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PHARMACEUTICAL APPLICATIONS OF LYOPHILIZATION: RECENT UPDATES AND ADVANCEMENTS

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ABSTRACT: For almost five decades, from its application in preserving blood plasma to stabilizing pharmaceuticals and biopharmaceuticals, lyophilization has been used in life sciences. Lyophilization is a popular drying technique for preserving various drugs, formulations and biological materials such as proteins, plasma, and living cells. The researchers have made significant efforts to develop lyophilized nanoparticulate drug delivery formulations. During lyophilization, biological products and vaccines could be increased by removing solvents and the shelf-life of injectables. Thus, lyophilization unstable or heat-sensitive drugs/biologicals can be dried at low temperatures without damaging their physical structure. Lyophilized products can be reconstituted quickly and easily, improving shelf life and easy transportation. Vast applications of lyophilization in the Pharmaceutical Area established the importance of lyophilization techniques in the pharmaceutical field. The present review aims to update recent and advanced applications of Lyophilization techniques in Pharmaceuticals and biological products such as Antibiotics, Vaccines, proteins, enzymes, and hormones.

INTRODUCTION: Long-term stability of formulations plays a crucial role in designing any drug delivery system. Formulators face chemical and physical instability during the formulation and storage of formulations. Drying is one of the popular methods for the preservation of drugs as well as formulation to prevail over these instabilities. Drying is one of the basic unit operations in pharmaceutical industries, permitting the removal of solvents from liquid drug formulations to transit them into solid forms.

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Lyophilization (freeze drying), a water removal method that involves the freezing and drying process, is well established and helps improve the stability of various drugs/formulations [1]. Lyophilization is considered to be the most accepted drying process for manufacturing pharmaceutical products that are thermo labile and unstable in an aqueous medium to provide stability.

Lyophilization offers many advantages over other drying processes, such as temperatures used during this process, the freeze-dried product's shelf life, reconstitution and the aseptic processing operation. Recent years witnessed the popularity of various novel techniques for the formulation and stabilization of various drugs, formulations, and biological materials such as proteins, plasma, and living cells, as well as nano drug delivery carriers

such as liposomes, niosomes, exosomes, nanoparticles [3, 4]. In addition, dry nano-formulations, enzymes and DNA-based formulations could be made via lyophilization, which improves long-term storage and helps avoid costly and tedious cold chain transportation [5, 6, 7]. This is a popular drying technique in developing solid-state protein therapeutics compared to liquid protein formulations because lyophilized proteins can overcome storage stability issues and are more convenient for transportation and delivery [8, 9]. Over the last ten years, the use of lyophilization in pharmaceutical and biopharmaceutical industries has escalated at a rate of more than 14 percent annually. Lyophilization is especially useful for parenterals formulations as the stable injectable reconstituted powder can be easily packaged and transferred as a finished drug product. This technique can also be used to generate stable intermediates in the development and manufacturing of pharmaceutical products [10].

Many researchers and pharmaceutical industries worked on developing and stabilizing Injectable, reconstituted powders and nano-delivery products. The various application of lyophilization as depicted in **Fig. 1** and **Table 1.**

