Chapter 6

Assessment of Cryoprotectant Concentration by Electrical Conductivity Measurement and Its Applications in Cryopreservation

Zhiquan Shu, Hsiu-Hung Chen, Xiaoming Zhou and Dayong Gao

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67747

Abstract

This chapter presents an important application of the electrical conductivity measurement in cryopreservation. Long-term cryopreservation of cells and tissues is essential in both clinical treatments and fundamental researches. In order to reduce the cryo-injury to the cells during cryopreservation, cryoprotective agents (CPAs) should be added before freezing, but also removed after thawing duo to the cytotoxicity. In these steps, severe osmotic stresses may result in injuries to the cells too. Therefore, monitoring the addition and removal of CPAs to the cell samples is critical in order to prevent the osmotic injury. In this chapter, the electrical conductivity measurement was applied to assess the CPA concentration in cryopreservation. Firstly, the standard correlations between the CPA concentration and the electrical conductivity of the solutions (including CPA-NaCl-water ternary solutions and CPA-albumin-NaCl-water quaternary solutions) were experimentally obtained for a few mostly used CPAs. Then a novel "dilution-filtration" system with hollow fiber dialyzer was designed and applied to remove the CPA from the solutions effectively. Measurement of electrical conductivity was validated as a safer and easier way to on-line and real-time monitoring of CPA concentration in cell suspensions. This work demonstrated a very important application of electrical conductivity in the biomedical engineering field.

Keywords: electrical conductivity (EC), cryopreservation, cryoprotective agent (CPA), addition and removal of CPA, dilution-filtration, hollow fiber dialyzer

1. Introduction

Cryopreservation of biological materials, including DNA/RNA, virus, bacteria, cells, tissues, and organs, both diseased and healthy samples, is essential for both fundamental researches and clinical applications. In cryopreservation, biological materials are cooled down to dormant state at low temperatures (such as −80 or −196°C, the temperature of liquid nitrogen) for long-term storage, and later thawed back to the normal physiological temperatures before usage with recovered viability and functionalities. However, there is a contradictory between the facts that the biological materials can be sustained at low temperatures, whereas their functions can be damaged during the cooling and thawing processes. There were very few successful cases of cryopreservation before 1940s.

Since glycerol was discovered to protect cells from cryoinjury in the late 1940s [1], the field of cryopreservation entered a new era. In order to reduce the cryoinjury to cells, cryoprotective agents (CPAs) should be added before freezing. Later, more and more CPAs have been proved effective for different cell types under diverse conditions, such as dimethyl sulfoxide (DMSO or Me_2SO), glycerol, propylene glycol (PG), ethylene glycol (EG), sugars (glucose, sucrose, trehalose), and macromolecules (dextran, hydroxyethyl starch (HES), polyvinylpyrrolidone (PVP)), which have indelible contributions to fundamental researches and clinical trials. However, CPAs might be toxic to cells, especially when cells and CPAs coexist at temperatures above 0°C for extended time. Meanwhile, if the added CPA is infused to patients together with the frozen-thawed cells, adverse reactions from mild to severe life-threatening problems may happen. For example, as the most widely used CPA, Me_2SO transplanted to patients may cause nausea, vomiting, chill, dyspnea, cardiac arrhythmia, hypotension, oliguric renal failure, and heart block, especially for pediatric patients [2–5]. The adverse effects of Me_2SO can even be cumulative when multi-dose cell therapies are implemented. Thus, generally, it is recommended to remove CPA from the cell suspension after thawing to an acceptable extent.

However, during the CPA removal, as well as its addition, osmotic injuries may happen to the cells if suboptimal procedures are applied. When a CPA with high concentration is added to or removed from cell suspensions, due to the osmolality difference between intracellular and extracellular solutions, cells will shrink or expand dynamically. This cell volume excursion can cause severe injury to the cells, that is, osmotic injury [6]. Therefore, fast and accurate assessment of the CPA concentration in the cell suspension during addition and removal is very important. In order to assess the CPA concentration in cell suspensions, a few methods have been applied by researchers, such as capillary zone electrophoresis [7]), high-performance liquid chromatography (HPLC) [8], and gas chromatography [9]. However, all these approaches are very complex, time-consuming, and expensive. In addition, special chemical agents, apparatuses, and expertise are needed. Inspired by the fact that the electrical conductivity of solutions depends on the composition and concentration of the ingredients, in this chapter, we demonstrated a method of electrical conductivity (EC) measurement to assess the CPA concentration [10, 11], which has been proved much simpler and cheaper, thus, more applicable for real-time monitoring of the CPA concentration during the CPA addition and washing procedures.

For the CPA removal method, nowadays, centrifugation is the most widely used method [12–16]. Briefly, isotonic washing solution is added to the cell-CPA suspension slowly. After equilibration

http://dx.doi.org/10.5772/67747

for a few minutes, the mixture is centrifuged, and then, CPA in the supernatant is removed. This dilution-centrifugation procedure usually needs to be repeated for a few times until the residual CPA concentration reaches an acceptable level. This method has many disadvantages, such as intense labor and time consumption, high possibility of contamination, osmotic and mechanical injury to the cells, clumping of cells due to centrifugation, and others. Thus, it would be highly desirable to find more reliable alternative methods for a CPA removal. Thus far, a few alternative approaches have been developed for CPA removal. Dialysis mass transfer in hollow fiber dialyzer has been proposed by some researchers [17–20]. However, the large osmolality gradient across the hollow fiber membranes can cause severe osmotic damage to the cells, especially at the beginning of the mass transfer process when cell suspension and diluent solution mix together in the dialyzer. To reduce osmotic injury to the cells, priming of the dialyzer with hyperosmotic solution first, which introduces extra complexity and time consumption, is generally required. Meanwhile, due to the non-uniform distribution of osmolality gradient across hollow fiber membranes along the fibers, mass transfer in the dialyzer is very complicated and hard to be well controlled. These problems prevent dialysis method from being an effective and reliable approach for CPA removal. Recently, Hubel et al. developed a method based on mass diffusion in microfluidic flows for CPA removal [21, 22]. However, therein the mass transfer rate is low since passive diffusion due to concentration gradient is the driving force for mass transfer. This system is hard to be scaled up for large volume samples.

In order to overcome the difficulties mentioned above, we proposed a method of "dilution-filtration" with hollow fibers for removing CPAs [11, 23, 24] (which is also applicable for CPA addition). The osmotic shock to cells when contacting with diluent and the removal rate of CPA can be well controlled by the dilution ratio and filtration rate, respectively. Compared to other methods, this "dilution-filtration" system can decrease cell loss, improve CPA removal effectiveness, easily manipulate the final sample volume, and diminish the possibility of contamination due the closed-loop system.

In this chapter, the electrical conductivity measurement was applied to assess the CPA concentration in cryopreservation. First, the standard correlations between the CPA concentration and the electrical conductivity of the solutions (including CPA-NaCl-water ternary solutions and CPA-albumin-NaCl-water quaternary solutions) were experimentally obtained for a few mostly used CPAs, including Me_2SO, EG, and glycerol. Then, the "dilution-filtration" system with hollow fiber dialyzer was applied to remove CPA from the solutions.

2. Electrical conductivity (EC) of the CPA solutions

2.1. Materials and methods

2.1.1. Measurements of EC of the Me_2SO-NaCl-water ternary solutions

The EC of Me_2SO-NaCl-water ternary solutions with different Me_2SO and NaCl concentrations was measured. In the solution preparation, NaCl-water solutions (NaCl: 99.6% pure, Mallinckrodt Baker, Inc., Phillipsburg, NJ) were first prepared with NaCl concentrations of 0.9, 1.8, 4.5, and 9 wt%, which were presented as r = 1, 2, 5, and 10, respectively, that is, r is the

relative concentration of NaCl compared to the isotonic NaCl solution. Then, Me_2SO (100% pure, Mallinckrodt Baker, Inc., Phillipsburg, NJ) was added to the NaCl-water solutions with volume percentages of 0, 2.5, 5, 7.5, 10, 20, 30, 40, and 50% (v/v). The EC data of the ternary solutions were measured with a conductivity meter (Orion 4-Star, Thermo Fisher Scientific Inc., Waltham, MA) at the AUTO-READ mode at room temperature (22 ± 0.5°C). After each measurement, the conductivity probe was rinsed with DI water and dried before the next measurement. Each individual solution was measured three times.

