Excipients used in lyophilization of small molecules

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ABSTRACT

This review deals with the excipients used in various lyophilized formulations of small molecules. The role of excipients such as bulking agents, buffering agents, tonicity modifiers, antimicrobial agents, surfactants and co-solvents has been discussed. Additionally, a decision making process for their incorporation into the formulation matrix has been proposed. A list of ingredients used in lyophilized formulations marketed in USA has been created based on a survey of the Physician Desk Reference (PDR) and the Handbook on Injectable Drugs. Information on the recommended quantities of various excipients has also been provided, based on the details given in the Inactive Ingredient Guide (IIG).

KEY WORDS: Lyophilization, excipients, bulking agent, small molecule, primary drying

INTRODUCTION

Lyophilization, or freeze drying, is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying). In this process, the moisture content of the product is reduced to such a low level that does not support biological growth or chemical reactions. The technique, therefore, finds special use in formulation development of drugs which are thermolabile and/or unstable in aqueous medium (1-3).

Lyophilization is based on the principle of sublimation of ice, without entering the liquid phase. The phase diagram of water (Figure 1) show that two phases coexist along a line under the given conditions of temperature and pressure, while at the triple point (0.0075 C at 0.61kPa or 610 Nm^{-2}; 0.01 C at 0.00603 atm), all three phases coexist. Lyophilization is performed at temperature and pressure

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conditions below the triple point, to enable sublimation of ice. The entire process is performed at low temperature and pressure, hence is suited for drying of thermolabile compounds.

Steps involved in lyophilization start from sample preparation followed by freezing, primary drying and secondary drying, to obtain the final dried product with desired moisture content (Figure 2). The concentration gradient of water vapor between the drying front and condenser is the driving force for removal of water during lyophilization. The vapor pressure of water increases with an increase in temperature during the primary drying. Therefore, primary drying temperature should be kept as high as possible, but below the critical process temperature, to avoid a loss of cake structure (4-6). This critical process temperature is the collapse temperature for amorphous substance, or eutectic melt for the crystalline substance (1, 7, 8).

During freezing, ice crystals start separating out until the solution becomes maximally concentrated. On further cooling, phase separation of the solute and ice takes place.

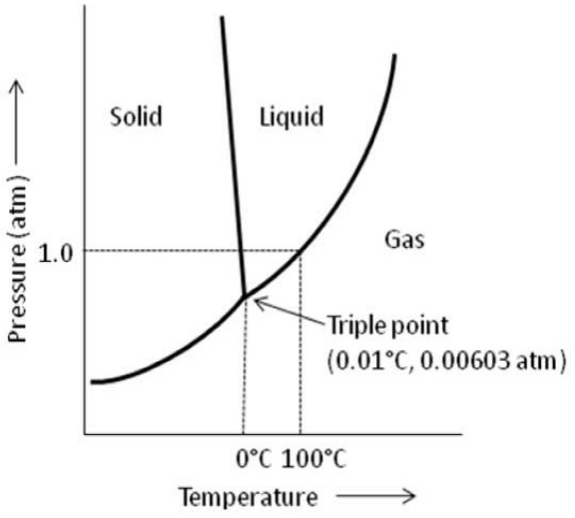


Figure 1 Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm. Lyophilization is carried out below the triple point to enable conversion of ice into vapor, without entering the liquid phase (known as sublimation).

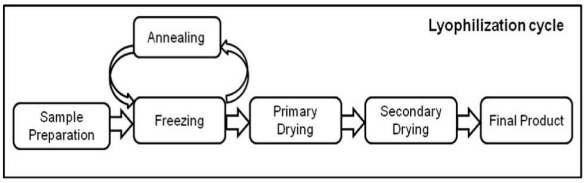


Figure 2. Steps involved in lyophilization from sample preparation to final product formation. Annealing is an optional step, occasionally used to crystallize the formulation component(s).

If the solute separates out in crystalline form, it is known as the eutectic temperature. In contrast, if an amorphous form is formed, the temperature is referred to as the glass transition temperature (T_g').

Determination of this critical temperature is important for development of an optimized lyophilization cycle. During primary drying, drying temperature should not exceed the critical temperature, which otherwise leads to 'meltback' or 'collapse' phenomenon in case of crystalline or amorphous substance respectively (Figure 3).

