2b}{3} + c - \frac{c^3}{3b^2}\right], \]

where \( V_{os} \) = volume of the oblate spheroid; \( V_{sk} \) = volume from \( b \) to \( c \) of the oblate spheroid; and \( V_L \) = total liquid volume.

**Dye Characteristics After Freeze Concentration**

To determine the properties of dyes after freeze concentration, we compared the dyeing ability and spectral absorbance of the dye before and after freezing. The dye solution was freeze concentrated at \(-20^\circ\text{C}\). The concentrated dye solution was then diluted to the same concentration as that before freezing. Both the original and freeze treated dye solutions were then used to dye cotton fabrics and to determine spectral absorbance.

The dyes used were CI direct red 81 and CI direct green 26. The fabric was #405 100% cotton sheerling (Testfabrics, Middlesb, NJ). The fabrics, weighing 1.5 grams each, were dyed with 2% owf dye and 10% owf sodium chloride at a 30:1 liquor-to-goods ratio for 30 minutes at 30 ± 0.2°C and then 60 minutes at 90 ± 0.2°C. The fabrics were dyed in a 50 ml Erlenmeyer flask (sealed with a rubber stopper) in a model 260 circulating water bath (Precision, GCA, Chicago, IL) to maintain constant temperature. After dyeing, the fabrics were rinsed twice in 10 ml ice water and then air dried at ambient temperature. Dye adsorption was calculated from the difference in dye bath concentration before and after dyeing. Color yield of the dyed fabrics, based on the parameters \( \Delta E^* \), \( \Delta a^* \), \( \Delta b^* \), and \( \Delta E \), was determined with a Color-Eye 3000 (Macbeth, Newburgh, NY). Fabrics dyed with the original dye solution were used as the control. Spectral absorbances of the dye solutions before and after freeze concentration were determined from 300 to 800 nm with a Cary 15 spectrophotometer (Applied Physics, Monrovia, CA).

**Results and Discussion**

**Void Formation**

Water crystals first formed at the vessel wall and then grew towards the middle of the container during the freezing process. The liquid shape, surrounded by the forming ice crystals, was oblate spheroidal (Figure 4). The voids grew until all of the liquid became solid at about 15 hours, although the whole solid was not continuous (compare Figures 4 and 5). For pure water, the center solid contained many voids. For the multicomponent systems, the center solid was a mixture of solvent (H2O) and solute with voids emanating from the central sphere.

Voids began to appear when ice, propagating from the wall of the container, occupied about 30% of the

![Diagram of an ellipse](image)

**Figure 3.** Schematic diagram of an ellipse. Rotation of the shorter axis (b) gives an oblate spheroid.

![Still freeze of water and its dye solutions at -10°C, 5 hours. Ice shell with oblate spherical shaped liquid in the middle.](image)

**Figure 4.** Still freeze of water and its dye solutions at \(-10^\circ\text{C}, 5\) hours. Ice shell with oblate spherical shaped liquid in the middle.

One direct and three acid dyes, *i.e.*, CI direct brown 74, acid orange 52, acid yellow 36, and acid red 111, were chosen for the freezing rate study. Each of the four dyes had an azo structure and linear molecular shape but varied in size, with acid orange 52 the smallest and direct brown 74 the largest (Table 1).
where \( V_L \) was the liquid volume (ml) left after \( t \) hours of freezing. The coefficient of \( r^2 \) depended on the shape, size, and material of the container, the temperature, and the volume and cooling efficiency of the freezing solution. There was no relationship between freezing rate \( \left( \frac{dV_L}{dt} \right) \) and size of the dyes studied.

**Dye Characteristics After Freeze Concentration**

A comparison of dye adsorption and color yield on cotton fabric using the dye solutions before (control) and after freeze concentration is summarized in Table III. There was no significant difference in both adsorption and color yield between fabrics dyed with the control and freeze concentrated dyes. The spectra of the control and freeze concentrated dye solutions (after dilution) were nearly identical (Figure 10) and thus showed that freeze concentration did not alter the visual absorbance characteristics of the dyes. The dye solution after freeze concentration therefore gives the same dyeing result as the original solution (Table III, \( \Delta E < 1.0 \).

