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# Heat Transfer Analysis on Progressive Freeze Concentration of Aqueous Lysozyme Solution

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In this study, operating conditions of progressive freeze concentration method were arranged to deal with heat transfer analysis. Progressive freeze concentration is also known as a method to remove water from the solution by freezing process. The aqueous solution of lysozyme needs a water removal technology system as the residual moisture content has a significant impact on the solid-state stability of this biopharmaceutical product. The process was carried out by a newly designed crystallizer called a multiple probe cryo-concentrator (MPCC) which is completely equipped with a controlled stirrer, adjustable cooling gas system, solution tank and a set of cooling probes. The operating condition involved in this system include operation time, t (20 to 60 min), coolant temperature, CT (-6 to -14 $^{o}$C) and stirrer speed, SS (200 to 400 rpm) at constant initial concentration of 10mg/ml. The result of overall heat transfer coefficient ($U_o$) at varied operating condition was analysed and obtained where coolant temperature of -8 °C, operation time of 20 min and stirrer speed of 350 rpm were found to give the highest $U_o$. All the operating conditions were found to contribute to the progress of ice development and removal of water in the MPCC system resulting in purity of ice and solution concentration accordingly.

## 1. Introduction

Chemical engineering separation technologies have always demanded for process efficiency, predictability, economic and simplicity aspect. Hence, increasing number of researchers are encouraged to study and keep a constant improvement on the existing separation technologies to develop some prominent innovation. In protein purification, one of the steps is to remove water from the protein aqueous solution. It is familiar that protein in aqueous solution is surrounded by water which has different properties from bulk water. The water called hydration water is more difficult to remove from the protein due to low activity (Rickard et al., 2010). Hydration water also has fewer degree of freedom and a long residence time (Rickard et al., 2010).

Freeze concentration is a process of concentrating a solution by freezing out the water content into ice crystals. By comparing to evaporation and membrane technology, freeze concentration has given some benefits compared to the others in producing a concentrate with high quality because the process occurs at low temperature where no vapour or liquid interface exist resulting minimal loss of volatiles (Jusoh et al., 2013). Recently, a new freeze concentration system has been introduced in the configuration of a one-step system, which is progressive freeze concentration (PFC) that is simpler than the conventional suspension freeze concentration (SFC). SFC and PFC exhibit different structure of ice formation in which SFC produces small ice particles while PFC yields a single block of ice, thus easier to separate.

Progressive freeze concentration method was applied in this research because the concept of this method can reduce the water content at almost 90 % (Miyawaki et al., 2012) and avoid protein denaturation contributed by vigorous heat. Progressive freeze concentration is a process that removes water from a solution to concentrate the solution through freezing out the water component into ice crystals. In PFC ice crystals are formed layer by layer to finally form a block of ice, which is quite different from suspension freeze concentration, where small ice crystals are formed suspended in the mother liquor. Hence the final separation

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of ice and the concentrate becomes easier. According to Yahya et al. (2017a), the cooled surface area to form the ice block influences the efficiency of the process. Other than that, supercooling, which is a phenomenon where ice crystal is not formed even below the freezing temperature of ice is also another factor to be overcome. Supercooling is phenomenon due to absence of first ice nuclei. Many researches have been conducted producing quite a number of designs for the experimental setup. However no research has been conducted to improve surface area in order to improve performance of the system.

A new design of ice crystalliser for a PFC process called multiple probe cryo-concentrator (MPCC) has been designed in this research. The MPCC has improved the surface area where the ice crystallisation should occur. However, in freeze concentration, heat transfer greatly influences the ice formation during the process and it depends on the surface area of the ice crystalliser. The process of heat transfer will be greatly affected by the change of phase and the boundary of solid-liquid interface movement (Fukosako and Seki, 1987). The three important points of heat transfer analysis are temperature distribution before the ice formation, during and after the change of phase in the system (Sahasrabudhe et al, 2011). Therefore, for this newly designed ice crystalliser, it is very important to investigate the heat transfer activity in the system. The aim of this study is to observe how the thickness of ice formed during the experiment as affected by the operation time, coolant temperature and stirrer speed impacted the heat transfer activity in the system.