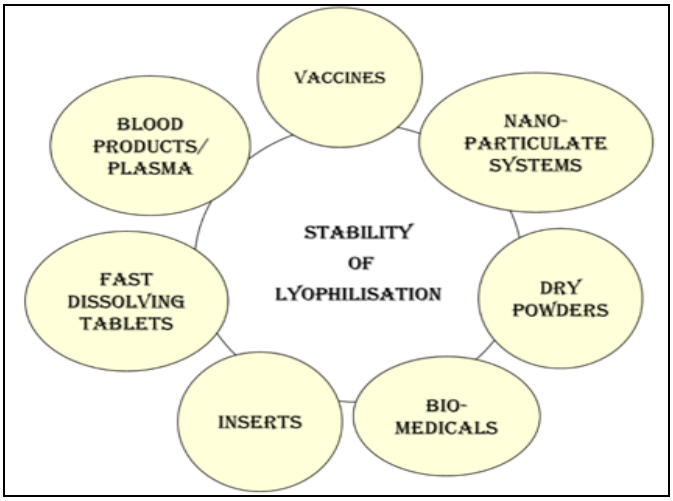

FIG. 1: APPLICATION OF LYOPHILIZATION IN PHARMACEUTICALS

TABLE 1: APPLICATION OF LYOPHILIZATION IN PHARMACEUTICALS

Product name	Category	Disease	Applications
Acetazolamide[11] (US20150061169A1)	Injection	Anti-glaucoma	Increases stability for longer period.
Acetylcystein [12] (CN101239037B)	Injection	Mucolytic & Antidote	Stable after being stored for a long period
Acyclovir[13] (CN101897672A)	Injection	Antiviral	Increases stability,faster dissolution
Amphotericin B[14]	Injection	Antifungal	Increases solubility and stability
Ambisome[15]	Liposome for injection	Fungal infection	Increased shelf life up to 36 months
Artesunate [16] (CN103705475A)	Injection	Antimalarial	Increases solubility and good stability
Azithromycin [17] US7468428B2)	Injection	Antibacterial, Antibiotic	Increases Stability
Bortezomib[18] (CN110314221B)	Injection	Cancer	Improves solubility and stability
Caspofungin[19] (EP 2 922 530 B1)	Injectable powder	Antifungal	Improves long-term stability
Chlorpheniramine[20] (Dave, Vivek et al 2016)	Tablet	Allergic rhinitis	Rapid dissolution
Clarithromycin[21] (CN104586791A)	Tablet	Antibiotic	Improves dissolution and bioavailability
Colistimethate	Injection	Antibiotic	Improves stability at a storage
Decitabine[22] (WO2013093934A1)	Injection	Myelodysplastic Syndrome	Increases stability
Dobutamin[23] (CN104706572A)	Injection	Cardiac	Improves stability for long term
Epirubecin[24] (CN103006586A)	Injectable	Anticancer	Improves patient drug safety and stability
Erythomycin[25] (CN104819622A)	Injection	Antibiotic	Improve stability
Esmoprazole[26] (CN102657650A)	Injection	Anti-Ulcer (Proton Pump Inhibitor)	Improves stability at a storage
FluphenezineDecanoate	Injection	Antipsychotic	Improves stability at storage
Flutamide[27]	Dispersion	Cancer	Increased dissolution rate
Gemcitabine[28] (CN102302462A)	Powder	Antibiotic	Improves stability
Glanciclovir[29] (CN104666303A)	Injection	Antiviral	Efficiency of product is increased
Glyburide[30] (CN104490755A)	Tablet	Hypoglycemic	Increased solubility

		agent	
Haloperidol Decanoate	Injection	Antipsychotic	Increase the stability of formulation
Hydralazin	Injection	Cardiac	Increase the stability of formulation
Itraconazole[31] (CN102499909A)	Nanosphere	Fungal infection	Increase the stability of formulation
Ketamine	Injection	Anaesthetic	Increase the stability of formulation
Ketoprofen[32] (CN1264505C)	Injection	NSAID	Improves solubility and stability
Lansoprazole[33] (CN102302463A)	Injection	Anti-Ulcer (Proton Pump Inhibitor)	Improves solubility of insoluble particles
Lignocaine[34] (CN105663105A)	Injection	Anaesthetic	Improve stability
Lornoxicam[35] (CN101327193A)	Injection	NSAID	Great improvement in stability
Midazolam	Injection	Anaesthetic	
Olanzapine[36] (EP1423124B1)	Injection	Antipsychotic	Improves stability
Omeprazole[37] (CN102512380B)	Powder injection	Anti-Ulcer (Proton Pump Inhibitor)	Excellent stability
Pantoptazole[38] (WO2009001163A1)	Injection	Anti-Ulcer (Proton Pump Inhibitor)	Good stabilty
Paclitaxel[39] (CN105055341A)	Dispersion	Cancer	Increase the stability
Pembrolizumab[40]	Injection	IgE Antibodies	Improves proyein stability
Pemetrexed[41] (CN105726492A)	Powder injection	Anti tumor	Good stability, good redissolution
Polymyxin B[42]	Powder	Antibiotic	Increases stability up 12 months
Rabeprazole[43] (CN102552178B)	Injection	Anti-Ulcer (Proton Pump Inhibitor)	Increase solubility and stability
Remdesivir	Powder	Antiviral	Increase the stability of formulation
Resperidone[44]	Nanosuspension	Psychotic disorder	Increased the physical stability
Rifampacin[45] (CN103976959A)	Injection	Anti-Tubercular Antibiotic	Increases stability and shelf life
Rocuronium[46] (CN1864667B)	Injection	Muscle Relaxant	Improves stability at storage
Sodium aescinate[47] (CN102836133A)	Powder injection	Antiinflamatory	Increases stability
Sirolimus[48]	Liposomes	Organ transplant rejection	Increased shelf life
Succinylcholine	Injection	Muscle Relaxant	Increase the stability of formulation
Thiopental	Injection	Anaesthetic	Increase the stability of formulation
Thiotepa[49] (EP0656211A1)	Injection	Anti tumor	Improves stability
Vancomycin[50] (US4885275A)	Injection	Antibiotic	Provides stability and increases solubility
Vecuronium[51] (CN103520121B)	Injection	Muscle Relaxant	Provides good stability
Voriconazole[52] (CN103251565A)	Injection	Antifungal	Improves solubility and stability
Zoledronic Acid[53] (CN102372741B)	Injection	Calcium Regulator	Increases stability

