The Mechanism Analysis of NaCl Solution Ice Formation Suppressed by Electric Field

Wei Sun[1*], Xiaobin Xu[2] , Hong Zhang[1], W. Sun[1], Chuanxiang Xu[1]
[1] School of Electrical Engineering, Xi'an Jiaotong University, Xian 710049, China
[2] School of Electrical and information Engineering, Jiangsu University, Zhenjiang 212013, China
*E-mail: sunwei65@sogou.com

Abstract: The cells and tissues usually may be subjected to cryo-injure, but caused to die during freezing process for cryo-preservation. Among them, the most direct reason is extra-cellular and intra-cellular ice formation. How to control, reduce, or eliminate the ice formation has been an important research topic in fundamental cryobiology. The objective of this study was to test a hypothesis that the alternated electric field could significantly influence the solution of 0.9% NaCl freezing property at a relative slow cooling rate. The test sample consisted of 0.9% NaCl solution and it was frozen in 0.26K/min cooling rate, simultaneously was exposed to alternating electric fields ranging from 50Hz to 5MHz. A system was built and utilized to measure the temperature of the test sample continually. It was founded that alternated electric fields strongly influenced the freezing property of the solution, included the undercooling point temperature of the solution and the latent heat produced by phrase transition during freezing process. The results were analyzed by using dielectric theory and ions moving phenomena, it indicated that the icons moving under alternated electric field is main factor influencing the property of 0.9% NaCl solution during freezing process.

INTRODUCTION

As we know, cells and tissues are usually preserved by low temperature freezing. Because all of biomaterials, include cells and tissues, contain abundant salt solution, therefore, during freezing process for cryo-preservation, it can be subjected to cryo-injure very easily and caused to die by ice crystal. Some studying results show that some injuries during cryo-preserved biomaterials process are caused directly by extra- cellular or intra-cellular ice formation.[1-2] How to reduce or eliminate the ice formation in freezing process for cryo-preservation, carrying out biomaterials vitrificated cryo-preservation, has been one of the most important research topic in fundamental cryobiology. Vitrifying technology is the good method to prevent biomaterials to be injured by ice crystal, there are some researched results about this problem to indicate that cells and tissues would be avoid to be injured if it were frozen in vitrification. Currently, there are two methods to be used to achieve cells and tissues vitrifying cryo-preservation. One is to adopt an ultra-rapid cooling rate(about more than 10^6K/min)[3] to freeze biomaterials, this method can make water in biomaterials complete phase transition in very short time, and H_2O molecules can't be ordered at definite direction and forming ordinary ice crystal, but forming glassy ice. Unfortunately, the ultra-rapid cooling rate for vitrifying biomaterials is very difficult to attain, especially for relatively large samples. This is because biological tissues have low thermal conductivities and high thermal capacities, therefore, the required cooling rate can be achieved for only very small specimens,[4] for large samples, it can not bring to success. The other method used to achieve vitrifying biomaterials involve the use of high concentration (about more than 6M) of cryo-protectants.[5-6] For example, SMDN, DMSO, etc.. But introduction of high concentration of cryo-protectants creates severe chemical toxicity and high osmotic stress, both of which can directly damage cells and tissues[7].

For these reasons, some novel technologies have been developed to vitrify biomaterials for cryo-preservation. Hanyu et al. introduced microwave into the experiment of the cryo-fixation of biomaterials for electron microscopy studies.[8] It was found that in comparison with samples cooled without microwave irradiation, in the presence of an intense microwave field, the rapidly cooled tissue samples contained a thicker layer (approximately 10 to 20 μm in thickness) of ice-free (glassy) region inlaid into the ice crystal region. The underlying mechanism of microwave effect was assumed to be the electric field component of electromagnetic radiation interacting with dipolar H_2O molecules and thereby disrupting ice nucleation phenomena. Thomas H. Jackson, *et al.* have reported that a combined use of microwave irradiation and cryo-protectant could significantly influence ice formation and enhance potential vitrification of biomaterials for long-term cryo-preservation at a relative slow cooling rate[9].

In the present study, we introduced electric field of sine-wave into the experiment of freezing 0.9% NaCl solution, using relatively low cooling rate. The purpose is to research the ice formation character of 0.9% NaCl solution during freezing process in above condition, and try to develop a new method of controlling or reducing ice formation.

