INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

COMPREHENSIVEREVIEW ON RECENT ADVANCES IN LYOPHILIZATION OF NANOMEDICINES

Rohit R. Bhosale[1*], Dhanashri D. Chavan[2], Amol S. Shete[3]

[1]*Department of Pharmaceutics, Krishna Foundation's Jaywant Institute of Pharmacy, Wathar, Tal. Karad- 415539, Dist. Satara, Maharashtra, India.*

[2]*Department of Pharmacology, Krishna Vishwa Vidyapeeth (KVV) Deemed to be University, Karad- 415539, Dist. Satara, Maharashtra, India.*

[3]*Department of Pharmaceutics, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth (KVV) Deemed to be University, Karad-415539, Dist. Satara, Maharashtra, India.*

ARTICLE INFO

Article history
Received 24/04/2023
Available online
01/06/2023

Keywords
Lyophilization;
Stability;
Nanomedicines;
Drug Delivery.

ABSTRACT

Lyophilization (also known as freeze-drying) has been considered as a good technique for improving the long-term stability of nanomedicines. Nanomedicines have found increasing applications in the targeted drug release or controlled drug release, and the development of Nanomedicines as potential candidates mainly aims towards harvesting the sufficiently small size of particle for proper administration, for preserving the drug potency significantly for a long time, and for restoring the bioactivity upon a quick re-dispersion. However, the poor stability in an aqueous medium of these systems forms a real barrier against the clinical use of nanomedicines. Most of the literature reflecting lyophilization technology for processing the nanomaterials falls under medical as well as pharmaceutical research, and this is perhaps due to the natural link existing between the lyophilization and the pharmaceutical development. The lyophilization of nanomedicines, when fully controlled could really revolutionize their use in the medical field thereby ensuring the stability of nanomedicines.This article mainly focuses on recent advances in lyophilization of nanomedicines.

Corresponding author
Dr. Rohit R. Bhosale
Department of Pharmaceutics,
Krishna Foundation's Jaywant Institute of Pharmacy, Wathar,
Tal. Karad- 415539, Dist. Satara, Maharashtra, India.
bhosalerohit707@gmail.com
(+91)7057224707

*Please cite this article in press as **Rohit R. Bhosale** et al. Comprehensivereview on Recent Advances in Lyophilization of Nanomedicines. Indo American Journal of Pharmaceutical Research.2023:13(05).*

INTRODUCTION

Nanomedicine is the nanobiotechnology application to the medicine.It has now emerged in the pastfew years as a promising tool for numerousdiagnosticsas well astherapeutic applications. In this point of view, one of the major challenges for the clinical use of nanoparticles is to maintain their initial physicochemical state during the entire formulation process, i.e., from the benchmark to the commercialization. Researches have focused on bio applications of nanoparticles(NPs) employing mostly the liquid suspensions. Yet from a commercial viewpoint, storage as well as transportation of liquid suspensions requires more logistic issues. Moreover, such suspensions of the NPsreflectnumerousinstability problemslike aggregation (depending on the nature of NPs), premature drug leakage, hydrolysis (in polymeric NPs), and denaturation or hydrolysis of the drug loaded.A simple way for overcomingsuch problems includes storage ofthe nanoformulations in a dry solid form by removing the water or the solvent, which is usually done by freeze-drying (also known as the lyophilization).Lyophilization is anextensive process employed in the pharmaceutical industry which is based on the water or solvent removal from a frozen sample by means of sublimation (primary drying) and then desorption (secondary drying) under vacuum. Consequently, lyophilization process aims to preserve the labile ingredients, and further results in a longer storage or shelf life of the final product/formulation [1].Most of the literature reflectinglyophilization technology for processing the nanomaterials falls under medical as well as pharmaceutical research, and this is perhaps due to the natural link existing between the lyophilization and the pharmaceutical development. NPs have found increasing applications in the targeted drug release or controlled drug release, and the development of NPsas potential nanomedicinesmainly aims towards harvestingthe sufficiently small size of particle for proper administration, for preserving the drug potency significantly for a long time, and for restoring the bioactivity upon a quick re-dispersion [2].The objective of this present piece of writing mainly focuses on reporting the recent advances in lyophilization of nanomedicines.