2.1.2. Measurements of EC of glycerol-NaCl-water and ethylene glycol-NaCl-water ternary solutions

Glycerol and EG are the other two types of CPAs that have been widely used in cryopreservation. Similar to the procedures mentioned above, the ternary solutions consisted of glycerol or EG (Mallinckrodt Baker, Inc., Phillipsburg, NJ), NaCl and DI water. When preparing the ternary solutions, NaCl crystal powder was first dissolved in DI water by weight to obtain final concentrations of 0.9, 1.8, 4.5, and 9 wt%, which were presented as r = 1, 2, 5, and 10, respectively. Second, glycerol or EG was added to NaCl-water solutions with different volume percentages from 0 to 50% (v/v). When preparing the glycerol solution, mass weighting was applied to precisely control the glycerol volume since small volume of glycerol was hard to be prepared due to its high viscosity. Then, the solutions with different NaCl and CPA concentrations were measured at room temperature for EC. For each solution, the measurement was performed at least for three times.

2.1.3. Effect of albumin on the electrical conductivity of the NaCl-albumin-water ternary solutions

Albumin usually exists in blood, culture medium, and cell products. In order to investigate the influence of albumin on the EC of the solution, NaCl-albumin-water ternary solutions were prepared. Similar to the procedure above, 0.9% (w/v) NaCl solution was prepared first. Then, bovine serum albumin (Sigma-Aldrich) was added to the NaCl solution with different final concentrations: 0, 2, 4, 6, 8, and 10% (w/v). Then, the EC of these solutions was measured at room temperature. Each solution was measured for three times.

2.1.4. Effect of Me_2SO on the electrical conductivity of the NaCl-albumin-Me_2SO-water quaternary solutions

In cryopreservation, cells may be in the NaCl (or other salts)-albumin-Me_2SO-water solution. During Me_2SO addition and removal, Me_2SO concentration increases or decreases, while albumin remains in the suspension. In order to apply the EC measurement to assess the Me_2SO concentration, we need to consider the influence of Me_2SO on the EC data.

NaCl-albumin-Me_2SO-water solutions with different Me_2SO concentrations were prepared and measured. Briefly, a ternary solution of 0.9% (w/v) NaCl-5% (w/v) albumin-water was prepared first. Here, 5% albumin concentration was chosen because this concentration was generally used in cell culture media. Then, this ternary solution was mixed with Me_2SO to make NaCl-albumin-Me_2SO-water solutions with different Me_2SO concentrations (0, 2.5, 5, 7.5, 10, 20, 30, 40, 50% (v/v)). When preparing these solutions, they were immersed in ice and the mixing was performed slowly such that the solution temperature was not heated up too

much. Then, the EC of these solutions was measured at room temperature. Each solution was measured at least for three times.

2.2. Results

2.2.1. Standard electrical conductivity data of Me_2SO-NaCl-water ternary solutions

The EC data of Me_2SO-NaCl-water ternary solutions are shown in **Figure 1**. Obviously, the EC depends on both the concentrations of NaCl and Me_2SO. The higher of NaCl concentration (larger r value) or the lower of Me_2SO concentration, the higher will be the EC of the solution. From the data, it shows that the dependence of EC on Me_2SO and NaCl concentrations can be written as an exponential function:

$$EC = A \times e^{B \cdot C} \tag{1}$$

where EC is the electrical conductivity of the ternary solutions (mS/cm); C is the concentration of Me_2SO (v/v, %); A and B are constants. It is interesting that B = −0.036, the same for different r values (different NaCl concentrations) (except B = −0.035 for r = 1, which difference may be due to the measurement accuracy). A is determined by NaCl concentration (shown in **Figure 2**) and can be estimated by:

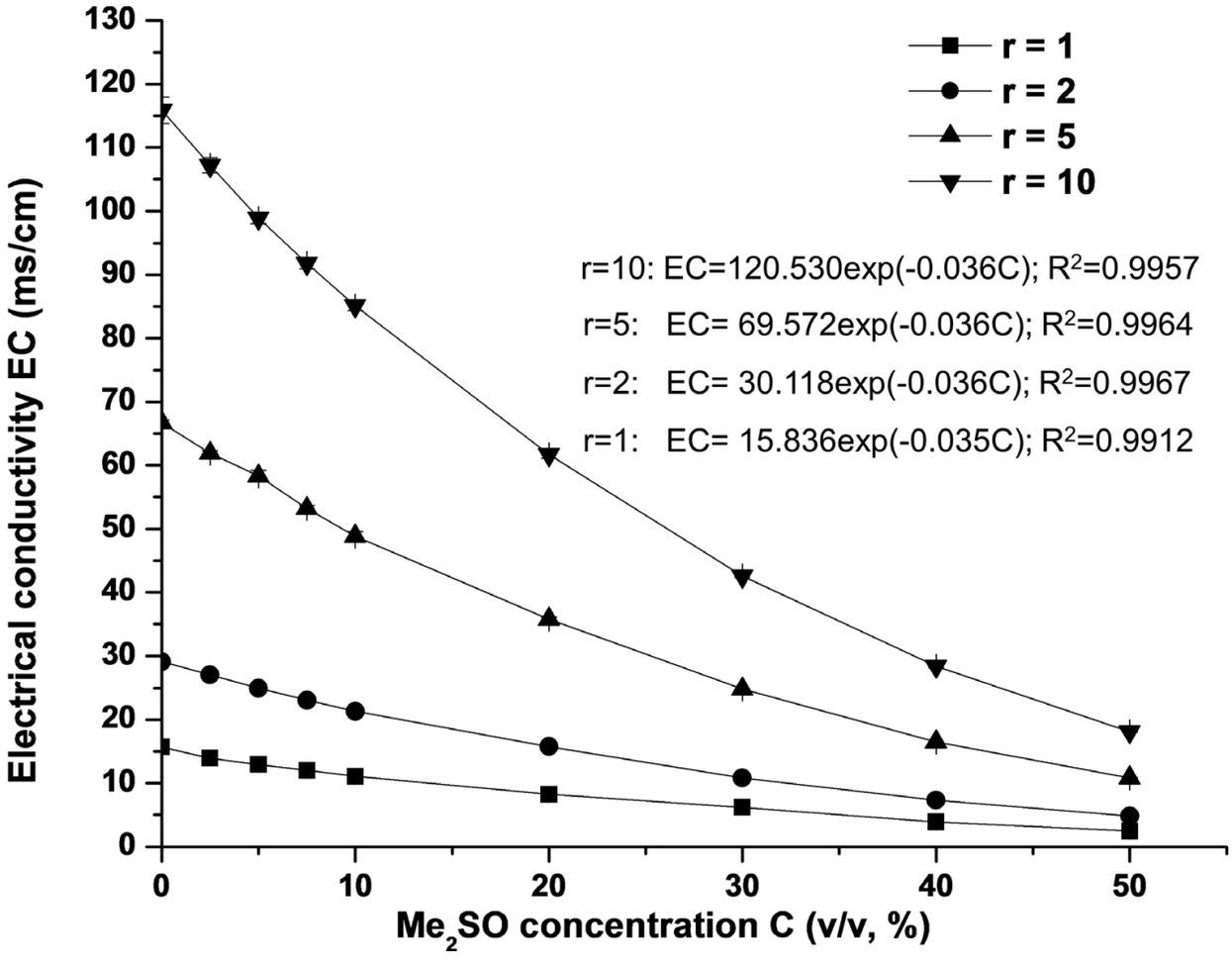

Figure 1. Electrical conductivity of Me_2SO-NaCl-water ternary solutions.

