

LYOPHILIZATION AND CRYSTALLIZATION PROCESS IN THE DEVELOPMENT OF NANOMATERIALS

Abstract

Since low solubility is typically associated with poor dissolving characteristics, which results in poor oral bioavailability, the majority of active pharmaceutical ingredients utilized in the therapy of disease have poor water solubility and provide challenging difficulties in drug formulation creation. To reduce solubility issues with current medication candidates, the development of a pharmaceutical product's novel formulation and drug delivery system is a significant challenge. Another significant obstacle to the development of pharmaceutical products is the limited drug-loading capacity, which necessitates a considerable amount of carrier material to obtain the proper encapsulation of the medication. This issue could be overcome by the production of nanocrystals.

Keywords: Nanoparticles, Freezing, Lyophilization, Nanocrystallization, Nanocrystals,

Authors

Mehak Aggarwal

Department of Pharmaceutics
Himachal Institute of Pharmacy
Paonta Sahib
H.P, India.
Mehakaggarwal2804@gmail.com

Dr. Esha Vatsa

School of Pharmaceutical Sciences
Himgiri Zee University
Dehradun
Uttarakhand, India.
esha.vatsa@hzu.edu.in

I. INTRODUCTION

Nanoparticles have drawn a lot of attention in the medical sciences during the past few decades, particularly in the areas of imaging, gene delivery systems, medication delivery, and sensing. The creation of polymeric nanoparticles as drug delivery systems has been the subject of numerous investigations. They are incredibly intriguing because of their capacity to encapsulate different medications, distribute them in a sustained and/or tailored manner, and lessen the toxicity of particular pharmaceuticals by shielding non-targeted tissues. Micron systems are nanoparticles. You can create nanoparticles, nanospheres, or nano capsules depending on the method utilized to prepare them.

Aqueous suspension versions of nanoparticles, and more especially nano capsules, are typically created. The main difficulty is the chemical and physical instability of such carriers in aqueous media. Their instabilities prevent them from being commercialized and do not guarantee long-term stability. It is necessary to immobilize the molecular mobility of the components of the nano capsule system in order to overcome the chemical and physical instabilities. After drying, this immobilization is possible to obtain.

One of the frequent unit operations in the pharmaceutical industry is drying, which is achieved by the process Lyophilization, which enables the removal of solvents (often water) from liquid medication formulations in order to solidify them through one or more phase transitions. Through the planned shelf-life, the dry form enables the preservation of their key qualities. Therefore, the primary goal of lyophilization is to obtain solid-state nano capsules since doing so causes the formulation's components, especially the active ingredients, nano capsules, and any ligands that may be on their surfaces, to become immobilized.

The process of crystallization occurs often in the majority, if not all, kinds of matter. Nanoparticles can be used as building blocks for new kinds of materials and are excellent systems for understanding and visualizing crystallization processes. Engineering complementary interactions, such as by affixing DNA ligands, has resulted in a stunning variety of nanoparticle crystals. During the assembly process, appropriate system mobility must be maintained in order to produce well-arranged nanoparticle crystals. It is generally agreed that the crucial nucleus size depends on the equilibrium between the nanoparticle cohesive energy stored within the nucleus and the nanoparticle interfacial contacts with the solvent at the earliest stages of the nucleation process. Expanding the processing window for nanoparticle crystallization has been accomplished using a variety of techniques, such as controlled solvent evaporation, interface-mediated assembly, emulsion-based assembly, and gradual cooling of concentrated/supersaturated solutions. Controlling the crystallization kinetics, however, is still difficult in order to produce high-quality nanoparticle crystals quickly and consistently.

Proteins and tiny molecules have been crystallized with the aid of precipitating agents such salts, solvents, and polymers. These additions can change the way molecules interact, stabilizing the intermediate phase or lessening the solubility of the species that crystallizes. This idea has not yet been experimentally tested, but it may enable control over the assembly rates and paths for nanoparticle crystallization.

The most popular procedure for creating pharmaceutical goods that are unstable and thermosensitive in aqueous medium is lyophilization, in order to assure stability and long-term storage in the dried condition. In the lyophilization process, the solvent is first frozen and then extracted by sublimation while under vacuum. Ice sublimation, primary drying, and secondary drying, or desorption of unfrozen water, are the three distinct steps of freeze drying.