Implementation of quality by design (QbD) in lyophilized product development

Quality by design (QbD) is a method of choice in formulation development to get robust and quality products followed by continuous improvement [3]. QbD is an organized approach that is based on predefined objectives and emphasizes product and process understanding and process control, based on thorough knowledge and quality risk management [4]. QbD is particularly helpful in designing robust processes with well-understood operational limits and their significance. Implementation of QbD is complex and challenging work in the pharmaceutical industry. The use of QbD in formulation development minimizes the trial cost of validation batches. Therefore, implementation of QbD principles, developing a better understanding, and possibly designing simple templates for the development of complex formulations is the current need.

From an operational standpoint, freeze-drying is an expensive and time-consuming process. As a result, the process must be brief, repeatable, and robust. To design a freeze-drying method, one must first define the essential formulation parameters and control them in the process design. The temperature of the heating fluid, pressure in the drying chamber, and timeframe of both drying stages throughout primary and secondary drying are some of the freeze-drying process parameters that have a major impact on the quality of finished products [5]. If such variables are not controlled, they can cause product denaturation, melting, or collapse of the dried cake. So, systematic development of the freeze-dried product by the implementation of QbD is most required to obtain a quality product. According to ICH Q8 (R2), different effective QbD tools can be used for the development of robust quality formulations.

Selection of lyoprotectants and their impact on the stability

Lyophilization is an industrial process that converts an aqueous nanoparticles suspension into an amorphous-crystalline solid via freezing, primary drying, and secondary drying [6]. These processes generate mechanical stress on the nanoparticles, causing aggregation and reducing nanoparticles' stability [7]. Lyoprotectants are inert excipients introduced exclusively into the aqueous suspension before lyophilization to provide physical and chemical stability to the nanoparticles [8]. It reduces the mechanical stress during freezing and drying steps and improves the stability of nanoparticles [9]. Bulking agents (mannitol, glycine, sucrose), buffers (Tris HCl, histidine, and phosphate), structure modifiers, tonicity adjusters (salts), stabilizers (glucose, dextran, sucrose, trehalose, lactose, mannitol, and alanine), collapse inhibitors (glucose, dextran, maltose, maltotriose, maltopentose and maltoheptose) are most commonly used inert excipients as lyoprotectants and cryoprotectant in the freeze-drying process [8]. Moreover, the lyoprotectants are also acting as cryoprotectants and vice versa [7].

Recent Advances in Lyophilization of Nanomedicines

Lipid NPs are being used increasingly to deliver thedrugsas well as genes, including the delivery of a small interfering RNA (siRNA). Pulmonary delivery of the drugs directly into the lungsvia lipid NPs may improve the lung disease management. The feasibility of aerosolizable lipid NPs dry powders prepared by thin-film freeze-drying (TFFD) was tested, wherein solid lipid nanoparticles (SLNPs) comprised of cholesterol, lecithin,and lipid-polyethylene glycol conjugate were prepared by the solvent evaporation technique, and then prepared the dry powders of SLNPs by TFFD, spray drying, or the conventional shelf freeze-drying. After SLNPs were subjected to TFFD and reconstitution, their particle size, polydispersity index and zeta potential were preserved; but not after subjectingthem to the conventional shelf freeze-drying and reconstitution. The TFFD prepared dry powder had shown better aerosol performance than that of prepared by the spray drying. SLNPs encapsulated with the siRNA can also be transformed successfully into the aerosolizable dry powder via TFFD. Subjecting those siRNA-encapsulated SLNPs to the TFFDdid not negatively affect the siRNA function, thereby representing the TFFD as a promising method for preparing the aerosolizable dry powder of lipid NPs [10].