## 2. Materials and method

### 2.1 Materials

Lysozyme powder was purchased from KONO Chem Co., LTD with original concentration of 10 mg/ml and used as a model of protein antibody. Water with 50 % ethylene glycol was used as coolant medium in water bath solution and gas R22 was used in the probe's coolant gas system. Meanwhile, a Bovine Serum Albumin (BSA) was used as a reference to plot the standard curve as shown in Figure 1 to determine the actual protein concentration in the lysozyme.

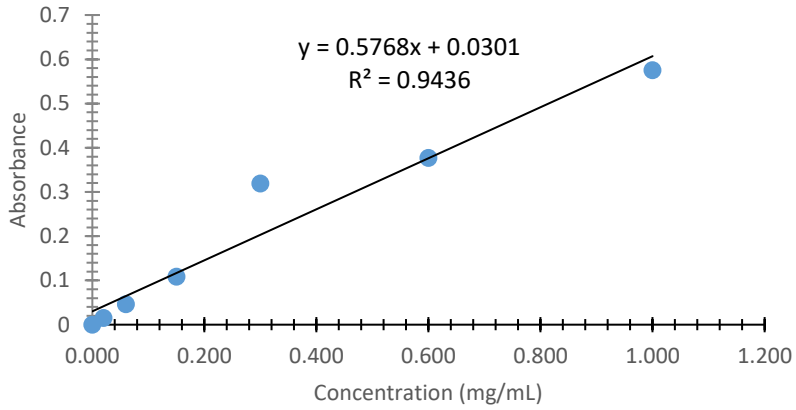
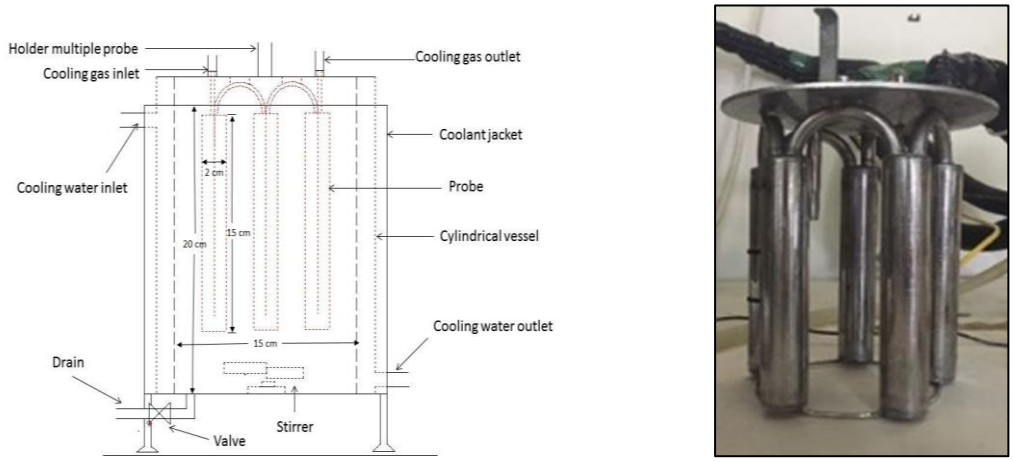