II. LYOPHILIZATION

The most popular technique for removing water and managing the long-term stability of nanoparticle compositions is freeze drying, also known as lyophilization. As an illustration, the need of developing a dry form of nanoparticle-based treatments during the latter two years of the COVID-19 pandemic has brought to light the significance of lyophilization studies to enhance the storage stability of lipid nanoparticle vaccines. There are three steps in the freeze-drying cycle.

- Freezing step
- Primary drying step
- Secondary drying step

1. Freezing: In the freeze-drying process, freezing is a crucial phase because the microstructure that is created during freezing impacts the end product's quality as well as its processing characteristics, such as the rate of primary drying and secondary drying. The product needs to be frozen at a low enough temperature to solidify entirely. By slowing down molecular motion, freezing the product reduces chemical activity. In general, the process of ice crystallization from extremely chilled water is referred to as freezing. Prior to freezing, the solution must be cooled until ice nucleation takes place. When ice crystals start to grow at a specific pace, the solution freezes, which causes the formation of either crystalline or amorphous solids or mixes. Lower than -35°C is the minimum temperature at which an aqueous pharmaceutical formulation can be frozen.

The phenomena that occur in the freezing step are as follows:

- **Super-cooling:** "Supercooling" is the retention of the liquid state below the equilibrium freezing point of the solution. It always happens when temperatures are below freezing, frequently at or above $10\text{-}15^{\circ}\text{C}$. Super-cooling comes in two varieties.
- **Global super-cooling:** It is the procedure in which a uniform amount of super cooling is present throughout the entire liquid volume.
- **Local super-cooling:** Only a small portion of the liquid gets super-cooled with this technique. Super-cooling is a metastable, non-equilibrium state that resembles the activation energy required for the nucleation process.
- **Ice Nucleation:** Water molecules cluster into patterns resembling those found in ice crystals as a result of density variations brought on by Brownian motion in the supercooled liquid water.

Nucleation comes in two forms:

- **Homogeneous nucleation:** The term “homogeneous nucleation temperature” refers to the apparent value of -40°C as the water’s limiting nucleation temperature. At this temperature, a pure water sample will have at least one active water nucleus that has developed on its own and is ready to start the formation of ice crystals.
 - **Heterogeneous nucleation:** In heterogeneous nucleation, layers of water are absorbed onto “foreign impurities” to form clusters that resemble ice.
 - **Ice crystal growth:** The quick ice crystallization that occurs once the critical mass of nuclei is reached results in the rapid creation of stable ice crystals throughout the entire system. The development of stable ice crystals is aided by the influx of molecules at the contact. The temperature of the final product rises quickly as crystallization starts and approaches the equilibrium freezing point. The solution is further cooled after the initial ice network has formed, which removes more heat, and the ice crystals that have already formed cause the remaining water to freeze. The quantity of newly generated ice nuclei, the rate of ice growth, and the size of the ice crystals are all dependent on the level of super-cooling.
2. **Primary Drying:** It is distinguished by the vial’s ice border layer retreating. Traditionally, this process is completed at shelf temperatures between -30°C and -10°C and chamber pressures between 40 and 400 Torr. By delivering vacuum into the product chamber, the chamber pressure is decreased during this phase to between 0.01 and 0.1mbar. The product is heated in order to melt the frozen mobile water. On a condenser’s surface, the water vapor is gathered. The condenser needs to have enough surface area and cooling power to keep the batch’s entire amount of sublimed water at a temperature lower than the temperature of the finished product. The drying process will halt if the ice on the condenser is warmer than the product, as this will cause water vapour to gravitate toward the product. Water vapor partial pressure and the sublimation front in the freeze-dry chamber. The product temperature must be higher than the condenser temperature since vapour pressure and temperature are connected. The water molecules travel from the chamber’s higher vapour pressure zone to the condenser’s lower vapour pressure region. It is crucial that the temperature at which a product is freeze dried strikes a balance between that which preserves the product’s frozen integrity and that which maximizes its vapour pressure. The secret to ideal drying is this equilibrium. Low temperatures are maintained in the condenser, often around -60°C . For best drying effectiveness, the product’s temperature should be maintained as close to the glass transition temperature as is practical.
 3. **Secondary Drying:** The product still has bound moisture after the initial freeze-drying procedure is accomplished and all the ice has melted. Even if the product appears to be dry, there could be as much as 7% to 8% of residual moisture. Therefore, dry must continue above a warmer temperature in order to reduce the residual moisture level to acceptable values. "Isothermal desorption" is the process by which the bound water is removed from the product. This process is finished by raising the shelf temperature exceed the surrounding air temperature. The shelf temperatures can be raised to $15\text{--}300^{\circ}\text{C}$