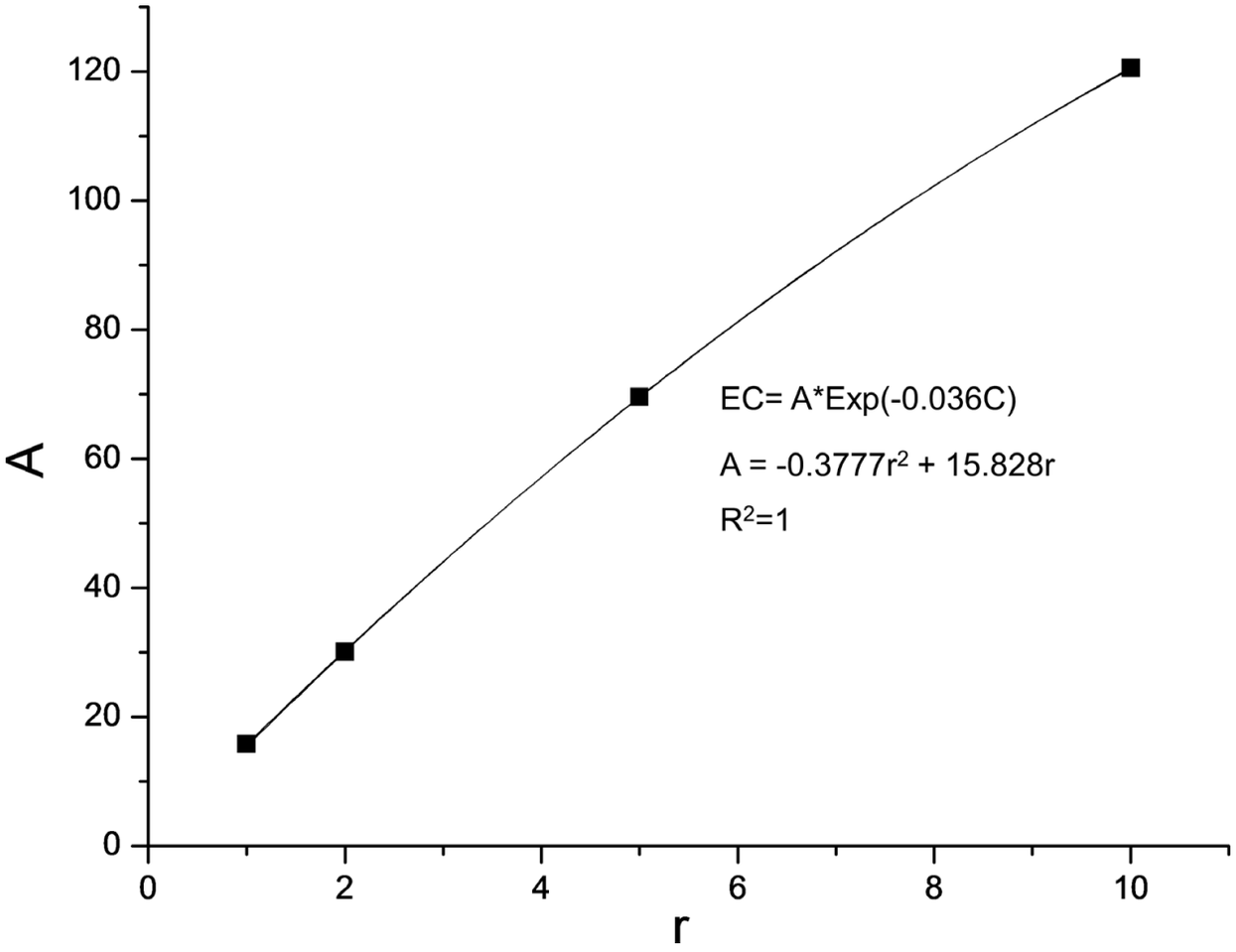

Figure 2. Dependence of A on NaCl concentration (*r*).

$$A = -0.3777r^2 + 15.828r \tag{2}$$

Accordingly, the EC of Me_2SO-NaCl-water ternary solutions can be estimated by:

$$EC = (-0.3777r^2 + 15.828r) \times e^{-0.036 \cdot C} \tag{3}$$

Specifically, for Me_2SO-0.9% NaCl-water ternary solutions (r = 1, the general case for cell suspension), the dependence of EC on Me_2SO concentration can be fitted by:

$$EC = 15.836 \times e^{-0.035 \cdot C} \tag{4}$$

2.2.2. Standard electrical conductivity data of glycerol-NaCl-water and ethylene glycol-NaCl-water ternary solutions

The EC results of glycerol-NaCl-water and ethylene glycol-NaCl-water ternary solutions are shown in **Figures 3** and **4**. Obviously, when CPA concentration increases or salt concentration decreases, the EC of the solution decreases. The dependence of CPA concentration on the EC can be also fitted by exponential equations.

http://dx.doi.org/10.5772/67747

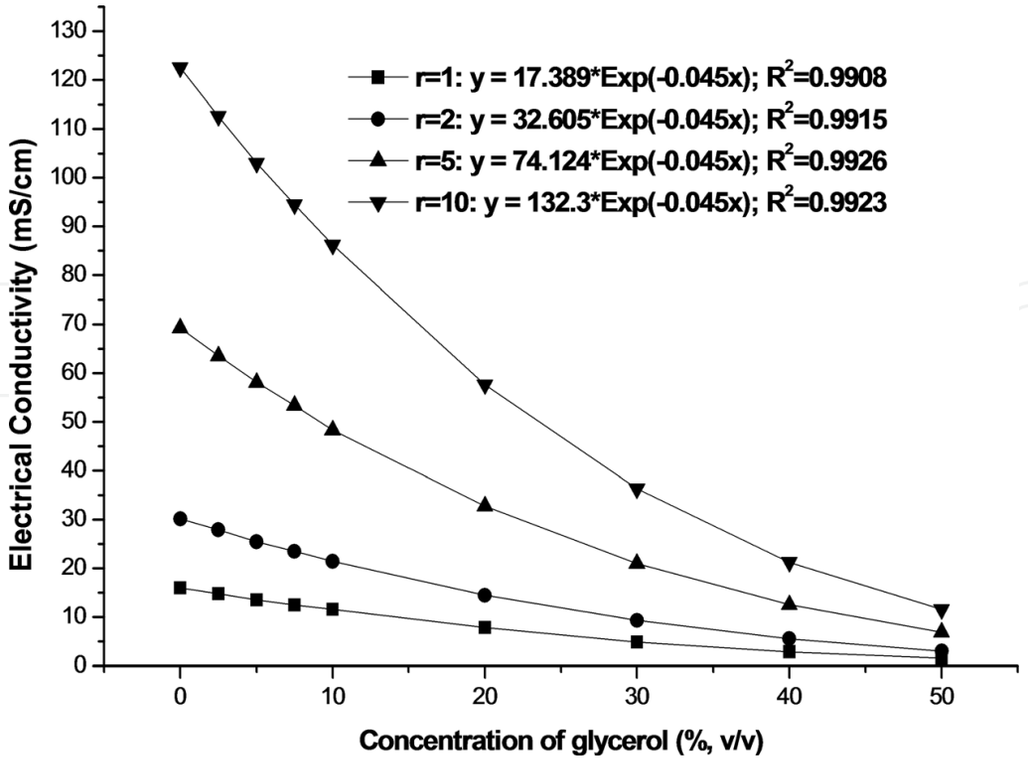

Figure 3. Electrical conductivity of glycerol-NaCl-water ternary solutions.