Nanoparticulate System: Nanoparticles have received much attention in the therapeutics field over the last few decades especially in imaging, sensing gene delivery systems, and novel drug delivery. Significant efforts have been made to develop nanoparticulate systems for drug delivery (*e.g.,* polyplexes, vaccines, and liposomes) [54]. This freeze-drying technique is frequently used as a primary strategy for reducing stability risks and increasing the shelf life of liposome-based drugs [55, 56]. Liposomal instability is a major drawback in developing a liposomal formulation for clinical use, and lyophilisation could be the better strategy for increasing liposomal product shelf life. Lyophilized drug product designs (e.g. lyo-product, lyo-cake) facilitate liposomal storage in dry state forms, extending shelf life and reducing cold chain custodial demands [57, 58]. Mohammady M. *et al*; elucidated the importance of various parameters involved in freeze-drying for the most common pharmaceutical NPs which include nanosuspensions, nanocrystals (NCs), cocrystals/ nanococrystals, nanoemulsions (NEs), nanocapsules (NCPs) and nanospheres (NSPs). They concluded that lyophilization could be used for developing different types of NPs like nanotubes, nanofibers, and nanoaggregates on an industrial scale [59] Rouquette, M. *et al.*; developed Squalene-adenosine (SQAd) NPs. Due to various long term stability issues these nanoparticles were lyophilised and the Long-term stability was found to be better for the prepared Nanoparticles [60]. Fonte

P *et al.* evaluated the influence of a freeze-drying process using different cryoprotectants on the structure of insulin loaded into poly (lactic-co-glycolic acid) nanoparticles and assessed the stability of these nanoparticles. They observed a marked improvement in the structural stability of insulin after the freeze-drying process [61]. Charoenviriyakul C. *et al.,* developed exosomes using lyophilization as preservation of exosomes is stable at -80 °C. The researchers concluded lyophilization to be an effective method for storing exosomes [62]. Xie C. *et al.*; used a polymerization-induced aramid nanofiber as a building block which helped modify the freeze-drying method for the preparation of *para*-aromatic-amide aerogels [63].

Luo, W. C. *et al;* evaluated the effects of freeze drying on solid lipid nanoparticles (SLNs), polymeric nanoparticles (PNs), and liposomes. They concluded that freeze drying increased the stability and quality of freeze-dried nanoparticles [64] Trenkenschuh, E., *et al.;* reviewed the stresses that occurred during the freezing and the drying step of lyophilization of polymeric, or vesicular Nanoparticles, and they formed NP lyophilizes which showed excellent colloidal stability [65]. Gandhi, N. V. *et al.;* developed a nanoparticulate (nanocrystals-loaded) or dispersible tablet with improved solubility and bioavailability. This study concluded that the prepared Nano sized Nitrendipime increased solubility and bioavailability [66].