EXPERIMENT METHODS

Test samples used in this study consisted of 0.9% NaCl solution. A set of experiment apparatus was built to

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provide low temperature environment for cooling the test samples, simultaneously put electric field of sine-wave on the samples, and measured the sample temperature continually during cooling process. A container used to contain the sample was made of perfluoro ethylene material, the container's specification was 20cm×5cm×10cm(L×W×H). there were two electrodes fabricated by the 0.2mm copperplate and were fixed on the two walls of container respectively. A thermal resistance was used to measure the samples temperature during freezing process, and fixed it in the center of the container. Fig. 1 shows the container structure.

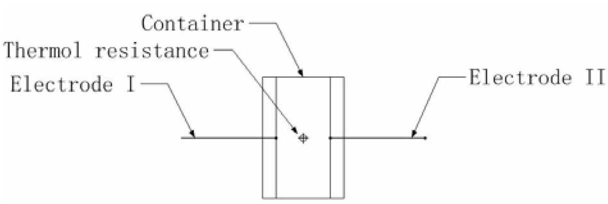


Figure 1. the structure of the container

The environment for cooling the samples was produced by a refrigerating cabinet, the most low temperature could drop to 243.15K(-30C). The environment initialization temperature value was set at 275.15K(2C), cooling rate was controlled at the value of 0.26K/min. According to Smith's expression,[10] it belong to a very slow cooling rate. In order to avoid the influence of the samples themselves initialization temperature on the test results, the test would be started after the test sample was putted in the low temperature environment about 2 hours. During all test process, an alternated electric field was applied to the copperplate electrodes with the peak voltage $V_{p\text{-}p}$ of 5V through a 20Ω resistance. The frequency of electric field was selected in the range from 50Hz to 5MHz.

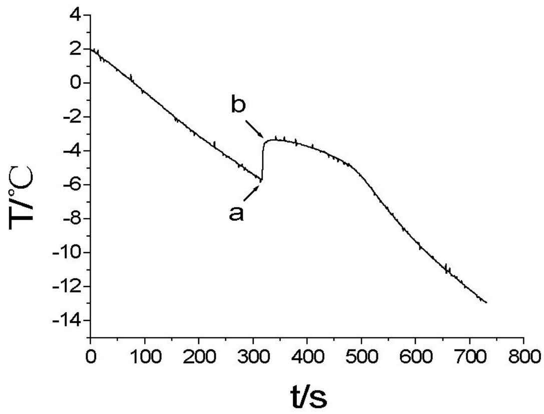
The system for monitoring the sample temperature was composed of computer, data acquisition cards and thermal resistance, measured and recorded automatically the sample temperatures, the sampling time interval value for acquiring data was set at one second.

EXPERIMENT RESULTS

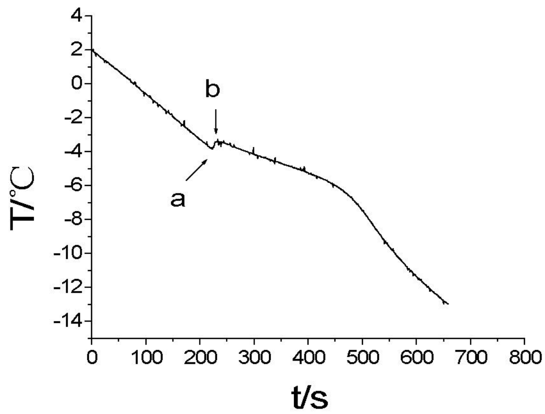
Fig. 2 shows the relationship curves of temperature and time (T-t) about the samples without electric field and with different frequency electric field during freezing process, respectively. Fig. 2(a) gives the T-t curve of the sample without alternated electric field. Fig. 2(b), (c) and (d) show the T-t curves with 50kHz, 500kHz and 5MHz alternated electric field respectively.

According to the Fig. 2, it is found that when the sample temperature decrease gradually along with low temperature environment applied by the refrigerating cabinet, the temperature of the sample happen to jumping up at the point "a", it changes from the point "a" to the point "b", these phenomenons were showed at Fig. 2(a), (b), (c), (d) respectively.

According to the freezing property of NaCl binary solution,[10] the point "a" indicate the start of phase transition of water in the solution, some H_2O molecules produce transition from liquid to ice, the temperature value at the point "a" is called undercooling point temperature of the solution.