In the majority of lyophilized formulations, excipients are included to improve the functional properties and stability of the

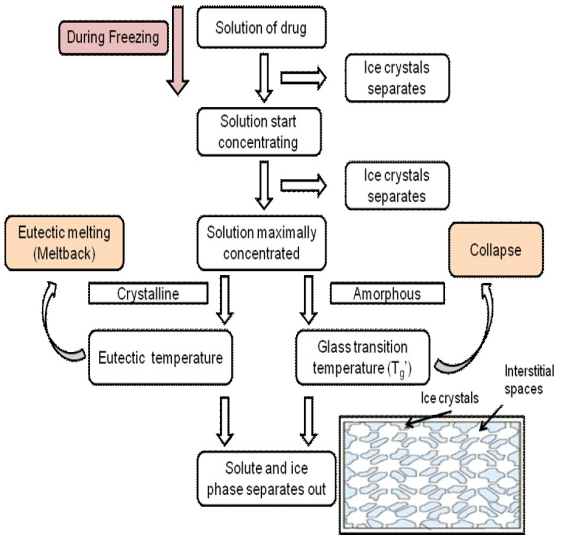


Figure 3 Flowchart showing the concept of eutectic temperature and T_g, and their importance during primary drying

lyophilized product. The International Pharmaceutical Excipients Council has defined excipients as: "…*substances other than the pharmacologically active drug or prodrug which are included in the manufacturing process or are contained in a finished pharmaceutical product dosage form"* (9). Excipients also provide an aesthetic appeal to the product in terms of good cake structure. Inclusion of excipients also helps in developing a robust and economical lyophilization process.

Neema *et al.* listed the excipients and frequency of their usage in marketed injectable formulations (10). Polwell *et al.* tabulated all the excipients used in parenteral formulation with reference to individual products (11). Strickley compiled the parenteral formulation of small molecules marketed in the United States (12-14). However, to our knowledge, a comprehensive analysis of the excipients used in lyophilization of small molecules does not exist in the literature, until now. This review therefore focuses specifically on the issues related to excipient selection in lyophilized formulations of small molecules. Proteins and peptides have not been included in the scope of this article. A comprehensive list of excipients used in lyophilized formulations of small molecules marketed in USA has been compiled from the Physician Desk Reference and Handbook on Injectable Drugs. Finally, the regulatory status of these excipients with respect to limits mentioned in IIG has been compiled.

Table 1 lists the excipients used in marketed lyophilized preparations, highlighting the frequency of their use in lyophilized formulations (Figure 4). About 67% of the lyophilized marketed preparations of small molecules contain excipient(s) in their formulation.

CLASSIFICATION OF EXCIPIENTS

The excipients commonly used in lyophilization of small molecules have been classified in Figure 5.

CRITERIA FOR SELECTION OF EXCIPIENTS

Selection of excipients in a lyophilized formulation employs a need based approach, to develop a simple, stable and elegant formulation, with an economical process. Figure 6 depicts a flow chart for selection of different excipients for lyophilization of small molecules.

EXCIPIENTS FOR LYOPHILIZATION OF SMALL MOLECULES

Bulking agent

Bulking agents, as the name implies, form the bulk of the lyophilized product and provide an adequate structure to the cake. These are generally used for low dose (high potency) drugs that *per se* do not have the necessary bulk to support their own structure. These are particularly more important when the total solid content is less than 2% (17). In such cases, a bulking agent is added to the formulation matrix (18, 19). The structure of the lyophilized

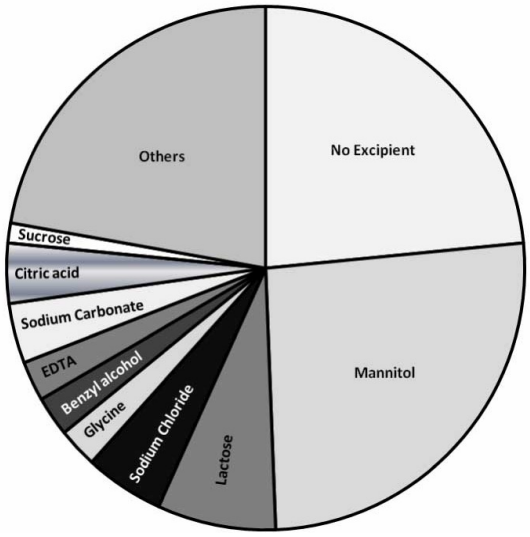


Figure 4 Distribution of commonly used excipients in marketed lyophilized formulations of small molecules. About 67% of marketed lyophilized formulations of small molecules contain excipients.