*Figure 1: BSA Standard curve*

### 2.2 Multiple probe cryo-concentrator (MPCC)

Multiple probe cryo-concentrator (MPCC) was used in this study as the apparatus for PFC method. Figure 2(a) shows the design and specifications of the design apparatus. The apparatus is composed of a cylindrical stainless-steel vessel (15 cm internal diameter, 20 cm height and 0.1 cm thickness), which can be occupied by 3000 ml of the lysozyme solution. This cylindrical vessel is jacketed where ethylene glycol-water solution is used as the coolant to maintain the temperature of the solution close to the freezing temperature (2 °C). The most outer layer of the cylindrical vessel is wrapped with an insulation layer (polyurethane foam) to avoid heat transfer of solution from the environment to the solution. A stirrer is placed at the bottom of the vessel controlled by a motor and its speed can be adjusted in order to control the radial velocity of the lysozyme solution. The probe as shown in Figure 2(b) has five fingers made of stainless steel which are hollow in structure and supplied with coolant gas (R22) on the inside. Refrigerant was supplied using copper tube to provide cooling to the probe to carry out ice crystallisation in the solution when immersed in the solution tank. The probe set was designed so that it is capable to rotate at 180° inter-directively with suitable speed to provide movement to the solution at the interface in order to maximize the formation of ice on the outer surface of the probe.

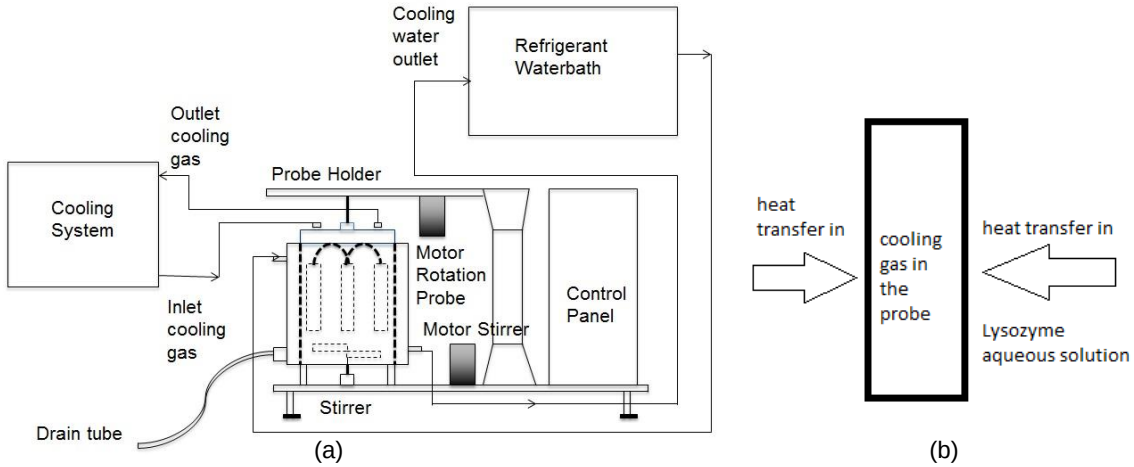



(a) (b)

*Figure 2: Schematic diagram for (a) multiple probe cryo-concentrator (MPCC) and (b) Five finger probe on the lid*

### 2.3 Experimental procedure

Before starting the experiment, the lysozyme solution needs to be cooled in a refrigerator at 2 °C which is near to the freezing temperature. After that, the lysozyme solution was measured by UV-Vis (UV-mini 1240, Shimadzu) to record the initial concentration (6 to 14 mg/ml). Then, the cool lysozyme was placed in the cylindrical vessel at a fixed volume of 3000 ml according to the limitation of vessel cavity. Ethylene glycol-water coolant was supplied in the jacket around the cylindrical vessel to keep the temperature of the solution maintained around temperature 2 °C. The stirrer at the bottom of the vessel was adjusted to the desired speed (200 to 400 rpm). Then, the MPCC was immersed in lysozyme solution in the cylindrical vessel and the MPCC started to rotate at 180° inter-directively. The cylindrical vessel was kept completely sealed with a lid attached to the MPCC to prevent heat loss to surrounding. The coolant gas temperature was then set to the desired value (-14 to -6 °C). When the designated temperature has been reached, the stopwatch was started immediately for the operation time to begin (20 to 60 min). The solution temperature inside the vessel was measured using a thermocouple data logger (TC-08) and was displayed online by PicoLog software throughout the process for easy process monitoring. Figure 3a and 3b illustrates the schematic diagram for the experimental setup of the process and heat transfer from lysozyme aqueous solution to the probe surface.