Lyophilization is a promising technology for improvingthe stability of nanoparticle (NP) suspensions, particularly during the long-term storage. It also offers the new routes of administration in a solid state. Basic mechanisms of colloidal stabilization of NPs in liquid and dried statehave been summarized. Also, the stresses that may occurwhile freezing and drying step of lyophilization have been studied, and practical advice has been provided for facilitating purposeful formulation and process development to get the NP lyophilizates possessing high colloidal stability [11].To repairthe damaged bone, manufacturing of the porous scaffoldby means of three-dimensional (3D) printing and lyophilization, is one of the best suitable techniquesas these two methods have been found acting as synergistic. Such techniques along with the bioactive ceramic and polymer rapidly improve the fracture and damagedparts for better healing of the bone lesions.Porous scaffolds having Gyroid shape were designed by using the solid work with proper porosityand then printed by using 3D printing machine with an Electroconductive Polylactic Acid (EC-PLA) followed by their coating with a polymeric solution containing various amounts of akermanitebioceramic(as reinforcement), and their mechanical as well as biological properties were investigated [12].

Bromelain, an enzyme extract derived from the stems of pineapples, holds numerous therapeutic activitiessuch as wound healing and improved blood circulation. It isusually unstable and undergoes autolysis, resulting in a decreased enzymatic activity as well as limited applications. However, encapsulatingthe bromelain intoNPs can overcome the problem such as its degradation by protease immobilization which further can increase its stability as well as efficacy. Chitosan, the natural polymer, can be nanostructured and can trap the enzyme, thereby maintaining the biodegradability, biocompatibility, and the natural source of formulation ingredients. In this viewpoint, chitosan-bromelain NPs (C-B-NPs) were formulated by means of ionic crosslinking, butthey were found unstable when stored in an aqueous suspension. Consequently, the formulation was subjected to the lyophilization thatresulted in improved bromelain and NPs stability, thereby further allowing *in vivo* applications as dried powder for the topical administration [13].

For improving the drug residence timein mucosa, promising method like designing nanoparticle (NP)-based mucosal drug delivery can be used.However, stability of polysaccharide-based NPs is limited in aqueous solutions, owing to the hydrolysis. Hence, the long-term stability of formulation is usually improved by lyophilization. In that context, effect of cryoprotection and lyophilization on both physical as well as chemical properties of mucoadhesive acrylated-chitosan NPswas investigated. Also,potential of these carriers for drug delivery was investigated, and the results have shown most effective cryoprotectionachieved using sucrose.Incorporation of dextran sulfate, hydrophilic macromolecular drug, resulted inincreased NP size and decreased zeta potential for both the fresh as well as lyophilizedNP formulations.Furthermore, lyophilized NPs presented penetration across the mucus gel layer, andflow through technique had revealed that short-term mucoadhesive properties were not impaired[14].

Novel sonochemical route was introduced by means of preparing graphene/TiO2/Ag nanocomposite for visible-light photocatalytic degradation of commonly used textile azo-dyecalledX6G, followed by extraction ofthat obtained nanocomposite from reaction solution by conventional centrifugation and lyophilization. Transmission Electron Microscopy (TEM) images have revealedbetter distribution of TiO2/Ag NPs within the graphene sheets inlyophilized nanocomposite as compared to conventional dried sample. Additionally, lyophilized nanocomposite reflected higher photocatalytic activity.Because of rapid freezing of sonicated sample by liquid N_2, TiO2/Ag NPs are kept between the graphene sheets followed by calcination process that attached and fixed them to the graphene, thereby preserving dispersion effect of the sonication.