Khattab, W. M. *et al.;* demonstrated the enhancement in oral bioavailability of a poor water-soluble antihypertensive drug Olmesartan Medoxomil (OM), due to the formulation of lyophilized oily-core nanocapsules. A comparative study of pharmacokinetics in rats was performed for oily-core polymeric nanocapsules after formulation, and the lyophilization tablet showed a significant improvement in oral absorption of OM. This concluded that the formulation of lyophilized ONC for OM had significantly enhanced oral bioavailability, therapeutic efficacy, and patient compliance [67]. Mohammed, A. R. *et al. investigated various liposomal vaccine techniques so* that they can be prepared in a freeze-dried state. They concluded the improvement in the long-term stability of liposomal vaccines by formulating freeze-dried products [68]. Wang, Y. *et al.;* reviewed an overview of liposome formulation-specific lyophilization approaches for parenteral use. They concluded that lyophilization methods preserve liposome formulation stability and product shelf-life [69]. Howard, M. D. *et al.*; optimized a lyophilization process for solid–lipid nanoparticles (SLNs) loaded with dexamethasone palmitate (Dex-P). They also compared the long-term stability of lyophilized SLNs and aqueous SLN suspensions at two different storage conditions. The results concluded that the lyophilized SLNs stored at 4°C exhibited the greatest stability, with no change in the particle size [70].

Muramatsu, H. *et al*; demonstrated that nucleoside-modified mRNA- LNPs could be lyophilized, with no significant change in properties for 12 weeks after storage at room temperature and at least 24 weeks after storage at 4°C. This study concluded a potential solution to overcome the long-term storage-related limitations of nucleoside-modified mRNA-LNP vaccines by lyophilization [71]. Hamaly, M. A., *et al.;* evaluated the colloidal stability of gold nanorods (GNRs) conjugated with rituximab as a model monoclonal antibody upon freeze-drying in the presence of various cryoprotectants (mannitol, trehalose, and sucrose. The researchers also concluded that Lyophilized Rituximab conjugated GNRs preserve typical lymphoma tissue binding [72].

Wong, C. Y. *et al.,* fabricated nanoparticles containing cationic chitosan and anionic Dz13Scr (Dz13 is a polyanionic oligonucleotide (DNAzyme)) using complex coacervation and lyophilized to preserve the bioactivity of entrapped insulin and they concluded that the stability of drug delivery system against enzymes was improved by entrapment of insulin within lyophilized nanoparticles [73]. Pena-Rodríguez, E. *et al.*; developed a Dexamethasone-loaded polymer hybrid Nanoparticles to treat alopecia areata. The lyophilized nanoparticles showed better resuspension and acceptable physicochemical parameters [74] Wang *et al.* reviewed about design strategies of lyophilized liposome-based parenteral drug development. Lipid nanoparticle (LNP)-formulated nucleoside-modified mRNA vaccines have proven to be extremely effective in combating the coronavirus disease 2019 (COVID-19) pandemic due to their safety. However, long-term

storage of mRNA-LNP vaccines without freezing remains a challenge. The study showed that nucleoside-modified mRNA-LNPs could be lyophilized and that the physicochemical properties of the lyophilized material do not change significantly after 12 weeks of storage at room temperature and at least 24 weeks of storage at 4°C. These mRNA-LNP vaccines were observed for 12 weeks of room temperature storage or at least 24 weeks after storage at 4°C. This finding suggested a potential solution to the long-term storage limitations of nucleoside-modified mRNA-LNP vaccine and can be transported easily [75].

Vaccines: Vaccination for disease prevention was first proposed in the late 1800s, and vaccines against tuberculosis, yellow fever, and influenza had been developed by the early 1900s. Vaccine liquid formulations are susceptible to instabilities caused by various physical and chemical degradation processes. Using the Lyophilisation method, various degradation pathways can be avoided or prevented in dried formulations.[76] Recently; vaccines are mostly developed as lyophilized or aqueous formulations; due to necessitates storage at sub-ambient temperatures and the inherent lack of vaccine thermostability to overcome these storage requirements in the developing world can be difficult; therefore, vaccines are frequently exposed to temperatures that cause vaccine efficacy losses.