in order to allow the molecules of water to desorb under vacuum. The shelf temperature shouldn't be raised over the product temperature so as to prevent product deterioration. Even though the product may seem dry at the end of the primary drying stage, there may still be 7-8% weight of moisture present. Up until the product's target moisture content is reached, secondary drying is conducted. A product's moisture content should be no more than 2% of its weight. In order to maintain the cake structure, the product should not be overdried and should not have a final moisture content below 1.5% weight. Some antibiotics and chemotherapeutic drugs can have moisture concentrations as low as 0.1 weight percent. The quantity and type of residual water in the product as well as the absorption, adsorption, and desorption processes serve as the basis for secondary drying parameters. The shelf temperature should not be elevated above the maximum amount of heat that the product can bear without degrading, which is another crucial consideration.

Some desirable characteristics of a freeze-dried nanoparticle include:

- The preservation of the product's fundamental physical and chemical properties
- An acceptable relative humidity
- Long-term stability

III. CRYSTALLIZATION

One of the major issues in nanoscience and nanotechnology today is the production of crystalline nanoparticles and nanostructures with well-defined sizes, morphologies, and hierarchical architectures. Although it is well known that a wide variety of particle morphologies and interior structures may be created by carefully regulating reaction conditions and adding additives like polymers, knowledge of the mechanisms by which these structures form is still lacking.

The creation of crystals that are solid from a liquid, from a solid melt, and directly from the gas phase is known as crystallization. By changing the soluble condition of the solute in the solvent, crystallization is accomplished while preventing precipitation brought on by a chemical reaction. This process is frequently employed in the production of pharmaceuticals.

A liquid inputs stream is often chilled to cause crystallization, or precipitants are added to make what is needed less soluble and crystallize. Well-formed crystals are often pure because each molecular or ion has to fit exactly into the structure of the lattice as it leaves the solution. Impurities incompatible with the lattice remain in solution.

Therefore, purification by crystallization operates on the concept of molecular recognition. However, occasionally contaminants mix into the lattice, lowering the purity of the finished product. Additionally, the solvent can stay in the lattice and create a solvate. Additionally, inclusion, a phenomenon where the solvent becomes trapped inside the crystal, is possible.

Nucleation and crystal growth are the two main phases of crystallization:

- Molecules of solute that have been dispersed in a solvent begin to accumulate into structures which are stable under the current operating circumstances. This is the process of nucleation. These stable clusters make up the nucleus. The clusters dissolve, nevertheless, if they are unstable. As a result, the clusters must grow to a certain size before they can serve as stable nuclei. The operating circumstances (for example, temperature, super-saturation) determine this essential size. The crystal structure (relative arrangement) is defined by the atoms' specified/periodic arrangement at the nucleation stage.
- The subsequent development of the nuclei called crystal growth. While the super-saturation is there, nucleation and growth continue to take place simultaneously. The catalyst for crystallization is super-saturation. This can be accomplished via a variety of techniques, including chilling, the addition of an anti-solvent, evaporation, Ph change, and chemical reaction. Crystals of various sizes and forms are produced as a result of nucleation or growth, depending on the circumstances. Unless the operating circumstances are changed, for as by supersaturating the solution once more, the solid-liquid system finds equilibrium once the super-saturation has been spent.