(a) (b)

*Figure 3: Schematic diagram for (a) multiple probe cryo-concentrator (MPCC) and (b) heat transfer from lysozyme aqueous solution to the probe surface.*

After the designated operation time was up, the cryo-concentration process was stopped. The MPCC was removed from the vessel and the thickness of ice layer formed on the fingers of the MPCC was measured. Immediately, the sample of the remaining solution in the vessel was drained out and sample of ice crystal formed was collected. After that, the experiment was repeated with different values of operating parameters, where for each batch of the experiments; only one parameter was manipulated while the others were kept constant. Both concentrated and thawed solution was analysed for concentration of lysozyme using UV-vis spectrophotometer.

### 2.4 Analysis Method

Due to the fact that the ice crystallisation process in the MPCC involves heat transfer, the calculation of overall heat transfer coefficient, $U_o$ is required to measure the capability of the apparatus to transfer heat from the lysozyme solution to the cooled probe. Overall heat transfer coefficient, $U_o$, is calculated by measuring the thickness of ice with varied coolant temperature, operation time and stirrer speed. The PFC process involved in this study generates ice crystal as a layer on the outer surface of the probe, therefore there is another heat transfer resistance involved which is the resistance of ice. R could then be expressed specifically for this design as stated in the Eq(1), assuming the resistance through the wall to be negligible:

$$R=\frac{1}{U_o A_m}=\frac{1}{A_i h_g}+\frac{x}{k_i A_m}+\frac{1}{A_o h_o} \quad (1)$$

$A_o$ ($m^2$) is the outer surface area, $A_i$ ($m^2$) is the inside surface area, $h_o$ ($W/m^2$. °C) is the heat transfer coefficient for lysozyme aqueous solution, $h_g$ ($W/m^2$. °C) is the heat transfer for gas (R22), $k_i$ ($W/m^2$. °C) is thermal conductivity of ice; and x (m) is thickness of ice, and $A_m$ ($m^2$) is a logarithmic area mean area as explained in Eq(2). Hence,

$$A_m=2\pi L\ \frac{x}{\ln\left(\frac{r}{r-x}\right)} \quad (2)$$

L (m) is a length of crystallizer, r (m) is a radius of crystallizer and x (m) is a thickness of medium wall.

## 3. Result and discussion

### Effect of operating conditions towards overall heat transfer

Generally, coolant temperature, operation time and stirrer speed will affect the overall heat transfer coefficient, which is very closely related to ice thickness (Yahya et al., 2017b). The higher the ice thickness, the greater the heat transfer resistance, thus resulting in lower $U_o$ (Cho and Lee, 2015). Besides that, the thickness of the crystallizer wall remains as a contributing factor of solid surface convection and conduction in the system. From Figure 3a, it is found that, $U_o$ highly accelerated with the increase in coolant temperature. The possible reason can be interpreted due to the temperature difference between the solution and coolant where at higher coolant temperature (-6 °C) the solution temperature was kept at 2 °C (near freezing point). Only thin layer of dendritic structured ice was formed on the outer wall of the probe at this condition which led to low resistance to the heat transfer, thus giving quite a high overall heat transfer coefficient, $U_o$. At coolant temperature of -8 °C, the $U_o$ becomes higher due to decreasing ice thickness compared to coolant temperature -6 °C, might be because of the erosion from the stirring effect which could wash away the unstable dendritic ice structure at this temperature. At this temperature the solution movement has defeated the advanced growth rate. However, at -10 °C, ice thickness increased again due to sufficient cooling and kept increasing until coolant temperature -14 °C was applied, giving lower and lower $U_o$. The highest $U_o$ was found to be 0.367 $W/m^2$.°C, at temperature -6 °C and the lowest $U_o$ is 0.281 $W/m^2$.°C at coolant temperature of -14 °C. This phenomenon can also be explained as the energetic reaction of lysozyme solution on heat transfer coefficient which is exothermic in nature. (Yu et al., 2012). Reaction of solution releases heat with supplication of convection force, in this case by the radial solution movement of the 3000 mL of solution, and the cooling down process required more energy from the coolant. Therefore, generally the heat from the solution was transferred to the coolant; and as the coolant temperature decreased, the temperature difference between the solution and coolant increased giving higher ice thickness. This is also supported by Yang et al. (2017) where it has been agreed that significant coolant rate directly affects the change of temperature difference between solutions. The formed ice will then promote further formation of ice crystals which will finally cause all water from the solution to become a block solid ice crystal (Hamid et al., 2015) attached on the probe wall. However, when the ice crystal growth rate is too high, entrapment of solute in the ice crystal layer is highly probable resulting in a decrease in the purity of ice and lower $U_o$ (Yahya et al., 2017b).

