

Creative Optimization and Industrial Research of Freeze Drying Process of the Cardiomyopeptidin for Injection

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Cardiomyopeptidin is a kind of biological drug used in the perioperative period of cardiac surgery, and the main ingredient of it can help to repair myocardial damage. In the production of cardiomyopeptidin for injection, vacuum freeze drying is a key technology. However, it has to face many difficulties such as the long cycle of freeze drying process (78 h) and large deviation of quality in group which reduce the efficiency of product. Currently, the study of freeze drying process of cardiomyopeptidin for injection is rare. The experiments in this paper were conducted by a miniature freeze dryer, cutting down the cycle of freeze drying process to an ideal value (45 h) and diminishing the deviation of quality in group. At present, many key factors of freeze drying process of the cardiomyopeptidin have been investigated, the optimized freeze drying program successfully cuts down the cycle to 36 h in the pilot freeze dryer. Luckily, the research also found a new auxiliary material which could greatly improve the efficiency of freeze drying. This paper provides a new way for the research of freeze drying process of biological drugs.

1. Introduction

Cardiomyopeptidin is a kind of biological drug of which the ingredients are active substance of polypeptide extracted from ventricular muscle of piglets, it is usually used in the perioperative period of cardiac surgery for the purpose to accelerate the renovation process of myocardial damage. The current situation is that the number of the patients of heart diseases increases year by year, which stimulates the demand for cardiomyopeptidin. The pharmaceutical method of cardiomyopeptidin for injection is freeze drying. Freeze drying is a process by which a solvent is removed from a frozen solution by sublimation of the solvent and by desorption of the solvent, generally under reduced pressure. This process involves the following three stages (Wang and Chen, 2012): freezing, primary drying, and secondary drying periods. Freezing stage is the core of freeze drying process, about 95 % of the solvent water is removed from the liquid formulation in the form of a pure solid ice phase in primary drying stage and the other water is removed in the secondary drying stage. Of all drying operations, freeze drying is the most expensive, both in terms of capital and operation costs (Hassan and Muhamad, 2017). There are a number of reasons why the freeze drying method is used to a great extent in industry. The most important one, common to all industrial sectors, is that the products freeze dried are the most often sensitive to heat and cannot be dried with other drying techniques due to their high operating temperatures (Bando et al., 2016). The research methods of freeze drying are mathematical models and freeze drying experiments, the former can predict the behaviour of the freeze drying process satisfactorily, but for most multivariable processes in which numerous potentially influential factors are involved as is the case for the freeze drying process, it is not always easy to determine the influence of each variable, as well as the interactions between them (Boss and Filho, 2004). The research has investigated many factors that may influence the freeze drying process to optimize the original program by freeze drying experiments though they are time consuming and are usually expensive to be carried out in all possible operating ranges (Tang and Pikal, 2004). The optimized freeze drying process can be directly applied into scale-up experiments to optimize the process in pharmaceutical factory.

2. Materials and method

In this work, many experiments were conducted to investigate various factors that may influence the freeze drying process of the cardiomyopeptidin. The experiments were conducted in a lab-scale freeze dryer from Tofflon with freeze drying area of 0.5 m², a freeze dryer is usually composed of a drying chamber, a condenser, a vacuum pump and a heater. The cardiomyopeptidin and mannitol that used as excipient in the freeze drying process were provided by Zhen-Ao pharmaceutical co. The process has a typical operating temperature range of -55 °C to 50 °C, a pressure range of 0.1 mbar to 0.6 mbar, and a quantity of entrained air range of 0.01 mbar to 0.1 mbar. Each experiment consumed about 30 h and the whole research conducted about 60 groups of experiments. All the experiments must be conducted in asepsis because bacteria may reduce the active ingredients of cardiomyopeptidin. The program of freeze drying of lab-scale freeze dryer should be different from pharmaceutical factory due to the difference of scale and performance between them. To get qualified cake (water content ≤1 % and “uniform and elegant” cake appearance), the process in pharmaceutical factory consumes about 66 h, but it only need 56 h in lab, so the researchers have to get the qualified cake with 35 h in lab because pharmaceutical factory wants to cut down the cycle to 45 h (Patel et al., 2017). This paper formulated a 35 h program of the freeze drying process as a program to be optimized in our lab. Each group of experiments consist of three steps:

(1) Preparation of solution: the researchers prepare 20 vials of solution each time according to the standard of pharmaceutical factory (in asepsis, quantity of polypeptide is 20 mg per vial, 9 %w/v of mannitol per vial), then put the vials on the scheduled location of shelf in the lab-scale freeze dryer.