The primary and most effective unit action for creating nanocrystals is crystallization. The process of producing nanocrystals, or crystalline particles of poorly soluble medicines, is known as nanocrystallization. Nanocrystals can be created using both conventional crystallization techniques, such as reactive crystallization and anti-solvent crystallization, and cutting-edge techniques, such as supercritical fluid crystallization and high-gravity controlled precipitation.

Reactive crystallization is the process by which supersaturation of a crystallizing substance is produced chemically. Reactions in reactive crystallization can happen quite quickly relative to the speeds of mass transfer and crystal formation. High local supersaturations are the result. In the pharmaceutical and fine chemical industries, anti-solvent crystallization, also known as precipitation, is a frequently used process to recover a product from solution in a solvent in which the product has a high solubility. When a supercritical or near-critical fluid is utilized as an anti-solvent, its dissolution into the solvent is often accompanied by a shift in the solvent's polarity, which lowers the fluid's solvation power in relation to the target substance. The density may also drop, further lowering the solvation capacity. As a result, the mixture becomes supersaturated, resulting in molecular nucleation and the growth of crystals that are well-ordered and extremely pure. In some circumstances, the density of the combination might even be higher than that of the neat solvent. In this instance, the mixture's shift in polarity is the main factor contributing to crystallization. HGCP (High Gravity Controlled Precipitation) technology platform was created based on fundamental mass transfer principles, wherein reaction phases are brought together in a high gravity environment and micro mixing of the reaction phases is accomplished in microseconds. For the synthesis of nano-particles with a limited size distribution in a precipitation process, a high level of super-saturation, uniform spatial concentration distributions, and equal development times for all crystals are necessary criteria. The HGCP technology is the best platform technology for developing and synthesizing a variety of nano-sized materials.

The key features of this process are:

- The input substance is a fluid that is either in solution or that is above the melting point of the solid phase. Multiple solvents may be present in a solution.
- Impurities in the combination could be either solid or dissolved. Some contaminants, particularly organic reaction byproducts, might resemble a solute in many ways.
- In the crystallization process, impurities may form distinct crystals, remain in solution, or combine with the completed crystals in some manner.
- The product's component is solid and available in a range of particle sizes.
- Usually, the product is surrounded by mother liquor.
- The process produces a fluid waste stream that contains pollutants and any dissolved product that is still present.

REFERENCES

- [1] M.R. Mozafari, "Nanomaterials and Nano systems for Biomedical Applications", Springer Science & Business Media: Dordrecht, The Netherlands, 2007.
- [2] H.K. Chan, P.C. Kwok, "Production methods for nanodrug particles using the bottom-up approach", *Adv Drug Deliv Rev.* vol. 63, pp. 406-416, May 2011.
- [3] J. Zhao, Y. Liu, L. Wang, Y. Zhou, J. Du, Y. Wang. "Functional and modified nanocrystals technology for target drug delivery". *J. Nanosci. Nanotechnol.* Vol.18, pp. 5207–5221, 2018.
- [4] S. P. Bhardwaj and R. Suryanarayanan. "Molecular Mobility as an Effective Predictor of the Physical Stability of Amorphous Trehalose". *Molecular Pharmaceutics*, vol. 9, pp. 3209-3217, 2012.
- [5] J. Zhai, Q. Li, H. Xu, T. Su, Yu-e Wang, W. Huang, Y. Ma, S. Guan. "An Aseptic One-Shot Bottom-Up Method to Produce Progesterone Nanocrystals: Controlled Size and Improved Bioavailability". *Molecular Pharmaceutics*. Vol. 16, pp. 5076-5084, 2019.
- [6] H.R. Constantino. "Excipients of use in lyophilized pharmaceutical peptide, protein, and other bioproducts, in: Constantino HR Ed.), *Lyophilization of Biopharmaceuticals*", AAPS Press, PP. 117-168, 2004.
- [7] F. Franks. "Freeze-drying of bioproducts: putting principles into practice". *Eur. J. Pharm. Biopharm*, vol. 45, pp. 221–229, 1985.
- [8] S.L. Nail, L.M. Her, "An improved microscope stage for direct observation of freezing and freeze drying". *Pharm Res*, vol. 11(8), pp. 1098-1100, 1993.