**Mechanistic Description of Freezing Process**

We developed a mechanistic description to make it possible to anticipate the extent of concentration we could obtain for a given solute-solvent pair. The mechanism of ice nucleation can be homogeneous, heterogeneous, or a combination of the two [6]. Below its melting point and above its eutectic point, water can form crystals with very high purity from its solution [17]. Heterogeneous nucleation needs foreign particles, which are larger than 10 nm, as initiators for the growth of ice. In comparison, homogeneous nucleation is the self-nucleation of water. In homogeneous nucleation, water molecules in the configuration of clusters promote the ice growth.

The thermodynamic relationship between homogeneous and heterogeneous nucleation can be expressed by Equation 5 [14],

\[
\Delta G_{heter}^* = \Delta G_h^* f(m, R),
\]

where \( \Delta G_{heter}^* \) and \( \Delta G_h^* \) are the free energy barriers to the growth of an ice nucleus, subscripts \( h \) = heterogeneous and \( h \) = homogeneous, and \( f \) is a function of the radius \( R \) of the solid particle and the interface parameter \( m \), which is given by

**Table III: Color yield and dye adsorption of cotton fabrics dyed before (control) and after freeze concentration.**

<table>
<thead>
<tr>
<th>Dye adsorption, mg/l</th>
<th>Color yield</th>
<th>Freeze Conc.</th>
<th>( \Delta \alpha^* )</th>
<th>( \Delta h^* )</th>
<th>( \Delta a^* )</th>
<th>( \Delta E )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl dye</td>
<td>Control</td>
<td>Direct red 81</td>
<td>6.8</td>
<td>-0.59</td>
<td>0.12</td>
<td>0.44</td>
</tr>
<tr>
<td>Direct green</td>
<td>26</td>
<td>4.2</td>
<td>4.3</td>
<td>0.28</td>
<td>-0.01</td>
<td>-0.16</td>
</tr>
</tbody>
</table>
total initial volume of the solution. We explain this as follows. As the volume of the inner liquid became smaller, the air dissolved in the liquid water became over-saturated. Thus, air generated from the degassing liquid formed pockets, i.e., voids, as seen in Figures 4, 5, and 6. This explanation is consistent with Figure 6, which compares water that was previously degassed by boiling (right) to water that was not degassed (left). The ice from the previously degassed water has fewer voids.

**TABLE II. Water solubility and concentration of solutes by freezing.**

<table>
<thead>
<tr>
<th>Solute</th>
<th>Solubility* in water at 25°C (mmole/L)</th>
<th>Conc. after freezing* initial conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Direct green 26</td>
<td>11</td>
<td>4.6</td>
</tr>
<tr>
<td>2 Direct red 80</td>
<td>22</td>
<td>5.4</td>
</tr>
<tr>
<td>3 Direct red 81</td>
<td>74</td>
<td>3.7</td>
</tr>
<tr>
<td>4 Direct blue 86</td>
<td>35</td>
<td>5.0</td>
</tr>
<tr>
<td>5 Reactive blue 4</td>
<td>126</td>
<td>3.6</td>
</tr>
<tr>
<td>6 Reactive orange 13</td>
<td>210</td>
<td>3.5</td>
</tr>
<tr>
<td>7 Basic blue 54</td>
<td>376</td>
<td>3.4</td>
</tr>
<tr>
<td>8 Basic red 29</td>
<td>304</td>
<td>2.2</td>
</tr>
<tr>
<td>9 Acid yellow 151</td>
<td>25</td>
<td>4.0</td>
</tr>
<tr>
<td>10 Acid red 151</td>
<td>77</td>
<td>4.1</td>
</tr>
<tr>
<td>11 NaCl</td>
<td>6100</td>
<td>6.8</td>
</tr>
<tr>
<td>12 Reactive blue 4/NaCl*</td>
<td>3.6/10</td>
<td></td>
</tr>
<tr>
<td>13 Glucose</td>
<td>5560</td>
<td>4.9</td>
</tr>
<tr>
<td>14 SDS</td>
<td>347</td>
<td>4.1</td>
</tr>
<tr>
<td>15 BSA</td>
<td>27</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Solubility data for NaCl, Glucose, SDS, and BSA are from reference 22. Solubility data for dyes are from the manufacturer.
† Initial concentration = 0.1 g/L, initial non-frozen solution volume = 60 ml. After freezing, total non-frozen solution volume = 5 to 10 ml.
‡ Concentration at boiling. *Dye/salt mixture, 100 mg/L each.