The importance of numerous parameters associated withthe lyophilization of common pharmaceutical NPs includingnanocrystals (NCs), nanosuspensions, nanoemulsions, nanocapsules and nanospheres was elucidated.Specific variables are of highest significance for each class of NPs, and should be optimizedas shown by the study. Freezing rate and antisolvent:solvent ratio should be considered for NCs, and for emulsified NPs, best results were obtained by 5-20% of saccharides as cryoprotectants. Consequently,suggesting thatfor obtaining a product with lowest aggregation and particle size, optimization of effective factors in formulation as well as lyophilization process are essential [15].Silica NPs (SiO_2NPs) were synthesized by using Stober-based process, followed by their characterization by various techniques like scanning electron microscopy (SEM), dynamic light scattering (DLS), and nitrogen adsorption/desorption isotherms. SiO_2NPs hydrodynamic diameters were compared pre- and post-lyophilization in presence or absence of carbohydrate protectants. Glucose was found as the most suitable protectant, and the minimum concentration of carbohydrate required to protect SiO_2NPs effectively from aggregation during lyophilization is influenced by NPs size and texture. Additionally, during storage, negligible aggregation was observed. Hence, carbohydrates can be used during SiO_2NPs lyophilization process for obtaining redispersable solids maintaining original size with no any residual aggregation [16].

Chitosan-coated polycaprolactone NPs were synthesized in microreactors and lyophilization of nanosuspension was focused for separating the particles from liquid phase. With respect to lyophilization process, effect of the lyoprotectants and steric stabilizers on the particle stability was investigated, and the best results were obtained with 2.5 % Poloxamer 388 as well as 5 % sucrose and the Zeta potential remained positive and greater than +30 mV in all cases [17]. Also, lyophilized wool NP powders derived from the wool fibers were extracted through neutralization method for developing protein NPs for the biotechnology field that requires size-controlled NPs [18]. Moreover, lyophilized keratinocyte-targeted nanocarriers loaded with locked nucleic acid modified anti-miR were developed for the topical application for full thickness burn injury [19].

In one of the studies, different paclitaxel (PX)-nanosuspensions were produced by using selected permeability enhancers (PEs), followed by their conversion into the dry NPs (PXNPs) throughlyophilization technique.PXNPs-based buccal tablets were then prepared, and it was observed that in-vitro disintegration time was found to be ≈ 36sec with rapid release rate >70% within first minute. Furthermore,as per the in-vivo bioavailability studies on rabbit models, bioavailability of PX was found to be increased six times as compared to the pure PX. All these outcomes have confirmed that NPs featured with PEs could be an effective solution to improve buccal permeability as well asthe absorption ofpoorly soluble chemotherapeutic agents for oral tumours [20].

Ibuprofen-loaded amorphous solid dispersions (ASDs) were prepared by means of emulsification-diffusion technique, wherein Eudragit L100-55 was used as a methacrylate co-polymer carrier. Later, lyophilization of NP suspensions was carried out, and ibuprofen-loaded Eudragit L100-55 NPs with different drug loadings were prepared. It was found that ASDs had exhibited good dissolution profiles, as indicated by sustained release of ibuprofen, followed by the build-up ofsupersaturation. Also, as per the physical stability tests carried out on ASDs, ibuprofen remained amorphous post 12 months of the storage, and hence, Eudragit L100-55 was found playing a dual role inASDs i.e., kinetic stabilization of ibuprofen in amorphous state while storage, drug release control, and supersaturated state stabilization [21].