(2) Freeze drying: first, the researchers set up the program of freeze drying and adjust the value of factor to be investigated, then the samples will experience three stages (freezing, primary drying, secondary drying) which will cost about 35 h.

(3) Date analysis: on the one hand, the researchers weigh the mass loss of each vial with ten-thousandth balance to measure the influence of each factor on water content, on the other hand, they grade each vial to measure the cake appearance under different conditions, finally, they get the best value of the factor investigated after analysing water content and cake appearance synthetically. After investigating all the factors scheduled, a time-saving and energy-saving freeze drying program will be achieved.

3. Results and discussion

In the research, more than twenty factors were investigated to test their influences on the freeze drying process, and the experiments of each factor were completely independent. The results of the experiments shown that seven factors of them were the key ones of the freeze drying process of cardiomyopeptidin for injection. The key factors were classified into three sections: preparation of solution, freezing stage, and drying stage.

3.1 Investigation of factors of preparation of solution

Preparation of solution is the most important step before the whole freeze drying process, the status of the solution to be freeze dried has great influence on the freeze drying process. The results of the experiments shown the following two factors were very important for the freeze drying process.

3.1.1 Filling quantity

Filling quantity is thought to be the most important factor that influences the freeze drying process, this paper researched the filling quantity from 3.2 ml (the lowest value can be accepted by pharmaceutical factory) to 4.0 ml (original value). To get a much more obvious conclusion, the vials were only freeze dried to the end of primary drying (totally about 26 h), the relationship between filling quantity and mass of cake is presented in Figure 1(a). The researchers found that water content increased with the increasing of filling quantity, filling quantity of 3.2 ml had the lowest mass of cake (0.78 g), besides, samples with filling quantity above 3.7 ml had skin formation on top on the cake and the cake color seemed uniform, but the ones below 3.6 ml had an “uniform and elegant” cake appearance. Synthetically, taking production allowance into consideration, filling quantity of 3.4 ml was optimal as an optimized value.

3.1.2 Excipients

In the freeze drying process, mannitol may function as skeleton for freeze dried cake (Wang, 2000), meanwhile, the researchers may use additional excipients to improve the freeze drying process. Different excipients in the solution may lead to different morphologies of ice which drastically influence the efficiency of freeze drying. This paper investigated six kinds of common excipients (tertiary butanol, saccharose, NaCl, glucose, sorbitol, lactose) to choose the best excipient for freeze drying process. The proportion of each excipients and mannitol is 1:3. Finally, it seemed that samples with tertiary butanol had the lowest water content and the best cake appearance, on the other hand, the ones with other excipients had unacceptable cake appearance such as

collapsed cake and melt back. It is reported that tertiary butanol can lead to needle type of ice which increases the area of drying, the next, the researchers investigated the influence on water content of the amount of tertiary butanol (Geidobler and Winter, 2013). The result is presented in Figure 1(b). The water content reduced as the volume fraction of tertiary butanol increased from 0 % to 7 %, the samples of volume fraction above 7 % had higher water content, besides, samples with different amount of tertiary butanol had good cake appearance. Therefore, 7 % was the best volume fraction of tertiary butanol for freeze drying process of the cardiomyopeptidin. Though tertiary butanol as excipient may improve the freeze drying process sharply, the safety of tertiary butanol for clinic must be reevaluated.

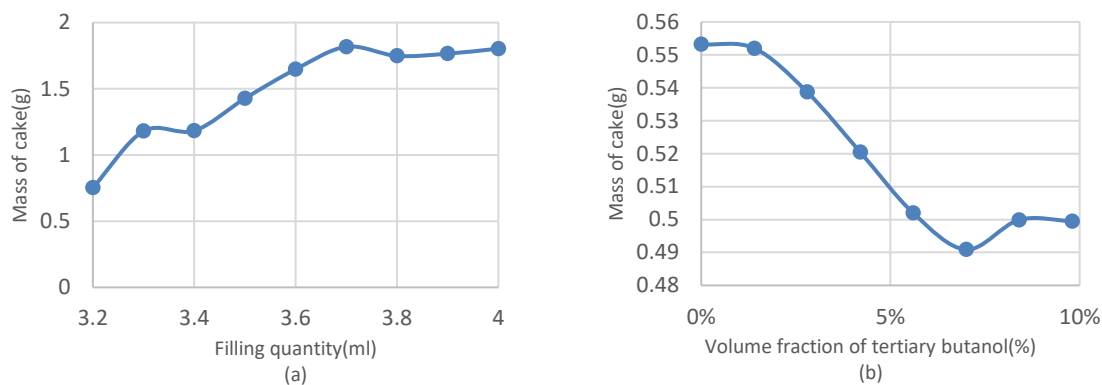


Figure 1: (a) Influence of filling quantity on water content. (b) Influence of volume fraction of tertiary butanol on water content

3.2 Investigating of factors of freezing stage

Freezing stage is the core of freeze drying process, because conditions of freezing stage determine the morphologies of ice which control the speed of drying. The results of the experiments shown the following three factors were key to the freezing drying process.