**FINAL SOLID ICE-SOLUTE MIXTURE**

During the crystallization of water, the inner solution increased its solids concentration. Freezing increased dispersion forces, which favored solute retention in the low concentration regions (i.e., ice). However, the solutes themselves tended to concentrate into the liquid core, since this minimized the free energy of the overall system. Franks estimated that if coagulated particles have radii larger than 10 nm, new heterogeneous nucleation seeds form [6], which promote freezing. Since the total interface between ice and liquid decreases, the latent heat produced per unit time also decreases, the rate of temperature drop increases, and the size and number of water clusters increase. This assumes freezing proceeds in the beaker or microfuge tube placed in an environment where the surrounding temperature is constant, such as in a freezer. The crystallization rate increases as the solid ice-solute mixture approaches a completely frozen state. Consequently, the freeze concentration process can be described as having four stages: continuous ice, discontinuous ice with a few air voids, solid ice-solute mixture with many air voids, and finally a concentrated liquid solution. If the freezing time is long enough, the last solution phase disappears to give a totally solid ice-solute mixture. For pure water, the solid phase is an ice having a concentration of voids at the core. For a solution, the solid phase would contain the solute as well.

**CONCENTRATION OF DYES BY FREEZING**

The dyes, and a detergent (SDS) were concentrated up to 500% after one freeze cycle, while salt was concentrated almost 700% (Table II). The data indicate
that both dyes and nondye components in a commercial dyebath will concentrate together. A higher molecular weight protein component BSA concentrated 160%. Results from the repetitive or "multifreeze" concentration of CI reactive blue 4 are presented in Figure 7. The initial dye concentration was 0.1 g/l with a volume of 100 ml. The first freeze cycle produced 15 ml of 0.504 g/l dye solution. Using this 15 ml for the second cycle produced 1.5 ml of 3.92 g/l dye solution. Again, using the 3.92 g/l solution for the third cycle freeze concentration, the dye was concentrated to 13.4 g/l with a volume of about 0.1 ml. Through the three-cycle multifreeze concentration, CI reactive blue 4 was concentrated 13,000%. In comparing water solubility data with concentration data (Table II), we found that the solubility of the dye influenced its ability to move toward the center liquid as freezing progressed. Dyes with higher water solubility do not concentrate as well as lower water solubility dyes (Figure 8). The attractive forces between dyes and water prohibit the freeze concentration. Since it is difficult to stop the freezing process to obtain the same amount of solution, different commercial dyes have different additives, and the solubilities of the dyes at supercooling temperatures (e.g., -10°C) are unknown, we did not obtain a mathematical model of solubility and extent of concentration upon freezing. The data also suggest that a large macromolecule such as BSA could concentrate differently, and they give an indication that separation of a protein from a salt or dye might be possible.

![Figure 7. Concentration of CI reactive blue 4 before and after three repetitive freeze cycles.](image)

**Figure 7.** Concentration of CI reactive blue 4 before and after three repetitive freeze cycles.

Recovery of Solutes

After freezing, the distribution of solute can be divided into three areas, the inner concentrated solution, the ice-solute mixture around the inner solution, and pure ice. If the first two areas are considered as the concentrated product, then the recovery is almost 100%, since the dye concentration in the outer ice is negligible. If only the dye in the concentrated solution is used as the product, then recovery of the dye after one freeze concentration cycle is about 75%. This is based on a five-fold concentration of the original solution and 15% of the original volume. The remaining 25% dye can be freeze concentrated again to increase the recovery.

**Freezing Rate**

The relation between freezing time and the volume of concentrated dye solution produced ($V_f$) is given in Figure 9. The solution began to freeze at about 1.5 hours after it had been transferred from a 22°C to a -28.5°C environment. The concentrated spheroidal solution, with a volume of about 200 ml or 10% of the initial solution, solidified in about 9 hours.

The freezing rate fit the polynomial regression curve ($R^2 = 0.960$).

$$V_f = (2.002 + 0.147t - 0.0931t^2 + 0.006t^3) \times 10^3$$ (4)