Folic acid NPs (FANPs) were prepared by combination of bead milling and lyophilization with a newly developed food additive transglycosylated naringin (Naringin-G), andexcellent dispersibility and photostability of prepared NPs were observed.Also, in this study, Polyvinyl pyrrolidon (PVP) was used to compare with Naringin-G, and water dispersibility as well as photostability of lyophilized formulations were assessed.FANPs with Naringin-G were found stable with no aggregation after re-dispersion of lyophilized FA formulations in water, andalso, adding PVP did not prevent aggregation of FANPs after re-dispersion of lyophilized FA formulations. Additionally, release rate of lyophilized FA formulation with Naringin-G was found exhibiting around five times enhancement as compared to the untreated FA, and the formulation was also found stable against the photodegradation under fluorescent lightdue to the antioxidant effectof Naringin-G and the scavenged radicals. Consequently, these outcomes have indicated the potential oflyophilized FA formulation with Naringin-G to improve its water-dispersibility as well as photodegradation[22].By lyophilization technique, carbon-coated VBO_3 on graphene sheets composite was synthesized as an anode material for the lithium-ion batteries. VBO_3NPs uniformly coated by amorphous carbonare either adhered to graphene sheets surface or covered by the graphene sheets, and the amorphous carbon and graphene not only prevent the growth of VBO_3 NPs but also provide an excellent conductive 3D networkbeneficial forbattery performance [23].In yet another study, lyophilization technique was evaluated for producing the calcium phosphates in a nanoscale, and the calcium phosphates phases were synthesized by wet chemical method. The results have shown thatlyophilizationdoes not alter samples crystallinity and morphological characteristics. However, particles size wasconsiderably reduced when compared with the other drying techniques, and hence, lyophilization is shown to be a better substitute for obtainingthe nanomaterials for the bone regeneration as well as tissue engineering [24].

Lyophilized highly concentrated chitosan/deoxyribonucleic acid (DNA) nanoparticle formulations were prepared for gene delivery at clinically relevant dosage. Chitosan and plasmid EGFPLuc were used to prepare chitosan/DNA NPs. These NPs were freeze-thawed to select the optimal concentration of lyoprotectants from mannitol, sucrose, dextran and trehalose in support with histidine buffer, lyophilized to prevent aggregation, evaluated its effects on polydispersity, zeta potential, and transfection efficiency of chitosan/DNA NPs. It was found that lyoprotectant combined with histidine buffer (pH 6.5) at ~ 0.5% w/v and ~ 3.5 mM could be useful for maintaining the stability and gene delivery of chitosan/DNA nanoparticles [25].

The effect of lyophilized ferulic acid-loaded polymeric NPs for ocular drug delivery was studied. Ferulic acid (FA) is a well-known antioxidant that has low bioavailability due to low aqueous solubility. The biodegradable polymer such as poly (lactic acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA) was used to prepare the ferulic acid-loaded and unloaded polymeric nanoparticles (FA-NPA and FA-NPB) using the nanoprecipitation method. The nanoparticles were lyophilized using hydroxypropyl-β-cyclodextrin (HP-β-CD) lyoprotectant and evaluated for stability studies. It was indicated that lyoprotectant could inhibit particle aggregation following rehydration and provide long-term stability [26]. Additionally, the effect of five lyoprotectants on the particle size and polydispersity index (PDI) value of mangiferin phospholipids complex loaded phytosomal soft NPs was studied. These nanoparticles, after lyophilization, showed smaller particle size and PDI. In this study, glucose, fructose (monosaccharides) and trehalose, maltose, and sucrose (disaccharides) were used as lyoprotectants at 0.5%, 1%, 2%, and 5% w/w, respectively, and findings of the study revealed that sucrose at a low concentration acted as a superior cryoprotectant and enhanced the nanoparticle's redispersibility and long-term stability via formation of hydrogen bonding interaction with developed NPs [27].Lyophilized curcumin-loaded polyethylene glycol (PEG)-PLA NPs were fabricated using flash nanoprecipitation technology and studied its effects on the long-term stability of curcumin nanoparticles. Curcumin is a highly lipophilic compound. Its nanoparticles in association with co-polymer stabilizer exhibited low stability attributes to weak binding with hydrophobic cores of the particles. Prepared nanosuspension was lyophilized using kleptose lyoprotectant. The study demonstrated that variables factors, i.e., mixing rate, the molecular weight of PEG-PLA, and the drug-to-stabilizer influenced the curcumin particle size and its distribution to a great extent [28].

Consequently, different studies reported have shown as well as proved that the lyophilization is truly a promising technology for the diverse applications in the area of nano- as well as bio-medical field.