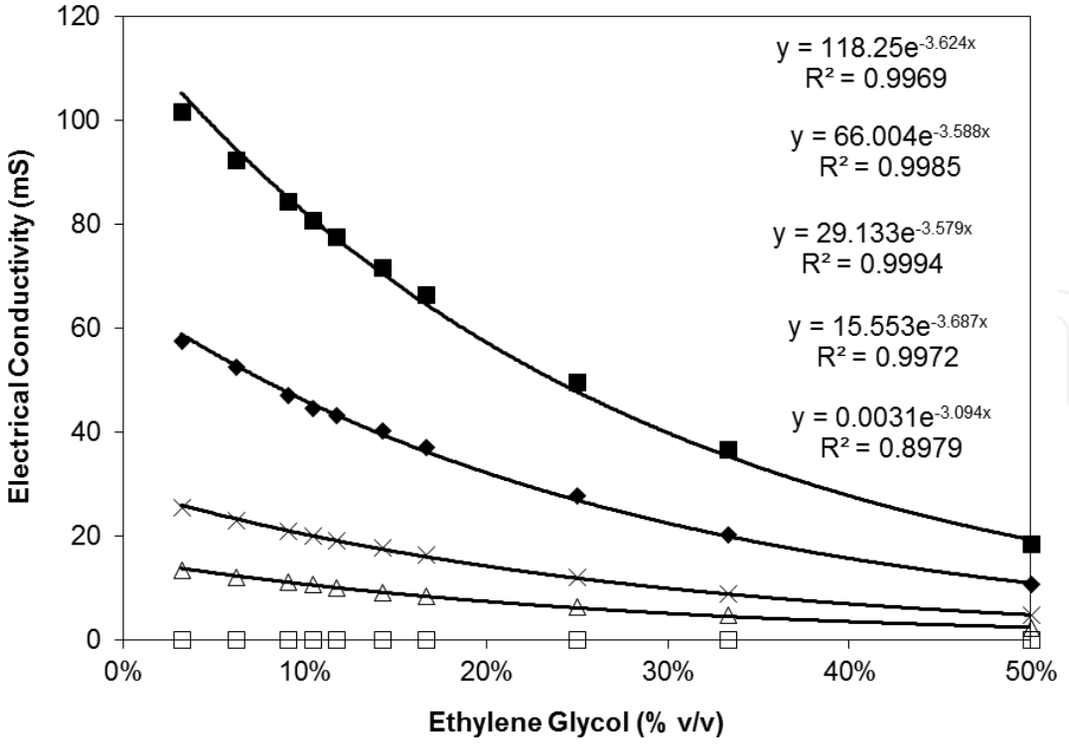

Figure 4. Electrical conductivity of ethylene glycol-NaCl-water ternary solutions.

For glycerol-NaCl-water ternary solutions, it can be presented as follows:

$$EC = A \cdot e^{-0.045C}, \quad (R^2 > 0.99) \tag{5}$$

For ethylene glycol-NaCl-water ternary solutions, the EC can be predicted as follows:

$$EC = A \cdot e^{-0.036C}, \quad (R^2 > 0.99) \tag{6}$$

A is determined by the salt concentration (r value).

2.2.3. Effect of albumin on the electrical conductivity of the 0.9% NaCl-albumin-water ternary solutions

The effect of albumin on the EC of albumin-NaCl-water ternary solutions is shown in **Figure 5**. In this experiment, the concentration of NaCl in the solutions was constant (0.9% w/v), and albumin concentration changed from 0 to 10% (w/v). Obviously, when the concentration of albumin increases, the EC of the solution decreases. The data can be fitted linearly as follows:

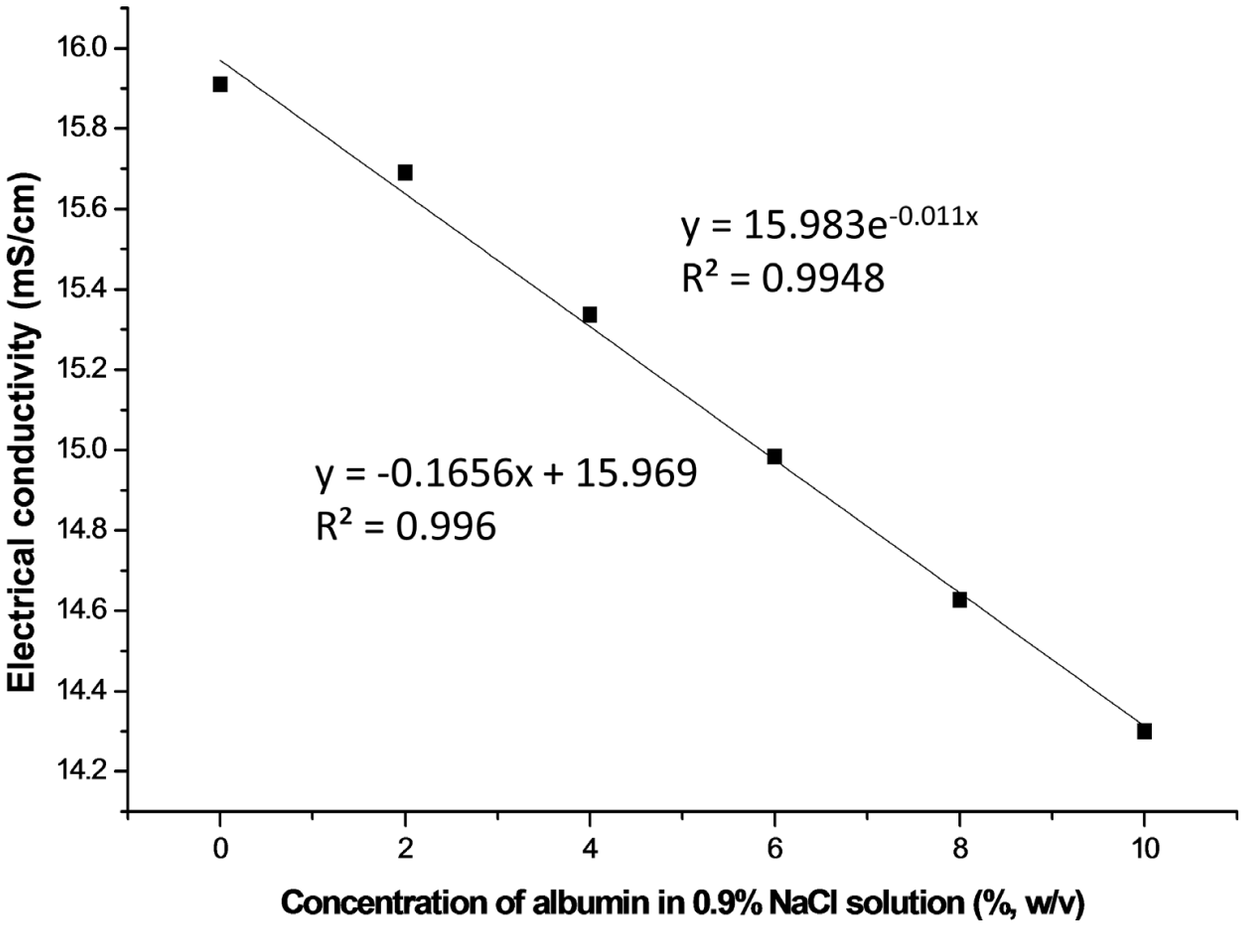

Figure 5. Effect of albumin on the electrical conductivity of albumin-0.9% NaCl-water ternary solutions.

http://dx.doi.org/10.5772/67747

$$EC = -0.1656 \cdot C + 15.969, \quad (R^2 = 0.996) \tag{7}$$

where C is the concentration of albumin (w/v%).

The data can also be fitted exponentially as follows:

$$EC = 15.983 \cdot e^{-0.011C}, \quad (R^2 = 0.9948) \tag{8}$$

Compared to Eq. (3), it implies that albumin and Me_2SO decrease the EC of the solutions with similar exponential ways, yet with different decreasing rates. For Me_2SO, the exponential constant B is −0.036, and for albumin, the constant is −0.011.

2.2.4. Effect of Me_2SO on the electrical conductivity of the 0.9% NaCl-5% albumin-Me_2SO-water quaternary solutions

In the Me_2SO-albumin-NaCl-water quaternary solutions, only the concentration of Me_2SO was changed. Its effect on the EC of the solutions is shown in **Figure 6**. Once again, we can see that the data can be well fitted by an exponential function as follows:

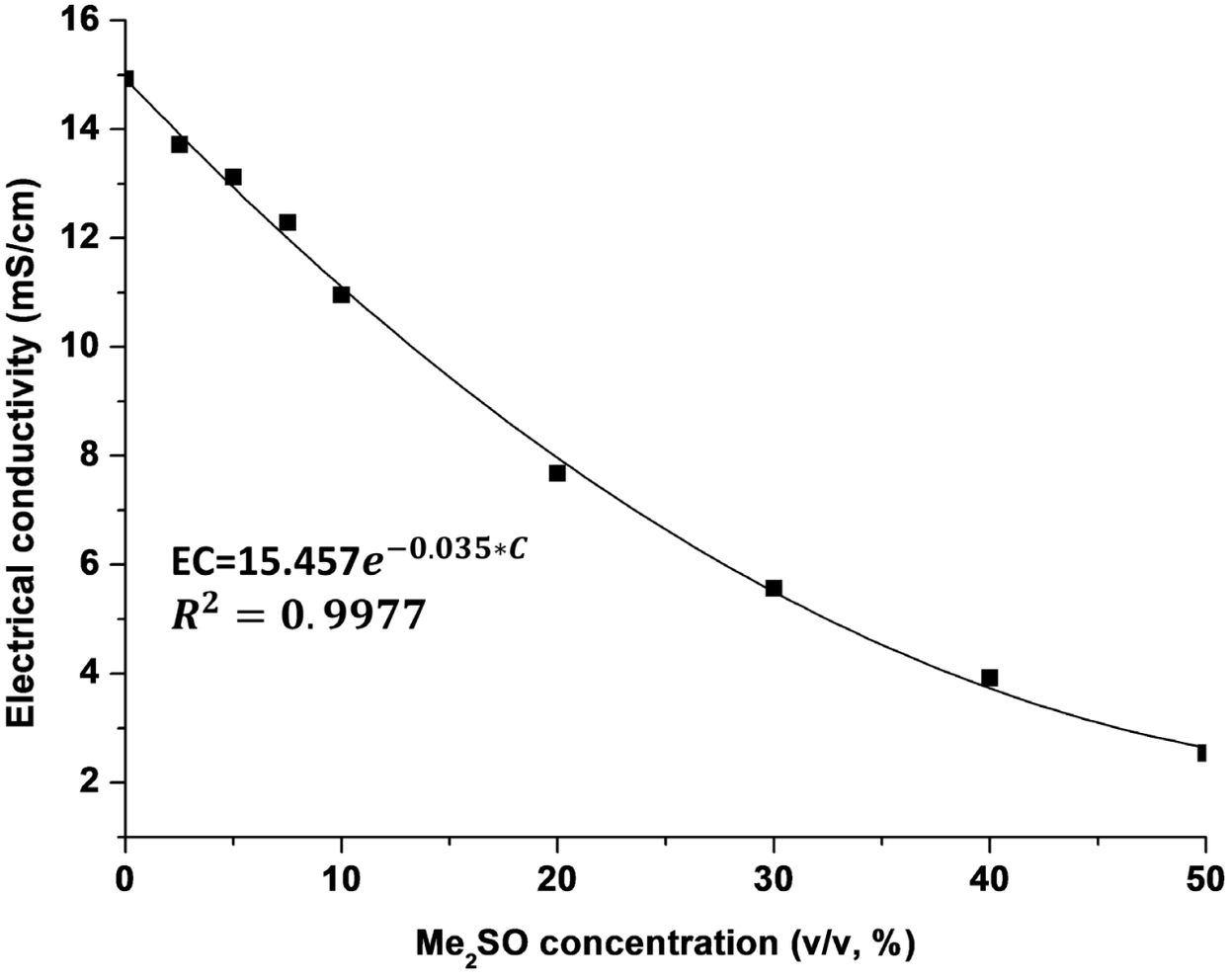

Figure 6. Effect of Me2SO on the electrical conductivity of Me_2SO-5% albumin-0.9% NaCl-water solutions.

$$EC = 15.457 \cdot e^{-0.035C}, \quad (R^2 = 0.9977) \tag{9}$$

where the exponential constant is −0.035, very close to the constant in Eq. (3).

3. Removal of CPA with dilution-filtration and assessment of CPA concentration with electrical conductivity measurement

3.1. Materials and methods

3.1.1. "Dilution-filtration" system for CPA removal

The "dilution-filtration" system for CPA removal is sketched in **Figure 7**. It mainly consists of three peristaltic pumps (400F/M1, Watson-Marlow, Wilmington, MA), one hollow fiber dialyzer (Hemoflow, F5HPS, Fresenius Medical Care, St. Wendel, Germany), a T-shape connector, and some silicon tubings (985-75, Pall, Port Washington, NY). Pump 1 and pump 3 cooperate to control the fluxes of diluent (q_d) and cell suspension (q_c). Diluent and cell suspension mix thoroughly in the T-shape connector and tubing, pass through the hollow fibers, and then, return to the cell suspension container. Pump 2 controls the filtration rate (q_u). Filtrated solution is collected in the waste solution container. Herein, the diameter of the tubings perfectly matches the pumps. When the peristaltic pump stops, it can also function to clamp the tubing loop, which prevents pressure loss inside the dialyzer. The dialyzer made of polysulfone was chosen in this work because of its large cross-membrane flux capability, high clearance efficiency of CPA, and low cost. Macromolecules (such as proteins and albumin) and cell debris cannot pass through the hollow fiber membranes, which leads to the simplicity of contents in the waste solution (only saline and permeable CPA, e.g., Me_2SO) and benefits the monitoring of CPA concentration.

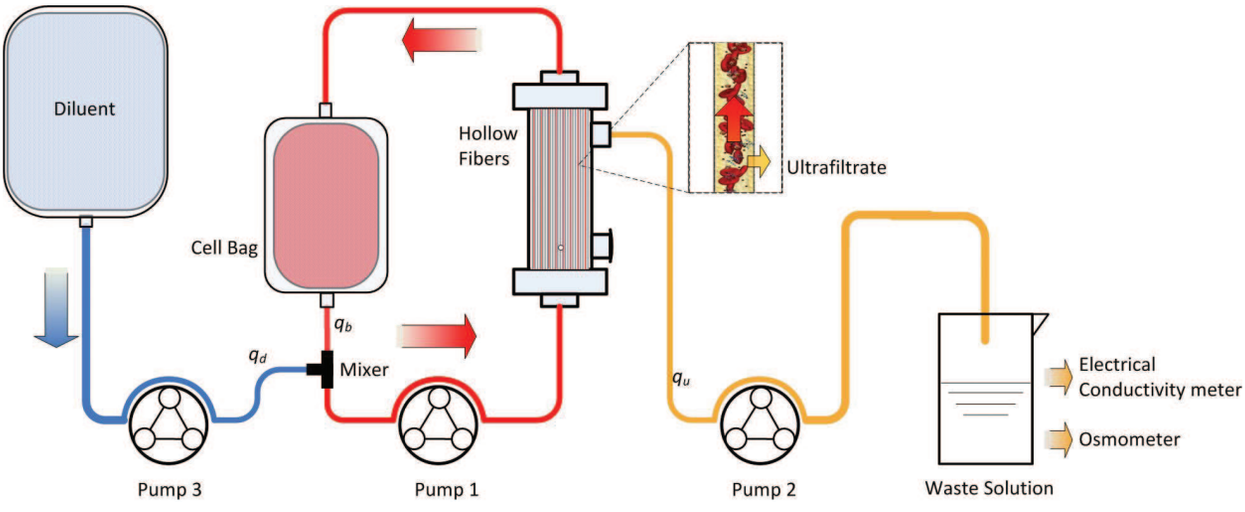

Figure 7. "Dilution-filtration" system for CPA removal.

3.1.2. Me_2SO removal with the "dilution-filtration" system

The 10% (v/v) Me_2SO-0.9% (w/v) NaCl-water ternary solution and 10% Me_2SO (v/v)-5% (w/v) BSA-0.9% (w/v) NaCl-water quaternary solution were used to mimic cell suspension with CPA. Isotonic NaCl solution (0.9%, w/v) was used as diluent. The actual pumping and filtration rates of the three pumps were calibrated before experiments. The "dilution-filtration" protocol was as follows:

Step 1 (priming): Pump 2 and pump 3 were shut down. Pump 1 was run at 100 mL/min for 1 min to drive the "cell suspension" to prime the hollow fibers (from bottom to top) and tubings.

Step 2 (dilution-filtration): Pump 1 and pump 3 were set to achieve q_c = 200 mL/min and q_d = 20 mL/min. Pump 2 was set to achieve filtration rate of q_u = 20 mL/min. Herein, $q_d = q_u$ such that the volume of "cell suspension" kept constant. The "cell suspension" container was kept agitating for better mixing. This step was run for 45 min. The osmolality and EC of both the "cell suspension" and waste solution were measured after every minute during the process.

Step 3 ("cell suspension" retrieval): Pump 2 and pump 3 were shut down. Pump 1 was set to run slowly (20 mL/min) in reverse direction for at least 4 min to retrieve the "cell suspension" in the tubings and dialyzer back to the cell suspension container.