Table 1 List of excipients used in lyophilized formulation of small molecules, as marketed in USA (15, 16)

Drug	Category	Excipients	Route of administration	Marketed name
Amifostine	Cytoprotective agent	-	IV infusion over 15-30 min	Ethyol® (MedImmune Oncology)
Amphotericin B cholesteryl sulfate	Antifungal	Sodium cholesteryl sulfate Lactose Tris EDTA	IV infusion at 3-4 mg/kg/hr	Amphotec® (Sequus Pharmaceuticals)
Amphotericin B	Antifunal	Hydrogenated soyaphosphatidylcholine Disteroylphosphatidly glycerol Cholesterol Alpha tocopherol Sucrose Disodium succinate	IV infusion at 3-5 mg/kg/hr	Ambisome® (Astellas)
Acyclovir sodium	Antiviral	-	IV infusion over 1 hr	Zovirax® (Glaxo Wellcome)
Allopurinol sodium	Anti-gout	-	IV infusion	Aloprim® (Nabi Biopharmaceuticals)
Alprostadil	Erectile dysfunction	α-cyclodextrin Lactose	Intracavernosal	Edex® (Schwarz Pharma)
Alprostadil	Erectile dysfunction	Lactose Sodium citrate Benzyl alcohol	Intracavernosal	Caverject® (Phamacia and Upjohn)
Azathioprine sodium	Immunosuppressive antimetabolite; management of severe rheumatoid arthritis	-	IV bolus, IV infusion	Imuran ® (Glaxo Wellcome)
Azithromycin	Antibiotic	Citric acid	IV infusion	Zithromax® (Pfizer)
Aztreonam	Antibiotic	L- arginine	IM, IV bolus, IV infusion	Azactam ® (Bristol Myers Squibb)
Carmustine	Antineoplastic	-	IV infusion	BiCNU® (Bristol Myers Squibb)
Cefazolin sodium	Antibiotic	-	IM, IV bolus, IV infusion	Kefzol® (Lilly)
Cefazolin sodium	Antibiotic	-	IM, IV bolus, IV infusion	Ancef ® (GlaxoSmith-Kline)
Chlorothiazide sodium	Diuretic and hypertensive	Mannitol Thiomersol	IV bolus, IV infusion	Diuril® (Merck)
Cisplatin	Antineoplastic	Mannitol Sodium chloride	IV infusion	Platinol® (Bristol Myers Oncology)
Colfosceril palmitrate	Prevention and treatment of respiratorydisease syndrome in low birth weight infants	Cetyl alcohol Tyloxapol Sodium chloride	Intratracheal	Exosurf neonatal® (Glaxo Wellcome)
Cyclophosphamide	Antineoplastic	Mannitol	IM, IV bolus, IV infusion, IP, Intrapleural	Cytoxan® (Bristol Myers Squibb)
Dactinomycin	Antibiotic	Mannitol	IV bolus, IV infusion	Cosmegen® (Merck)
Dantrolene sodium	Muscle relaxant	Mannitol	IV bolus, IV infusion over 1 hr	Dantrium® (Procter & Gamble)
Daunorubicin HCl	Antibiotic	Mannitol	IV infusion	Cerubidine® (Bedford)
Dexrazoxane	Cardioprotective agent	-	IV	Zinecard® (Pharmacia & Upjohn)
Diltiazem	Antianginal	Mannitol	IV bolus, IV infusion	Cardizem® (Hoechst Marion Roussel)
Doxorubicin HCl	Antineoplastic	Lactose Methyl paraben	IV	Rubex® (Bristol Myers Squibb)
Etoposide phosphate	Antineoplastic	Sodium citrate Dextran 40	IV infusion over 30-60 min	Etopophos® (Bristol Myers Squibb)
Epoprostenol sodium	Antihypertensive	Mannitol Sodium chloride Glycine	IV infusion	Flolan® (Glaxo Wellcome)
Ethacrynate sodium	Diuretic	Mannitol	Slow IV bolus, IV infusion	Sodium edecrin® (Merck)
Fludarabine phosphate	Antineoplastic	Mannitol	IV infusion over 30 min	Fludara® (Berlex)
Ganciclovir sodium	Treatment of CMV retinitis in immunocompromized patient	-	IV infusion at 5mg/kg over 1 hr	Cytovene® (Roche)