(a)



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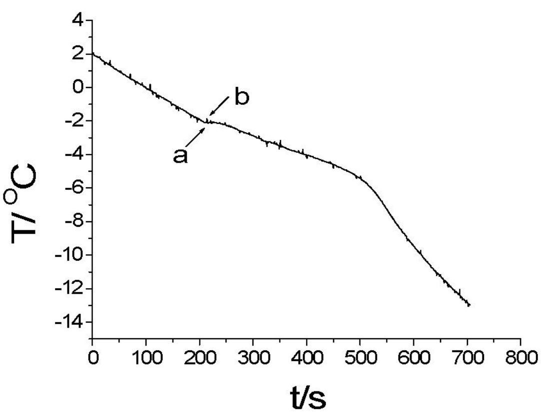

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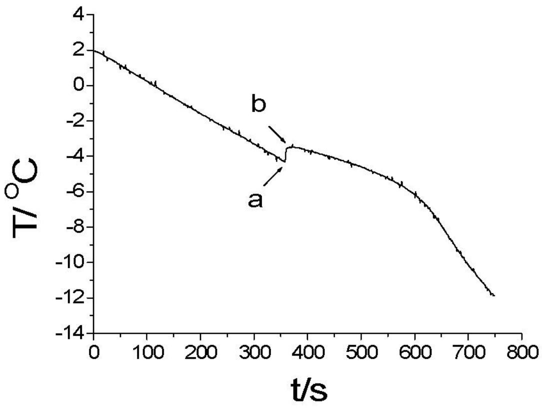

(d)

Figure 2. Show the relationship curves between temperature and time about the sample without electric field and with different frequency electric field in freezing process. (a) no electric field, (b) the frequency of alternated electric field is 50kHz, (c) 500kHz and (c) 5MHz.

As we know, when water state changes from liquid to ice, some quantity of heat will be produced, we call it latent heat, the quantity of latent heat will make water temperature up. In Fig. 2, the temperature difference value from the point "a" to the point "b", we call it "ΔT", is decided by the quantity of latent heat produced by phase transition in the solution. So we may consider that the value of ΔT and the quantity of the latent heat have certainly direct proportion relation.

About the results of Fig. 2, there are two aspects which should be noted. One is the change of undercooling point temperature of solution under different condition; the other is ΔT.

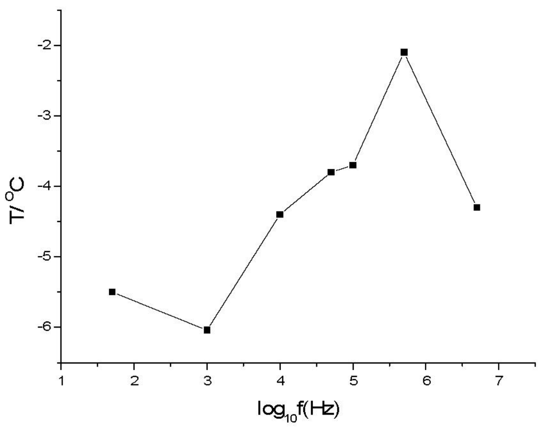

Figure 2. The relationship curve between the undercooling point temperature and the frequency of electric field applied to the sample.

Fig. 2 shows the dependence of undercooling point temperature on frequency of electric field applied to the sample. It is very obvious, when an alternated electric field is applied to the solution and the frequency of electric field increases gradually, it is found that the undercooling point temperature increases along with the frequency increasing and reaches the maximum value at the frequency of 500kHz. If the frequency continues to increase from 500kHz, on the contrary, the undercooling point temperature starts to decrease gradually.

Fig. 3 gives the dependence of ΔT on the frequency of electric field applied to the sample. We can know from it, when an alternated electric field is applied to the sample and the frequency of electric field increases gradually, it is found that ΔT decreases along with the frequency increasing and reaches the minimum value at the frequency of 500kHz. If the frequency continues to increase from 500kHz, on the contrary, ΔT starts to increase gradually.

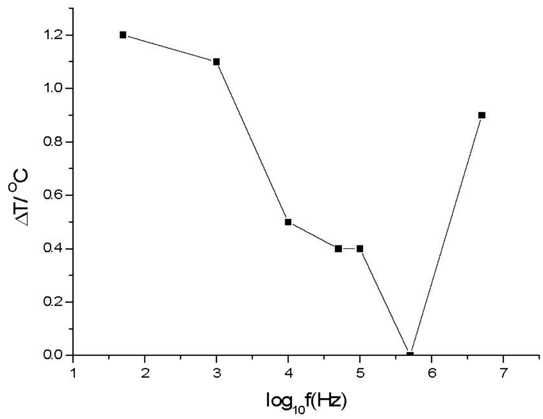

figure 3. The relationship curve between ΔT and the frequency of electric field applied to the sample.