3.1.3. Real-time monitoring of Me_2SO concentration

In order to real-time monitor the Me_2SO concentration during processing, EC measurements of the filtrated product (waste solution) were implemented. In Step 2 of the procedure (dilution-filtration) described above, after every minute, the EC of the newly collected waste solution (volume: ~20 mL) was measured. For verification, the osmolality of the waste solution was also measured by an osmometer (Wescor Inc., Logan, UT) with the working mechanism of vapor pressure assessment. The EC and osmolality of the "cell suspension" in the experiments were also measured after each minute of dilution-filtration for comparison with those of waste solution. All the measurements were conducted twice for each data.

3.2. Results

3.2.1. Me_2SO removal from Me_2SO-0.9% NaCl-water ternary solution by "dilution-filtration"

The experiment results of Me_2SO removal from Me_2SO-0.9% NaCl-water ternary solution by "dilution-filtration" system are shown in **Figure 8**. After processing for 45 min, the volume of "cell suspension" was 196 mL, which was very close to the original volume (200 mL). Totally, 860 mL isotonic NaCl solution was used as diluent. **Figure 8A** shows the EC and osmolality of the waste solution that was achieved every minute. According to Eq. (4), EC data were converted to Me_2SO concentrations of the waste solution, shown in **Figure 8B**. The Me_2SO concentration decreased to <1% (v/v) after 35 min. The results also show that Me_2SO concentrations estimated

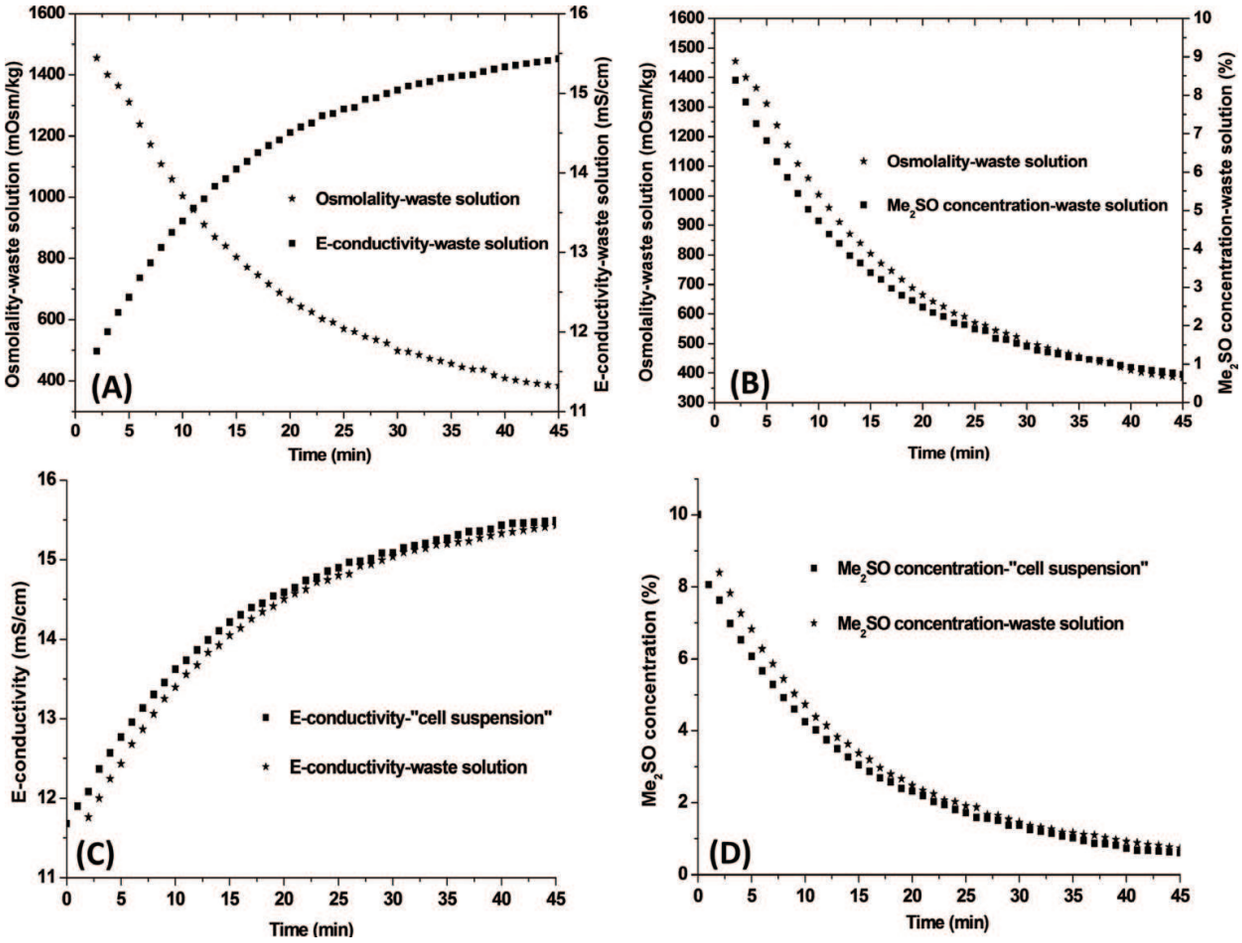

Figure 8. Me_2SO removal from Me2SO-NaCl-water ternary solution by "dilution-filtration." (A) Conductivity and osmolality of waste solution; (B) Me_2SO concentration and osmolality of waste solution; (C) conductivity of "cell suspension" and waste solution; and (D) Me_2SO concentration of "cell suspension" and waste solution.

by electrical conductivity measurements match the osmolality data very well, which implies EC measurement can be used to monitor the Me_2SO concentration during processing.

The EC data and converted Me_2SO concentrations of "cell suspension" and waste solution are shown in **Figure 8C** and **D**. After about 10 min, the difference between "cell suspension" and waste solution was very small. After 20 min, they were almost identical to each other. The discrepancy between "cell suspension" and waste solution in the beginning was caused by the experiment design and sample procurement method. In the first 2 min, waste solution was cumulated in the dialyzer head part for priming and therefore, the Me_2SO concentration was high. Only after the 3rd minute, waste solution sample could be procured and measured; however, herein, the waste solution sample was actually the mixture of that achieved in the first 3 min. Therefore, it had higher Me_2SO concentration and lower EC than "cell suspension." After a few minutes, the cumulative effect disappeared, and the readings of "cell suspension" and waste solution became identical.

The theoretical prediction of the concentration of Me_2SO in the "cell suspension" is a mixing-dilution problem. The Me_2SO concentration (C_{Me_2SO}) can be estimated by the governing equation:

$$V_{Cell} \cdot \frac{dC_{Me_2SO}}{dt} = -\frac{m}{m+1} \cdot f_{Diluent} \cdot C_{Me_2SO} \tag{10}$$

where V_{Cell} is the volume of "cell suspension" (mL), t is time (min), m is the flux ratio of cell suspension to diluent, and $f_{Diluent}$ is the flow rate of diluent (mL/min).

Solving this equation, the concentration of Me_2SO can be estimated as follows:

$$C_{Me_2SO}(t) = C_{Me_2SO}(t=0) \cdot e^{-\frac{f_{Diluent}}{V_{Cell}} \cdot \frac{m}{m+1} \cdot t} \tag{11}$$

In our experiment, the initial concentration was $C_{Me_2SO}(t=0) = 10\%$, flux of diluent was 20 mL/min, m = 10, and volume of "cell suspension" was 200 mL. So, theoretically, the Me_2SO concentration in "cell suspension" was theoretically predicted as follows:

$$C_{Me_2SO}(t) = 10 \cdot e^{-\frac{20}{200} \cdot \frac{10}{10+1} \cdot t} = 10 \cdot e^{-0.091 \cdot t}(\%) \tag{12}$$

According to the results presented in **Figure 8D**, actually the Me_2SO concentration in "cell suspension" during the whole removal process (45 min) can be fitted as $C_{Me_2SO}(t) = 10e^{-0.07t}$ (R^2 = 0.9741), which was close to but a little different with theoretical prediction. The discrepancy may be caused by the fact that the fluxes of "cell suspension," diluent, and filtration cannot be precisely controlled as programed after longer time running since the engagement between tubing and pumps may get worse due to fatigue of the plastics. This hypothesis can be proved by the fact that in the first 10 min of the experiment (with good engagement and precise flux control), the Me_2SO concentration data of "cell suspension" can be fitted as follows: $C_{Me_2SO}(t) = 10e^{-0.091t}$ (R^2 = 0.9372), which perfectly matches the theoretical prediction.