CONCLUSION

Lyophilization is a multidisciplinary field that requires deep knowledge of formulation, physical and chemical characterizations of the product and also a deep knowledge of the complete process. Formulation and lyophilization are intimately related and one impacts the other. Lyophilization of nanomedicines is quite delicate owing to the process generating numerous stresses on these fragile systems. The lyophilization of nanomedicines, when fully controlled could really revolutionize their use in the medical field, thereby ensuring the stability of nanomedicines and future applications.

Funding
Nil

Authors Contribution
All Authors have contributed equally.

Conflict of Interests
Author declares no conflict of interests.

REFERENCES

1. Picco AS, Ferreira LF, Liberato MS, Mondo GB, Cardoso MB. Freeze-drying of silica nanoparticles: redispersibility toward nanomedicine applications. Nanomedicine. 2018;13(2):179-190.
2. Chen G, Wang W. Role of freeze drying in nanotechnology. Dry Technol. 2007;25:(1):29-35.
3. Mahajan R, Gupta K. Food and drug administration′s critical path initiative and innovations in drug development paradigm: Challenges, progress, and controversies. J Pharm Bioallied Sci. 2010;2:307.
4. Q8(R2) Pharmaceutical Development. United State Food Drug Adm. 2009. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q8r2-pharmaceutical-development
5. Cheng HP, Tsai SM, Cheng CC. Analysis of heat transfer mechanism for shelf vacuum freeze-drying equipment. Adv Mater Sci Eng. 2014;2014:1-7.
6. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: Formulation, process and storage considerations. Adv Drug Deliv Rev. 2006;58:1688-713.
7. Fonte P, Reis S, Sarmento B. Facts and evidences on the lyophilization of polymeric nanoparticles for drug delivery. J Control Release. 2016;225:75-86.
8. Sadikoglu H, Ozdemir M, Seker M. Freeze-drying of pharmaceutical products: Research and development needs. Dry Technol. 2006;24:849-61.
9. Fangueiro JF, Andreani T, Egea MA, Garcia ML, Souto SB, Silva AM, Souto EB. Design of cationic lipid nanoparticles for ocular delivery: Development, characterization and cytotoxicity. Int J Pharm. 2014;461:64-73.
10. Wang JL, Hanafy MS, Xu H, Leal J, Zhai Y, Ghosh D, Williams RO, Smyth HDC, Cui Z. Aerosolizable siRNA-encapsulated solid lipid nanoparticles prepared by thin-film freeze-drying for potential pulmonary delivery. Int J Pharm. 2021;596:120215.
11. Trenkenschuh E, Friess W. Freeze-drying of nanoparticles: How to overcome colloidal instability by formulation and process optimization. Eur J Pharm Biopharm. 2021;165:345-360.
12. Dong X, Heidari A, Mansouri A, Hao WS, Dehghani M, Saber-Samandari S, Toghraie D, Khandan A. Investigation of the mechanical properties of a bony scaffold for comminuted distal radial fractures: Addition of akermanite nanoparticles and using a freeze-drying technique. J Mech Behav Biomed Mater. 2021;121:104643.
13. Ataide JA, Geraldes DC, Gérios EF, Bissaco FM, Cefali LC, Oliveira-Nascimento L, Mazzola PG. Freeze-dried chitosan nanoparticles to stabilize and deliver bromelain. J Drug Deliv Sci Technol. 2021;61:102225.
14. Eliyahu S, Almeida A, Macedo MH, Neves J, Sarmento B, Bianco-Peled H. The effect of freeze-drying on mucoadhesion and transport of acrylated chitosan nanoparticles. Int J Pharm.2020;573:118739.
15. Ashraf MA, Liu Z, Peng WX, Jermsittiparsert K, Hosseinzadeh G, Hosseinzadeh R. Combination of sonochemical and freeze-drying methods for synthesis of graphene/Ag-doped TiO2 nanocomposite: A strategy to boost the photocatalytic performance via well distribution of nanoparticles between graphene sheets. Ceram Int. 2020;46(6):7446-7452.
16. Mohammady M, Mohammadi Y, Yousefi G. Freeze-drying of pharmaceutical and nutraceutical nanoparticles: The effects of formulation and technique parameters on nanoparticles characteristics. J Pharm Sci. 2020;109(11):3235-3247.
17. Zelenková T, Onnainty R, Granero GE, Barresi AA, Fissore D. Use of microreactors and freeze-drying in the manufacturing process of chitosan coated PCL nanoparticles. Eur J Pharm Sci. 2018;119:135-146.
18. Lyu P, Zhang C, Wang Y, Li C, Xiang X, Zhou J, Xu W, Liu X. A simple way of fabricating lyophilized wool nanoparticle powders using neutralization method. Adv Powder Technol. 2020;31(1):87-93.
19. Li J, Ghatak S, El Masry MS, Das A, Liu Y, Roy S, Lee RJ, Sen CK. Topical lyophilized targeted lipid nanoparticles in the restoration of skin barrier function following burn wound. Mol Ther. 2018;26(9):2178-2188.
20. Abdelhaleem Ali AM, Abo El-Enin HA. In-vitro/in-vivo evaluation of Paclitaxel Freeze-Dried micellar nanoparticles intended for buccal delivery. J Drug Deliv Sci Technol. 2021;62:102424.
21. Hassouna F, El Dahab MA, Fulem M, Haiek ADL, Laachachi A, Kopecký D, Šoóš M. Multi-scale analysis of amorphous solid dispersions prepared by freeze drying of ibuprofen loaded acrylic polymer nanoparticles. J Drug Deliv Sci Technol. 2019;53:101182.