According to above the results, it can be concluded that alternated electric field can affect the freezing property(including undercooling point temperature and ΔT) of the 0.9% NaCl solution as the solution is cooled and water is frozen forming ice crystal. Especially, in the case of 500kHz, the undercooling point temperature reaches the maximum value, and the value of ΔT reaches the minimum. It is indicated that the alternated electric field with the frequency of about 500kHz has a maximum effect on the freezing property of the 0.9% NaCl solution.

ANALYSIS AND DISCUSSION

It is generally believed that there are two steps for phase transition of water from liquid to ice formation. The first step is to form nucleation of the ice crystal in the undercooling water; the second step is the growth of ice crystal from the nucleus. As we know, NaCl is a kind of strong electrolyte, in the dilute NaCl solution, the NaCl can be dissolved in water and can be converted totally into ions. Therefore, there are three kinds of elements in the dilute NaCl solution, include H_2O molecule, Na ion(Na^+), Cl ion(Cl^-). Na^+ and Cl^- are distributed averagely between H_2O molecules. When an alternated electric field is applied to the solution, consequentially, there are two effects to occur in the solution. One is the dipole polarization of H_2O molecules; the other is ions moving.

In this experiment, the frequency of electric field applied to the sample is selected in the range from 50Hz to 5MHz, though the loose frequency of water is of the order of magnitude of 10^{10} Hz, but the loose frequency of ice is 10^4 Hz. Therefore, it is assumed that the effects of alternated electric field on the freezing property of the solution are concerned with the H_2O molecular dipole polarization and relaxation properties during the ice forming process, and the effect of alternated electric field perhaps directly acts on the nucleation process.

For verifying this assumption, the other experiment is designed, de-ionized water is selected for test sample, the test process is as the same as above. It is found that the freezing property of de-ionized water with alternated electric field is almost the same as that without alternated electric field. The results show that the H_2O molecules dipole polarization and relaxation properties are not the main reason to affect the freezing property of the NaCl solution during the freezing process.

Beside the H_2O molecular dipole polarization and relaxation properties, there is the other effect, it is the ions moving. As we know, Na^+ and Cl^- are distributed averagely between H_2O molecules. The ions will be moving back and forth along the direction of the alternated electric field applied to the solution, similar ions vibration. The ions vibration must disturb the original alignment structure of the H_2O molecules, and affect the H_2O molecules to combine mutually, and then, the change of the relative position of the ions maybe make the variety of the internal electric field inside the solution. According to above analysis, some assumptions below will be brought forward. (1) The distribution of internal electric field inside the solution is changed by ions moving, resulting in a non-uniform distribution of electric field. Stronger electric field is probably formed at some sections and at some time, and acts on the H_2O molecules. The polarization induced by the stronger electric field during the ice forming process results in rapid reorientation of H_2O dipole, which is assumed as one of candidate factors to enhance the aggregation of H_2O and the forming of nucleus in the undercooling water. (2) Because of forming a lot of nucleus in the solution, the ions inevitably come into collision with nucleus, and the more forming nucleus, the more colliding odds. As the ions and the H_2O molecules collide mutually, some H_2O molecules aggregating to nucleus maybe get rid of nucleus attracting, and return into free state. In this case, ions moving are against some H_2O molecules aggregating to nucleus and against nucleus growing up into big ice crystal. It is supposed that some sections of all forming ice area are not in ordinary pure ice, maybe there are some glassy regions to be inlaid into the ice crystal.

CONCLUSION

The 0.9% NaCl solution freezing property under alternated electric field has been investigated. Attention was focused on the undercooling point temperature and latent heat produced by phase transition of water when the solution was in a slowly cooling rate(the value is 0.26K/min) and different frequency of electric field(from 50Hz to 5MHz). It was found alternated electric field significantly affected the freezing property, and at 500kHz the effect was the most obvious. H_2O molecule polarization theory and ion moving were sued to analyze, the analysis indicated that the main factor of affecting freezing property of the solution was not dipolar H_2O molecules, but ions moving.

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