Figure 3b signifies that the longer the operation time, the lower the $U_o$. The highest $U_o$ (0.351 W/m$^2$.°C) was observed at 20 min of operation time, which is the shortest operation time and thus providing only a short residence time for contact between the cooled probe surface and the lysozyme solution. This would not give enough time for all water to become ice thus reflecting the lower resistant of heat transfer. Other design of freeze concentration in progressive freeze concentration has also defined that the operation time during the process would need more than 20 min to provide high average crystal growth rate which then would increase the mass and heat transfer resistance. When the operation time increases, the solute concentration in the ice-liquid interface also becomes higher and thus forming increasing ice thickness (Amran et al., 2015). In year 2009, Jusoh et al. had conducted a research and found that the ice started to form after 23 min, and the first ice formed during this period of time was not in a solid phase but more to a dendritic structure (Jusoh et al., 2009). This occurs due to incomplete freezing process giving lower ice thickness and thus higher $U_o$. In this research, when time reached 60 min, the probe was covered with solid ice and the growth rate become slower with increasing ice thickness over time.

Figure 3c shows that $U_o$ decreases with increasing stirrer speed, up until 350 rpm. This occurred due efficient heat transfer at stirrer speed of 200 rpm to 350 rpm. Jusoh et al. (2008) mentioned that heat will be transferred from solution to the coolant until the temperature of solution reaches the freezing point of water and increasing solution movement rate will promote heat transfer which would give speedy ice crystals formation (Wakisaka et al., 2001). However, at the speed of 400 rpm, the sheer force provided by the radial movement of the solution might have been too high and caused erosion of the ice formed on the probe, hence decreasing its thickness. Therefore, at this condition, $U_o$ is higher compared to 350 rpm. The increase in sheer force at higher stirrer speed will also enhance the liquid-liquid or solid liquid interaction and remove the solute in the solution. In other word the movement has brought away the stagnant solid ice layer giving lower ice thickness, thus resulting in higher $U_o$.