22. Semba K, Kadota K, Arima H, Nakanishi A, Tandia M, Uchiyama H, Sugiyama K, Tozuka Y. Improved water dispersibility and photostability in folic acid nanoparticles with transglycosylated naringin using combined processes of wet-milling and freeze-drying. Food Res Int. 2019;121:108-116.
23. Cheng B, Zhuo H, Luo L, Zhu J, Chen S, Zeng X. Synthesis of carbon-coated VBO3 nanoparticles on graphene sheets as anode material for lithium-ion batteries by freeze-drying method. J Alloys Compd. 2019;780:49-54.
24. Piazza RD, Pelizaro TAG, Rodriguez-Chanfrau JE, La Serna AA, Veranes-Pantoja Y, Guastaldi AC. Calcium phosphates nanoparticles: The effect of freeze-drying on particle size reduction. Mater Chem Phys. 2020;239:122004.
25. Veilleux D, Nelea M, Biniecki K, Lavertu M, Buschmann MD. Preparation of Concentrated Chitosan/DNA Nanoparticle Formulations by Lyophilization for Gene Delivery at Clinically Relevant Dosages. J Pharm Sci. 2016;105:88-96.
26. Romeo A, Musumeci T, Carbone C, Bonaccorso A, Corvo S, Lupo G, Anfuso CD, Puglisi G, Pignatello R. Ferulic acid-loaded polymeric nanoparticles for potential ocular delivery. Pharmaceutics. 2021;13:1-19.
27. Telange DR, Sohail NK, Hemke AT, Kharkar PS, Pethe AM. Phospholipid complex-loaded self-assembled phytosomal soft nanoparticles: evidence of enhanced solubility, dissolution rate, ex vivo permeability, oral bioavailability, and antioxidant potential of mangiferin. Drug Deliv Transl Res. 2021;11:1056-83.
28. Chow SF, Wan KY, Cheng KK, Wong KW, Sun CC, Baum L, Chow AH. Development of highly stabilized curcumin nanoparticles by flash nanoprecipitation and lyophilization. Eur J Pharm Biopharm. 2015;94:436-49.

5487847845**1230411**