Table 1 List of excipients used in lyophilized formulation of small molecules, as marketed in USA (15, 16)

Drug	Category	Excipients	Route of administration	Marketed name
Gemcitabine HCl	Antineoplastic	Mannitol Sodium acetate	IV infusion over 30 min	Genzer® (Lilly)
Hemin	Treatment of acute intermittent porphyria related to mensuration	Sorbitol Sodium carbonate	IV infusion	Panhematin® (Abbott)
Hydromorphone HCl	Opioid analgesic	-	IV, IM, SC	Dilaudid-HP® (Abbott)
Indomethacin sodium	NSAID	-	IV bolus	Indocin I.V. ® (Merck)
Lansoprazole	Proton pump inhibitor	Mannitol Meglumine Sodium hydroxide	IV	Prevacid® (TAP)
Levothyroxine sodium	Hormone relacement	Mannitol Sodium phosphate tribasic	IM, IV	Synthrod® (Knoll)
Melphalan HCl	Antineoplastic	Povidone Diluent: Water, propylene glycol, ethyl alcohol, sodium citrate	IV infusion over 15-20 min	Alkeran® (Celgene)
Methohexital sodium	Anesthetic	Anhydrous sodium carbonate	IV, IM	Brevital sodium® (KING)
Methyl prednisolone succinate sodium	Hormone replacement	Sodium phosphate Lactose Benzyl alcohol	IM, IV bolus, IV infusion	Solu-Medrol® (Pfizer)
Metronidazole	Antibacterial	Mannitol	IV bolus, IV infusion	Flagyl® (Pfizer)
Mitomycin	Antineoplastic	Lactose	IV infusion	Mutramycin® (Bristol Myers Squibb)
Pamidronate disodium	Inhibition of bone resorption	Mannitol	IV	Aredia® (Novartis)
Pentostatin	Antineoplastic	Mannitol	Slow IV bolus, IV infusion	Nipent® (Supergen)
Phentolamine mesylate	Antihypertensive	Mannnitol	IM, IV bolus, IV infusion	Regitine® (Novartis)
Pipecuronium bromide	Long acting neuromuscular blocking agent	-	IV bolus	Arduran® (Oryannon)
Pralidoxime chloride	Antidote for overdose due to anticholinesterase	-	IV bolus, IV infusion	Protopam® (Baxter Healthcare)
Remifentanil HCl	Analgesic	Glycine	IV infusion	Ultiva® (GlaxoWellcome)
Streptozocin	Antineoplastic	Citric acid	IV bolus, IV infusion	Zanosar® (Pharmacia & Upjohn)
Tazobactam sodium and Piperacillin sodium	Antibacterial combination	EDTA Sodium citrate	IV infusion	Zosyn® (Lederle)
Thipental sodium	Short acting anesthetic	Sodium carbonate	IV infusion	Pentothal sodium® (Baxter)
Thiotepa	Antineoplastic	-	IV bolus, Intracavitary, Intravesical	Thioplex® (Immunex)
Thiothixene HCl	Antipsychotic	Mannitol	IM	Navane® (Pfizer)
Ticarcillin disodium	Antibacterial	-	IM, IV bolus, IV infusion	Ticar® (Smith Kline Beecham)
Tigecycline	Antibacterial	-	IV infusion	Tygacil® (Wyeth)
Topotecan	Antineoplastic	Mannitol Tartaric acid	IV infusion	Hycamtin® (Smith Kline Beecham)
Trimetrexate glucuronate	Treatment of pneumonia	-	IV infusion	Neutrexin® (U.S. Biosciences)
Vancomycin HCl	Antibiotic	-	IV infusion	Vancocin HCl® (Lilly)
Vecuronium bromide	Muscle relaxant	Mannitol Citric acid Sodium phosphate dibasic	IV bolus, IV infusion	Norcuron® (Organon)
Vinblastine sulfate	Antineoplastic	-	IV bolus	Velban® (Lilly)
Warfarin sodium	Anticoagulant	Mannitol Sodium chloride Sodium phosphate,monobasic, monohydrate Sodium phosphate, dibasic, heptahydrate	Slow IV over 2 min	Coumandin® (Bristol Myers Squibb)