3.2.2. Me_2SO removal from Me_2SO-5% BSA-0.9% NaCl-water quaternary solution by "dilution-filtration"

The results of Me_2SO removal from the Me_2SO-5%BSA-0.9% NaCl-water quaternary solution by "dilution-filtration" system are shown in **Figure 9**. **Figure 9A** shows the EC and osmolality of the waste solution that was achieved every minute. According to Eq. (4), EC data were converted to Me_2SO concentrations of the waste solution, shown in **Figure 9B**. The Me_2SO concentration decreased to <1% (v/v) after 35 min. The EC data and converted Me_2SO concentrations of "cell suspension" and waste solution are shown in **Figure 9C** and **D**. ECs of "cell suspension" were always lower than those of waste solution due to the existence of

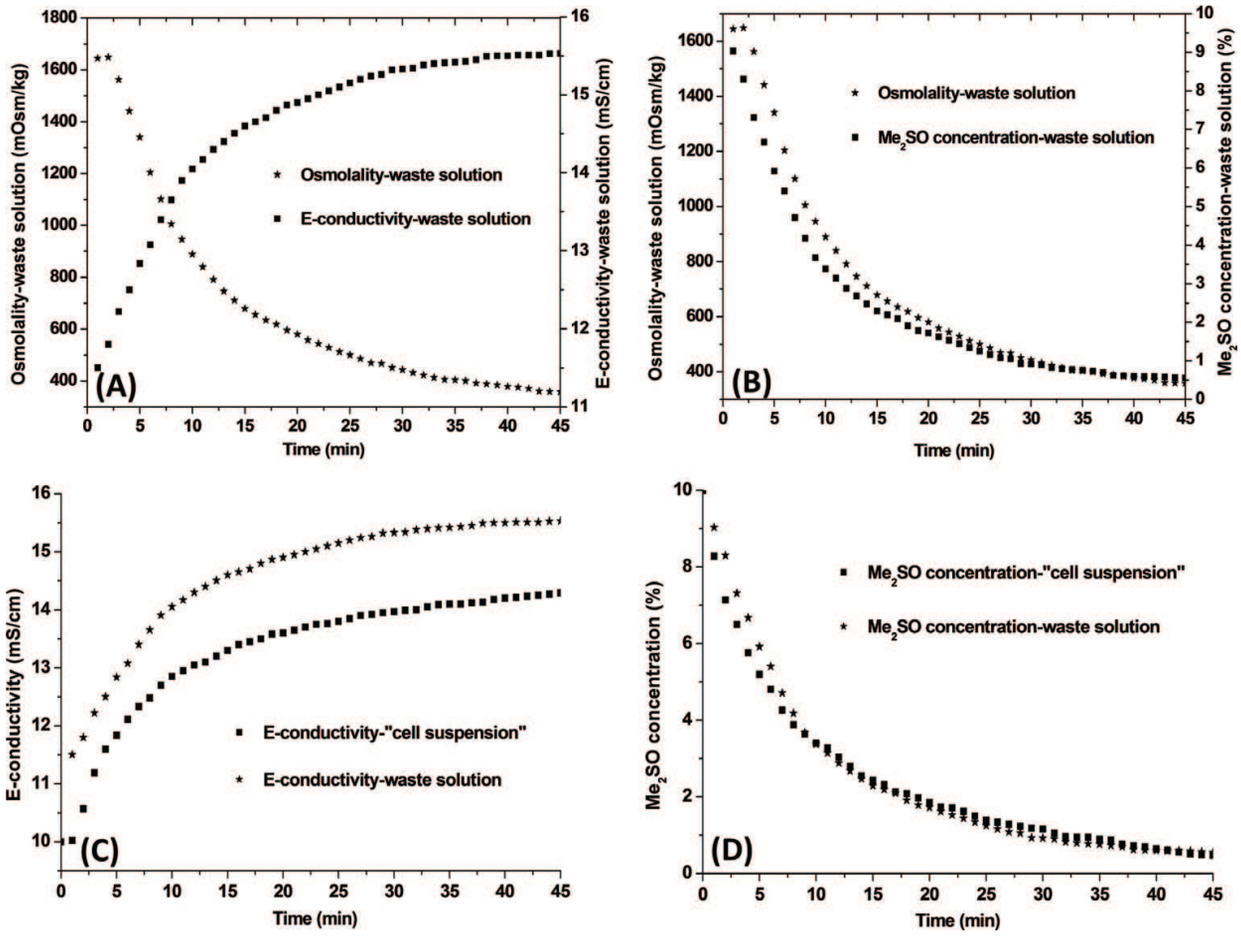

Figure 9. Me_2SO removal from Me_2SO-BSA-NaCl-water quaternary solution by "dilution-filtration." (A) Conductivity and osmolality of waste solution; (B) Me_2SO concentration and osmolality of waste solution; (C) conductivity of "cell suspension" and waste solution; and (D) Me_2SO concentration of "cell suspension" and waste solution.

BSA in "cell suspension." After certain time of processing (~10 min), the difference of Me_2SO concentration between "cell suspension" and waste solution was very small, which implies that measurements of waste solution can be applied to monitor the status of "cell suspension."

4. Discussion

Since the EC of a solution is determined by the solution composition, this fact can be applied to assess the solute concentration. In this chapter, an application of EC measurement in biomedical engineering is presented. In cryopreservation, CPA is needed to eliminate the cryoinjury to cells, which should be added before cooling and removed after thawing. EC measurement of the solution can be used to assess the CPA concentration during CPA addition and removal.

In order to evaluate the CPA concentration (Me_2SO, glycerol, and ethylene glycol), the standard curves of "CPA concentration-EC of the CPA solutions" were obtained experimentally first. For

http://dx.doi.org/10.5772/67747

CPA-NaCl-water ternary solutions, EC can be well represented by exponential equation: $EC = A \cdot \exp(B \cdot C)$. Interestingly, A is determined only by salt concentration, and B is constant for any salt concentrations (B = −0.036, −0.036 and −0.045 for Me_2SO, glycerol and ethylene glycol, respectively. Concentration unit: %, v/v). A similar effect of albumin on the EC of albumin-NaCl-water ternary solutions was also found with an exponential constant of −0.011. This indicates that the effects of CPA, albumin and salt on the EC values are not coupled. This might be due to the fact that CPA, albumin, and salt cannot combine or interact in the solutions.

To demonstrate the application of EC measurement for CPA concentration assessment, a "dilution-filtration" system was successfully applied to remove Me_2SO from solutions efficiently. Compared to the traditional centrifugation method of CPA removal, the "dilution-filtration" can decrease labor and time consumption, eliminate mechanical injury due to centrifugation, avoid cell packing and clumping, and prevent contamination. The volume of diluent solution needed for CPA removal is also decreased dramatically in the "dilution-filtration" method. Compared to the method of dialysis using hollow fibers, the "dilution-filtration" method also has many other advantages: (1) in the beginning of the dialysis process, the cell suspension has to be exposed to diluent in the dialyzer. This process is generally hard to control, and severe osmotic injury can happen. In order to decrease the osmotic shock to cells, sometimes hyperosmotic non-permeable solutions are applied to prime the dialyzer first. This can improve cell recovery but cause complexity, and this non-permeable material eventually needs to be removed. In "dilution-filtration" method, the mixing of cell suspension and diluent can be well controlled in the "dilution" step (adjust the m value). (2) In dialysis method, CPA clearance is due to the passive diffusion transport across the fiber membranes caused by the CPA concentration gradient, while in "dilution-filtration" method, CPA is removed by active filtration. So the CPA removal efficiency can be improved dramatically. (3) In dialysis method, the CPA gradient across the membranes is not uniform along the fibers. So mass transport is not uniform and cells experience different osmotic stresses along the fibers. This increases complexity and thus makes it harder to achieve optimal conditions. (4) It is much easier to control and manipulate the final cell suspension volume and cell concentration with the "dilution-filtration" method.