3.2.1 Feeding method

A modification of the ice nucleation temperature was observed by placing the vials on a pre-cooled shelf in the freeze drying process. While cooling from room temperature to the shelf set point, it was observed that the average nucleation temperature was increased compared to standard ramp freezing. The researchers converted the feeding method from standard ramp freezing to pre-cooled shelf method because increasing the ice nucleation temperature can significantly reduce primary drying duration. The researchers changed the temperature of shelf from $-10\text{ }^{\circ}\text{C}$ to $12\text{ }^{\circ}\text{C}$ (standard ramp freezing). The result is presented in Figure 2(a). The water content reduced with reducing from $12\text{ }^{\circ}\text{C}$ to $0\text{ }^{\circ}\text{C}$ of the temperature of shelf, but increased when the temperature continued to reduce to $-10\text{ }^{\circ}\text{C}$, maybe it was because the cold shelf mist over with ice due to contact with humid air. Besides, samples of $0\text{ }^{\circ}\text{C}$ and $5\text{ }^{\circ}\text{C}$ had a better cake appearance and the others whose color was not uniform and had skin formation on the top. Finally, the researchers decided to apply pre-cooled shelf method to our optimized program and the temperature of shelf was $0\text{ }^{\circ}\text{C}$.

3.2.2 Pre-freezing temperature

Pre-freezing temperature is the terminal temperature of the freezing stage of freeze drying process of cardiomyopeptidin for injection and it is the lowest temperature in the freeze drying process. The pre-freezing temperature low enough can ensure solution in the vials utterly frozen, it may lead to acceptable cake appearance and water content. The freezing stage with pre-freezing temperature low excessively leads to a waste of energy and the samples with a high pre-freezing temperature will have undesirable appearance such as fusion and skin formation. Taking energy saving and quality of products into consideration, pre-freezing temperature is thought to be a key factor of the freeze drying process. This research adjust pre-freezing temperature from $-56\text{ }^{\circ}\text{C}$ to $-43\text{ }^{\circ}\text{C}$ ($-50\text{ }^{\circ}\text{C}$ is original value) to investigate its influence on freeze drying process. The result is presented in Figure 2(b). It could be found that water content became lower when pre-freezing temperature got down, meanwhile, appearance of cake got better too. In view of refrigerating capacity of pharmaceutical factory, the researchers selected $-53\text{ }^{\circ}\text{C}$ as the optimized value of pre-freezing temperature (Kasper and Friess, 2011).

3.2.3 Pre-freezing duration

Be similar to pre-freezing temperature, enough pre-freezing duration can ensure solution in the vials utterly frozen. In this paper, pre-freezing duration ranged from 120 min to 360 min (240 min is original value), investigating its influence on freeze drying process. The result is presented in Figure 2(c). What most surprised us was that samples' appearance and water content had little to do with pre-freezing duration. Allowance for difference of performance between lab-scale freeze dryer and industrial scale ones, the researchers reduced the pre-freezing duration slightly to 180 min as the optimized value.