Preston, K. B. *et al.,* described the development and characterization of monovalent and trivalent filovirus vaccines with a squalanein-water emulsion adjuvant. After lyophilization and reconstitution, they found that the adjuvant particle diameter and zeta potential were preserved in the single-vial presentation. Further, they concluded that these findings promote the development of a single-vial trivalent filovirus vaccine, which would make vaccine distribution and administration in resource-constrained places easier [77]. Preston, K. B. *et al.,* reviewed variously lyophilized and spray-dried vaccines for various pathogens, as well as some of the assays, were used to quantify their stability.. Recent trends include needle-free dry powder delivery *via* nonparenteral administration methods and the introduction of improved vaccine adjuvants into formulations. They concluded that lyophilization and spray drying are the most common methods of stabilizing vaccines through drying [78]. Ameri, M. *et al.;* Formulated trivalent influenza split vaccine at high concentration and it was coated on the transdermal microneedle system. In this study, they used three influenza strains, out of which two influenza A strains and one influenza B strain which was then diafiltrated, concentrated and lyophilized as a monovalent vaccine. This study revealed that the transdermal microneedle technology is an appealing alternative for influenza vaccine delivery, with major benefits such as preservative-free storage and storage at room temperature [79].

Hammerling, M. J. *et al.*; studied and compared lab-developed and commercial SARS-CoV-2 diagnostic RT qPCR mixes for the ability to be lyophilized and thus stabilized against high temperatures. This comparison concluded that the commercial mix had maintained activity and sensitivity after storage for at least 30 days at ambient temperature after lyophilization [80].

Dry Powders: Pulmonary delivery is promising for treating many lung diseases, including Cystic fibrosis because it allows for noninvasive and direct drug deposition in the lungs. Lyophilisation was used to convert them to dry powders in order to improve the stability of nasal delivery [81]. Wanning *et al.*; outline the development of flowable lyophilized powders and conclude that lyophilized powder with small particle size showed significant particle size distribution for needle-free injection or nasal delivery of protein and peptides [82]. Zhang *et al*; expressed the development of PEGylated chitosan/CRISPR-Cas9 dry powders for pulmonary delivery through thin-film freeze drying and concluded that TFFD processing of CRISPR-Cas9 polymer nanocomplexes showed higher transfection efficiency and improved the aerodynamic performance of dry powder formulations. This technique showed the encouraging way to prepare polymer-based nucleic acid nanocomplex dry powder [81]. Pozzoli, M. *et al.;* developed an amorphous solid dispersions/solution (ASD) of a poorly soluble drug, budesonide (BUD), with a novel polymer Soluplus® (BASF, Germany) using a freeze-drying technique to improve dissolution and absorption through the nasal route [83]. Liu, D *et al.;* performed formulation screening and freeze-drying process optimization to

produce ginkgolide B (GB) lyophilized powder for injection with outstanding appearance and consistent quality. And they concluded that GB lyophilized powder for injection was prepared with improved solubility more than 18 times [84].

Li, Z. *et al.*; studied the effective strategies for the drug development of α9α10 Nicotinic Acetylcholine Receptor Antagonist α-Conotoxin GeXIVA. According to this study, drug development ofα9α10 Nicotinic Acetylcholine Receptor Antagonist α-ConotoxinGeXIVA for clinical use has been limited due to its instability. To overcome this instability, the drug was lyophilized, which improved the stability of the [85]. Liawrungrueang, W. *et al.;* aimed to evaluate the elution characteristics of gentamicin-impregnated PMMA made with lyophilized liquid gentamicin, which was compared with PMMA; which was made from commercial gentamicin powder. This study concluded that the Gentamicin-impregnated PMMA made with lyophilized liquid gentamicin showed a higher rate of antibiotic elution in preliminary in vitro studies when compared with PMMA made with premixed gentamicin powder [86].

Solid Dosage Form: Orally disintegrating tablets, due to their added advantages, gained popularity amongst other solid dosage forms [87]. Liu, T. *et al.;* formulated Meloxicam nano suspensions which were prepared using three different methods: high-pressure homogenization, wet bead milling, and a combination strategy of freeze-drying and high-pressure homogenization. According to this study, Freeze-dried meloxicam powder has highly improved the size reduction efficiency compared to the unmodified drug, and the particle size of the freeze-dried sample was reduced to 342 nm after only one homogenization cycle at 1000 bar. This study concluded that the tablets made using homogenizer nanosuspensions and a combination method disintegrated faster in the first 20 minutes than bead milling nanocrystal tablets [88]. Alami-Milani, M. *et al.;* prepared and optimized a fast disintegrating tablet of isosorbide dinitrate using lyophilization. The results demonstrated a faster disintegration rate of the lyophilized preparation [87]. Vanbillemont, B. *et al*; Prepared and compared Four ODTs with diverse properties using lyophilisation technique. They concluded that Orally disintegrating tablets (ODTs) produced by lyophilization have a unique porous structure which leads to a favorable orodispersable functionality. They also possess ultra-fast disintegration kinetics, with acceptable mechanical strength, and also give a smooth mouth texture [89].