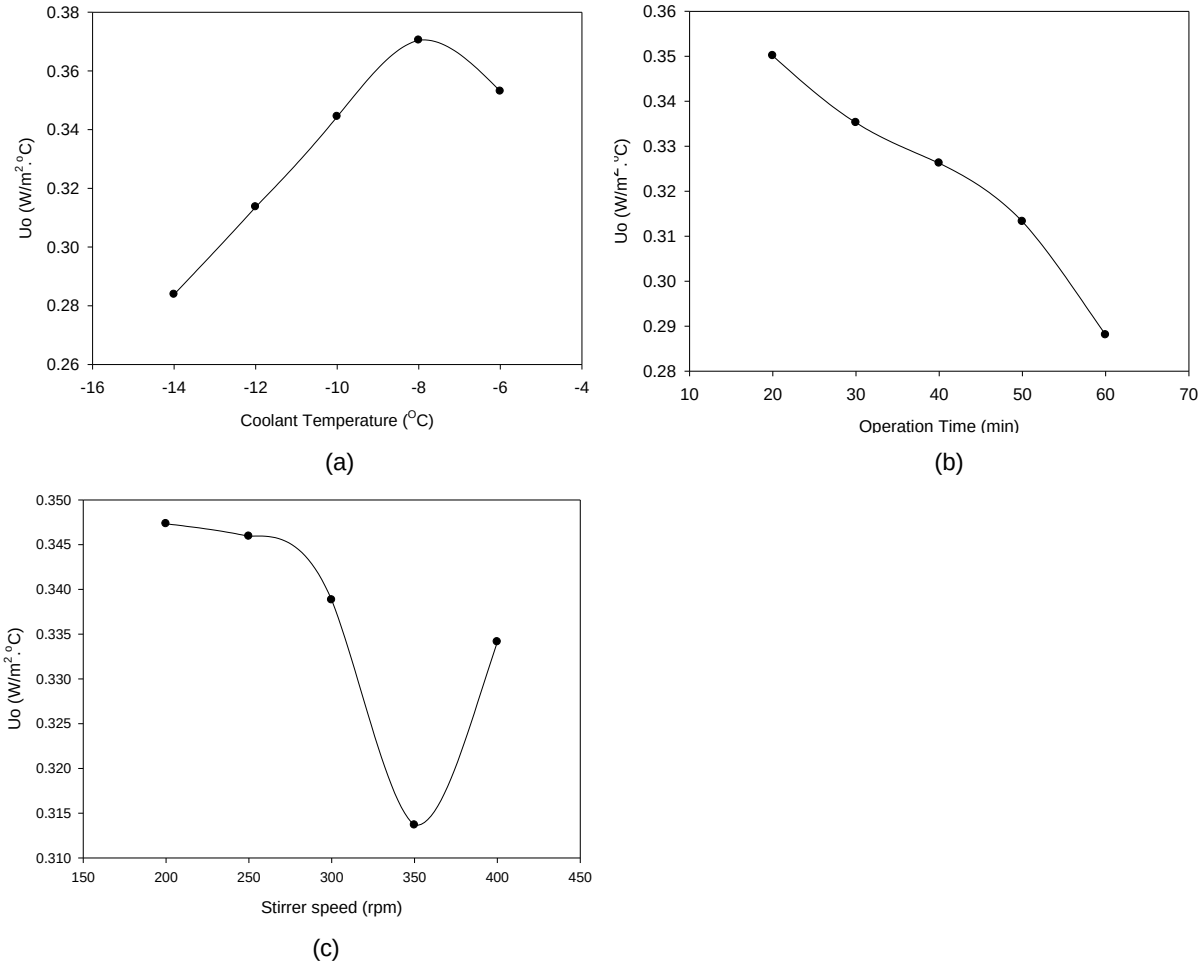



*Figure 3: (a) $U_o$ vs Coolant temperature (°C) vs $U_o$ (b) $U_o$ vs Operation time (min) (c) $U_o$ vs Stirrer speed (rpm)*

## 4. Conclusions

Ice formation for the MPCC system is indeed influenced by coolant temperature, operation time and stirrer speed, in which the coolant temperature of -8 °C, operation time of 20 min and stirrer speed of 200 rpm gave the highest $U_o$. However, although the highest $U_o$ is desirable in any heat transfer process, it might not be the case in progressive freeze concentration. This is because highest $U_o$ means low ice thickness hence less impact on the concentration process. As evident from the investigation parameters resulting in high ice thickness, which is desirable in a freeze concentration system, gave low $U_o$. Therefore, a careful selection of operating parameters ranges has to be done in order to produce successful freeze concentration process.

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