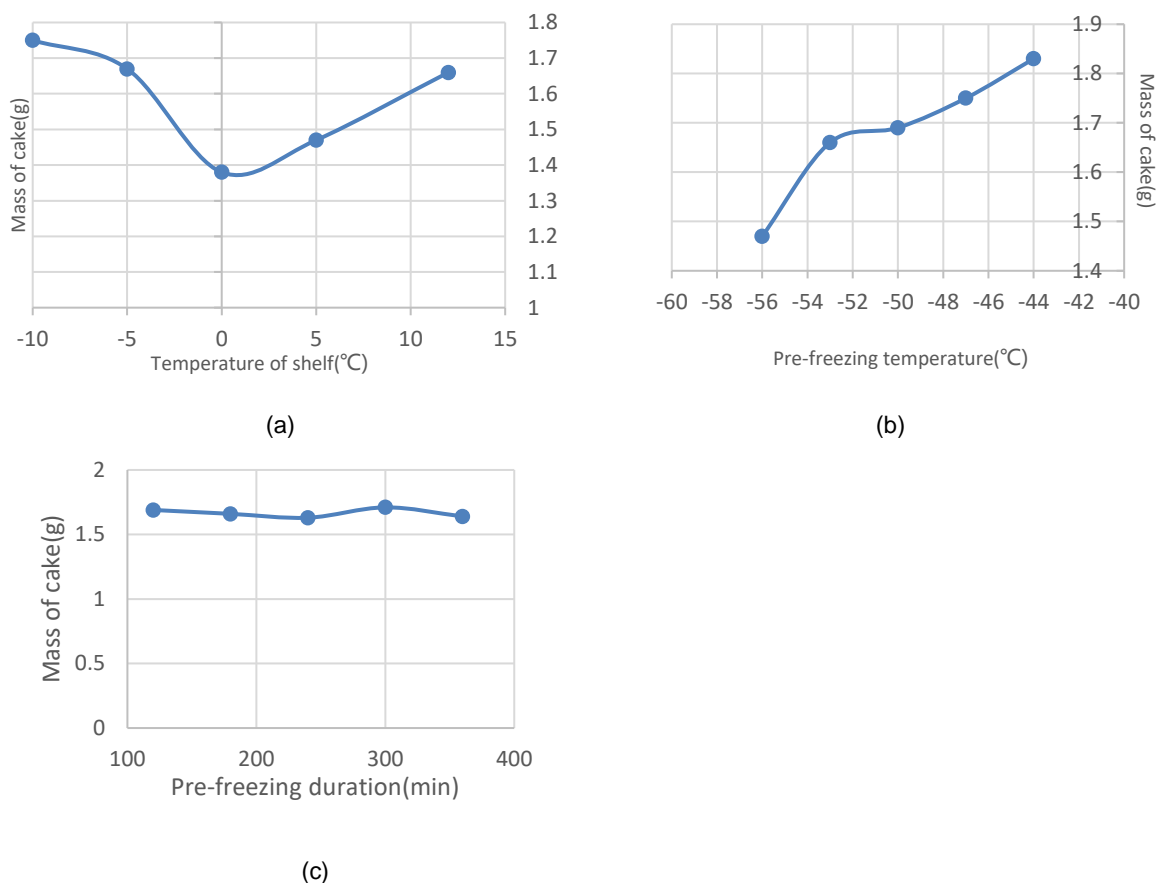


Figure 2: (a) Influence of the temperature of shelf on water content. (b) Influence of pre-freezing temperature on water content. (c) Influence of pre-freezing duration on water content

3.3 Investigation of factors of drying stage

During drying stage, different temperature intervals have different drying speed, so the research divided the drying stage into several small stages to study how each small stage influenced the freeze drying process. The results of the experiments shown the following two stages influenced the whole process greatly.

3.3.1 Duration time of high speed stage

According to experience achieved from pharmaceutical factory, the researchers divided the whole primary drying into two stage: low speed stage (-40 °C to -28 °C) and high speed stage (-23 °C to 0 °C). Most water sublimates during high speed stage, they adjust the duration time of high speed stage from 9 h to 15 h (11 h is the original value) to investigate how it affects the freeze drying process. The result is presented in Figure 3(a), they found the duration time of high speed stage didn't affect the total drying rate, but during the freeze drying process, they found water sublimated faster at -8 °C than the temperature interval from -23 °C to -15 °C and samples with high speed stage under 13 h had an unacceptable cake appearance. Synthetically, they extended 4 h at -8 °C and reduced 2 h at -23 °C to -15 °C stage, to sum up, the research extended 2 h of the duration time of high speed stage as the optimized program.

3.3.2 Duration time of secondary drying stage

During secondary drying, most bound water is removed. Enough duration time of secondary drying stage may ensure qualified water content. This paper adjust duration time of secondary drying stage from 6.5 h to 18.5 h (totally time of freeze drying process ranged from 36 h to 48 h), finding out how long of duration time of secondary drying stage can be applied to the optimized program. The result is presented in Figure 3(b). The water content reduced with the increasing of drying time, but water content of 36 h (0.668 %) is far below standard (1 %). Finally, the researchers cut down the duration time of secondary drying stage to 6.5 h as the optimized program.