Lal, M. *et al.* prepared fast-dissolving tablets (FDTs) using the freeze-drying in blister method and confirmed the stability of the formulations [90]. Zhu, C. *et al.*; Reviewed that postpartum hemorrhage is a leading cause of maternal mortality and morbidity in various underdeveloped nations. They recommended treatment that includes oxytocin delivery; however, because oxytocin is a heat-labile protein, it must be administered as an intramuscular injection by trained medical personnel. So, they created a freeze-dried oxytocin fast-dissolving tablet (FDT) for needle-free sublingual (SL) delivery to solve these issues [91]. Rautiola, D. *et al.*; demonstrated the use of avizafone (AVF), a prodrug for diazepam, as a stabilizer to reduce APB inactivation during lyophilisation. Lyophilization of the APB+AVF+trehalose formulation was subjected to a 6-month accelerated stability study, with negligible activity reduction observed at the end. They concluded that lyophilisation with substrate and trehalose provides a greater stabilizing effect [92].

Gene Therapy: Gene therapy is a method of treating disease that involves delivering gene coding or editing material. Lyophilization has been used in gene therapy to facilitate refrigerated storage of biologics that are not stable in liquid form [93]. Zhang, Y. Z. *et al.;* created a lyophilized (freeze-dried) Adeno-associated viruses (AAV) formulation and concluded that the lyophilized formulation prepared was stable for 24 months at 2 to 8°C, which showed that a dried formulation for AAV gene therapy is feasible after lyophilization [93]. Mohammed saeid *et al.;* suggested the *in-vitro* transfection of gemini surfactant-lipoplexes and the influence of lyophilization on critical physiochemical properties and also appraised the viability of lyophilization as a method for producing long-lasting lipoplexes. Furthermore, they concluded that when compared to liquid formulations, gemini surfactant-based lipoplexes were much more physically stable after lyophilization [94].

Kasper *et al.*; recommended lyophilization of gene carrier systems for long-term storage stability and concluded that the lyophilized products showed higher transfection efficiency and long-termed physical stability and improved shelf life of products [95].

Del Pozo-Rodriguez *et al.*; outline the influence of lyophilization on the morphological properties and transfection capacity of solid lipid nanoparticles (LyoSLN) and SLN-DNA vectors (Lyo(SLN-DNA)). The Researchers concluded that lyophilization can generate physically stable dried SLNs [96]. Wang W. studied that developing recombinant protein pharmaceuticals proved to be very challenging because of both the protein production and purification complexity and the limited physical and chemical stability of proteins. To overcome the instability barrier, proteins often have to be made into solid forms to achieve an acceptable shelf life as pharmaceutical products. The most commonly used method for preparing solid protein pharmaceuticals is lyophilization (freeze-drying). This concluded that lyophilization has efficient and minimal adverse effects on protein stability [97].

Inserts, Needles and Microneedles: Abdelmonem, R. *et al.*; formulated a novel Bioadhesive granisetron hydrochloride (GH) spanlasticin gels and inserts. They lyophilized the prepared inserts for intranasal delivery, thereby increasing GH bioavailability and brain targeting for the prepared bioadhesive [98]. Sabri, A. *et al.;* presented the development of a composite pharmaceutical system that was composed of hydrogel-forming microneedles (MNs) in tandem with CFZ dry reservoirs. They created two distinct CFZ-loaded dry reservoirs, which were then evaluated based on directly compressed tablets (DCT) and lyophilized (LYO) wafers. They concluded that the dry reservoir systems showed fast dissolution, dissolving in phosphate buffer saline pH 7.4 in less than one minute [99].

CONCLUSION: This review concluded that lyophilization in the pharmaceutical formulation had gained major advantages for improving the stability, storage, and making transportation easy for Pharmaceuticals. The Lyophilization technique can generate physically stable dried pharmaceutical formulations, including solid lipid nanocarriers, parenteral, vaccines, and many more. This technique could also improve the dissolution of reconstituted productalong by efficiently processing a liquid formulation.

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