EC measurement can be a very good method to assess CPA concentration. Compared to direct osmolality measurement by osmometer, its advantages include low cost, ease of operation, real-time and online monitoring, and broad working range (CPA concentration).

For the CPA-salt-water ternary solution, once the salt concentration is fixed, the CPA concentration can be determined by its EC. This is generally the case of CPA removal after cell cryopreservation with fixed salt concentration. The hollow fibers selected in this work can block macromolecules from crossing the fiber membranes, such that the waste solution is CPA-NaCl-water ternary solution. Meanwhile, salt concentrations in cell suspension and diluent are isotonic, and this leads to the fact that salt concentration everywhere, including in waste solution, is isotonic. Accordingly, EC change of the waste solution is determined only by the CPA concentration change. In order to further evaluate the validity of predicting CPA concentration in cell suspension with the data of waste solution, the measurements of "cell suspension" were conducted and compared with those of waste solution. The results show that after a short period

of priming solution removal, the EC, CPA concentration, and osmolality of "cell suspension" and waste solution were almost identical to each other. This proves that assessment of waste solution is a good measure of the real-time state of the cell suspension. Measuring the waste solution, instead of cell suspension, has at least two advantages: First, waste solution is generally simpler than cell suspension without effect of proteins, cell debris, etc. Second, this can prevent direct contact of the EC probe with the cell suspension, keep the cell loop closed, and reduce the risk of contamination. A probe can be mounted in the waste solution loop to achieve real-time, online monitoring of CPA concentration during CPA removal.

5. Conclusion

A simple approach based on electrical conductivity measurements was developed for the quantification and monitoring of the CPA concentration in cryopreservation. Standard data of a few CPAs solutions (Me_2SO, glycerol, ethylene glycol) were obtained. Coupled with the "dilution-filtration" system, this method can be used to measure the EC of waste solution and predict the real situation in cell suspension. This way can help to prevent contamination and achieve on-site and real-time monitoring of the CPA concentration effectively.

Acknowledgements

This work was supported by the Bill & Melinda Gates Foundation (OPP1032522), U.S. National Institutes of Health (NIH) (UM1AI068618), and a Supplement to R33AI094412 funded by National Institute of Allergy and Infectious Diseases (NIAID).

Author details

Zhiquan Shu[1,2]*, Hsiu-Hung Chen[3], Xiaoming Zhou[4] and Dayong Gao[1]

*Address all correspondence to: zqshu@u.washington.edu

1 Department of Mechanical Engineering, University of Washington, Seattle, WA, USA

2 School of Mechanical and Materials Engineering, Washington State University, Everett, WA, USA

3 Department of Mechanical and Aerospace Engineering, University of Missouri, Columbia, MO, USA

4 School of Mechatronics Engineering, University of Electronic Science and Technology, Chengdu, Sichuan, China

References

[1] Polge C, Smith AU, Parkes AS: Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature* 1949, 164(4172):666.

[2] Okamoto Y, Takaue Y, Saito S, Shimizu T, Suzue T, Abe T, Sato J, Hirao A, Watanabe T, Kawano Y et al: Toxicities associated with cryopreserved and thawed peripheral-blood stem-cell autografts in children with active cancer. *Transfusion* 1993, 33(7):578–581.

[3] Davis JM, Rowley SD, Braine HG, Piantadosi S, Santos GW: Clinical toxicity of cryopreserved bone-marrow graft infusion. *Blood* 1990, 75(3):781–786.

[4] Shu Z, Heimfeld S, Gao D: Hematopoietic SCT with cryopreserved grafts: adverse reactions after transplantation and cryoprotectant removal before infusion. *Bone Marrow Transpl* 2014, 49(4):469–476.

[5] Stroncek DF, Fautsch SK, Lasky LC, Hurd DD, Ramsay NKC, Mccullough J: Adverse reactions in patients transfused with cryopreserved marrow. *Transfusion* 1991, 31(6):521–526.

[6] Gao DY, Liu J, Liu C, Mcgann LE, Watson PF, Kleinhans FW, Mazur P, Critser ES, Critser JK: Prevention of osmotic injury to human spermatozoa during addition and removal of glycerol. *Hum Reprod* 1995, 10(5):1109–1122.

[7] Calmels B, Houze P, Hengesse JC, Ducrot T, Malenfant C, Chabannon C: Preclinical evaluation of an automated closed fluid management device: Cytomate (TM), for washing out DMSO from hematopoietic stem cell grafts after thawing. *Bone Marrow Transpl* 2003, 31(9):823–828.

[8] Rodriguez L, Azqueta C, Azzalin S, Garcia J, Querol S: Washing of cord blood grafts after thawing: high cell recovery using an automated and closed system. *Vox Sang* 2004, 87 (3):165–172.

[9] Perotti CG, Del Fante C, Viarengo G, Papa P, Rocchi L: A new automated cell washer device for thawed cord blood units. *Transfusion* 2004, 44(6):900–906.

[10] Chen HH, Zhou XM, Shu ZQ, Woods EJ, Gao D: Electrical conductivity measurements for the ternary systems of glycerol/sodium chloride/water and ethylene glycol/sodium chloride/water and their applications in cryopreservation. *Biopreserv Biobank* 2009, 7 (1):13–17.

[11] Shu Z, Fang C, Zhou X, Gao D: Cryoprotective agent (CPA) removal with dilution-filtration method and CPA concentration monitoring with electrical conductivity measurements. In: *ASME 2014 International Mechanical Engineering Congress and Exposition: November 14–20, 2014; Montreal, Quebec, Canada*. ASME 2014.

[12] Valeri CR, Ragno G, Pivacek L, O'Neill EM: In vivo survival of apheresis RBCs, frozen with 40-percent (wt/vol) glycerol, deglycerolized in the ACP 215, and stored at 4 degrees C in AS-3 for up to 21 days. *Transfusion* 2001, 41(7):928–932.

[13] Valeri CR: Simplification of methods for adding and removing glycerol during freeze-preservation of human red blood-cells with high or low glycerol methods — biochemical modification prior to freezing. *Transfusion* 1975, 15(3):195–218.

[14] Rowe AW, Eyster E, Kellner A: Liquid nitrogen preservation of red blood cells for transfusion — a low glycerol-rapid freeze procedure. *Cryobiology* 1968, 5(2):119–&.

[15] Meryman HT, Hornblower M: Simplified procedure for deglycerolizing red blood-cells frozen in a high glycerol concentration. *Transfusion* 1977, 17(5):438–442.

[16] Meryman HT, Hornblower M: Method for freezing and washing red blood-cells using a high glycerol concentration. *Transfusion* 1972, 12(3):145.

[17] Ding WP, Zhou XM, Heimfeld S, Reems JA, Gao DY: A steady-state mass transfer model of removing CPAs from cryopreserved blood with hollow fiber modules. *J Biomech Eng-T* Asme 2010, 132(1):011002.

[18] Ding WP, Yu JP, Woods E, Heimfeld S, Gao DY: Simulation of removing permeable cryoprotective agents from cryopreserved blood with hollow fiber modules. *J Membr*ane *Sci* 2007, 288(1–2):85–93.

[19] Castino F, Wickramasinghe SR: Washing frozen red blood cell concentrates using hollow fibres. *J Membr*ane *Sci* 1996, 110(2):169–180.

[20] Arnaud F, Kapnik E, Meryman HT: Use of hollow fiber membrane filtration for the removal of DMSO from platelet concentrates. *Platelets* 2003, 14(3):131–137.

[21] Mata C, Longmire EK, McKenna DH, Glass KK, Hubel A: Experimental study of diffusion-based extraction from a cell suspension. *Microfluid Nanofluid* 2008, 5(4):529–540.

[22] Glass KKF, Longmire EK, Hubel A: Optimization of a microfluidic device for diffusion-based extraction of DMSO from a cell suspension. *Int J Heat Mass Transfer* 2008, 51(23–24): 5749–5757.

[23] Zhou XM, Liu Z, Shu ZQ, Ding WP, Du PA, Chung J, Liu C, Heimfeld S, Gao DY: A dilution-filtration system for removing cryoprotective agents. *J Biomech Eng-T Asme* 2011, 133(2):021007.

[24] Shu Z: Development of optimal biopreservation methods and technology for cellular therapy and clinical diagnosis [Thesis]. Seattle, WA, USA: University of Washington; 2013.