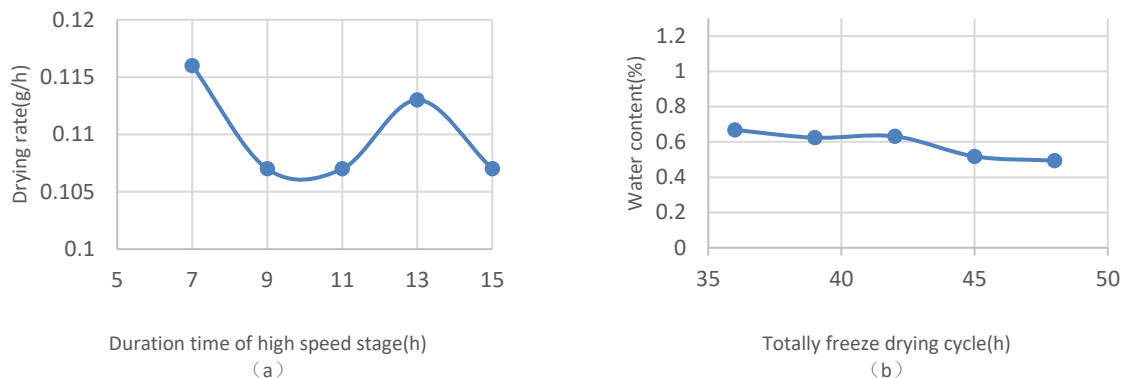


Figure 3: (a) Influence of the duration time of high speed stage on water content. (b) Influence of duration time of secondary drying stage on water content

3.4 Comparison of cycles of freeze drying process

The researchers have investigated seven key factors above that greatly influence the freeze drying process of cardiomyopeptidin for injection and got a group of optimized parameters through the freeze drying experiments. Applying the optimized parameters into the original freeze drying process, the researchers got an optimized freeze drying cycle that is presented in Figure 4 in comparison with the original process. According to Figure 4, the cycle of the original freeze drying is successfully cut down by nearly 20 h, the production efficiency increases greatly. The researchers conducted freeze drying experiments with the optimized freeze drying process and found that the samples' appearance was quite good and water content was far below 1 %.

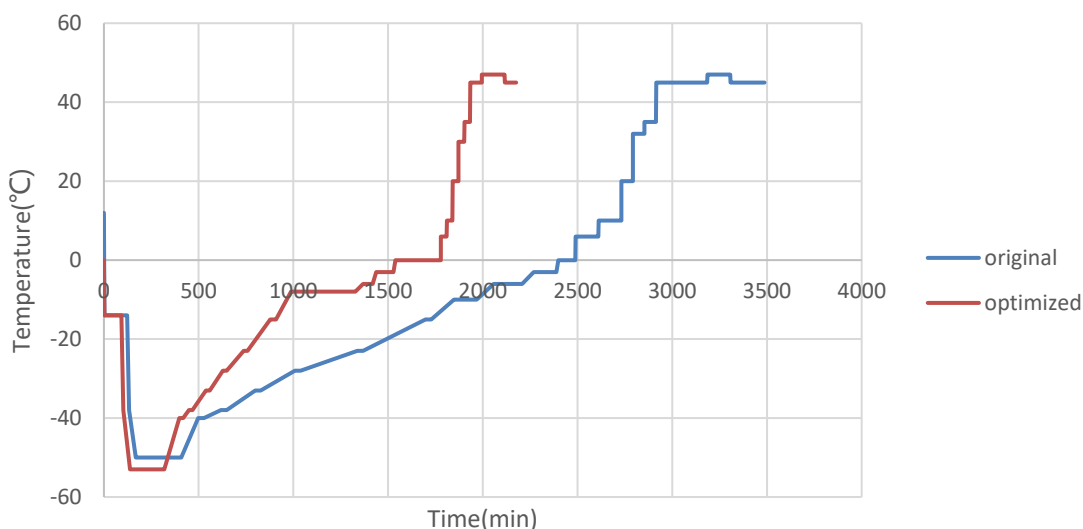


Figure 4: Comparing of original process and optimized process

4. Conclusions

After investigating the factors above, the researchers get an optimized program of the freeze drying process. Decreasing of filling quantity directly reduces the water to be freeze dried, meanwhile, the process of heat and mass transfer is improved, it is in favor of freeze drying process, too (Brulls and Rasmuson, 2002). In the research, the researchers found tertiary butanol may sharply cut down the cycle of the freeze drying process by helping forming needle type of ice, due to converting of the feeding method from standard ramp freezing to pre-cooled shelf method, duration of primary drying was reduced a lot. With qualified water content and acceptable cake appearance, the cost of time of new freeze drying process is shorter than the original one by nearly 20 h. Subsequently the researchers conducted scale-up experiments and successfully realized the industrialization of the optimized freeze drying process for cardiomyopeptidin. Applying the optimized freeze drying process, pharmaceutical factory saves about 20 hours in the freeze drying process for cardiomyopeptidin, it means that the energy consumption of freeze drying process and the cost of production are reduced. The authors think that the process integration of freeze drying process in this paper contributes to energy saving and pollution reduction of pharmacy industry.

Acknowledgments

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