HCl – hydrochloric acid; **i.v.** – intravenous; **i.m**. – intramuscular; **s.c.** – subcutaneous; **PDR-** Physicians Desk Reference; **EDTA** – ethylenediaminetetraacetic acid

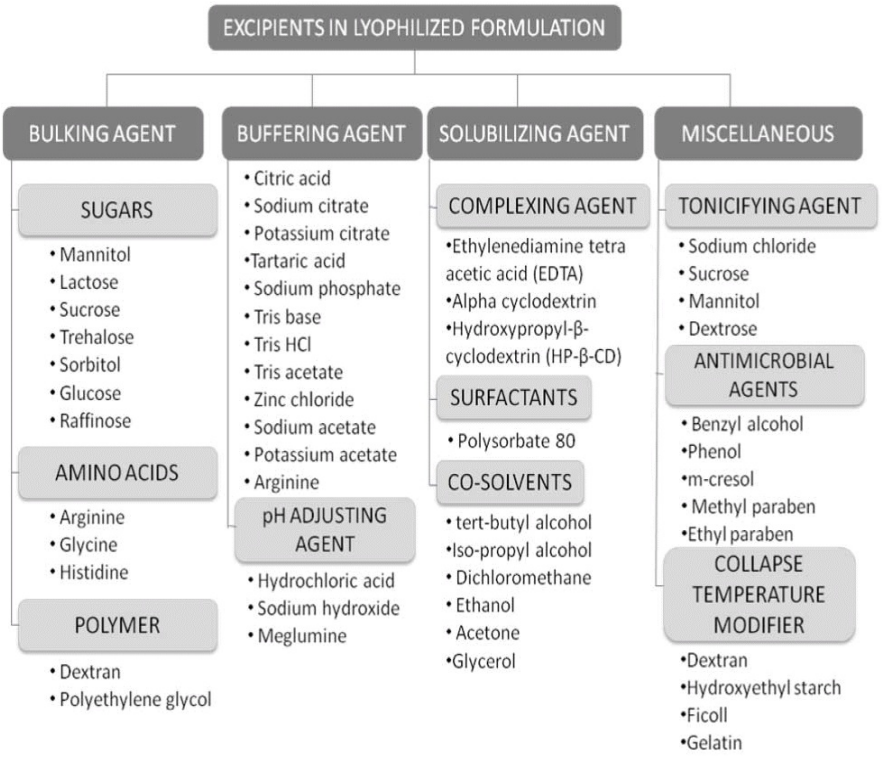


Figure 5 Classification of commonly used excipients used in lyophilization of small molecules. A need based approach is utilized to select the appropriate excipient(s) for lyophilization.

cake is important, since proper cake formation leads to proper pore formation that provides the means for vapor to escape from the product during the drying cycle. Loss of product structure blocks the path for vapor removal leading to an increased resistance in moisture removal and thus higher moisture content in the final product (Figure 7). Such localized high moisture content may lead to degradation of the active pharmaceutical ingredient during the shelf life. Kovalcik and Guillory demonstrated the need of a bulking agent for cyclophosphamide. They also showed that the moisture containing cake showed better stability with mannitol, than with lactose as bulking agent (20). Mannitol and glycine, are the most commonly used bulking agents, followed by glucose, sucrose, lactose, trehalose and dextran (21).

The bulking agent may appear as crystalline and/or amorphous solid at the end of lyophilization process. Crystallization of the bulking agent, however, might adversely affect the physical stability of the product in certain instances, for which, an amorphous bulking agent would be preferred. Herman *et al.* studied the rate of hydrolysis of methylprednisolone sodium succinate in the presence of mannitol and lactose as the bulking agents, and observed that formulation with mannitol showed a faster degradation in comparison to that with lactose. It was hypothesized to be due to the crystallization of mannitol during lyophilization, unlike lactose, which remained in the amorphous state (22). The non-hygroscopic nature of crystalline mannitol, led to an increase in the amount of water available with the drug. In contrast, the hygroscopic amorphous lactose held water

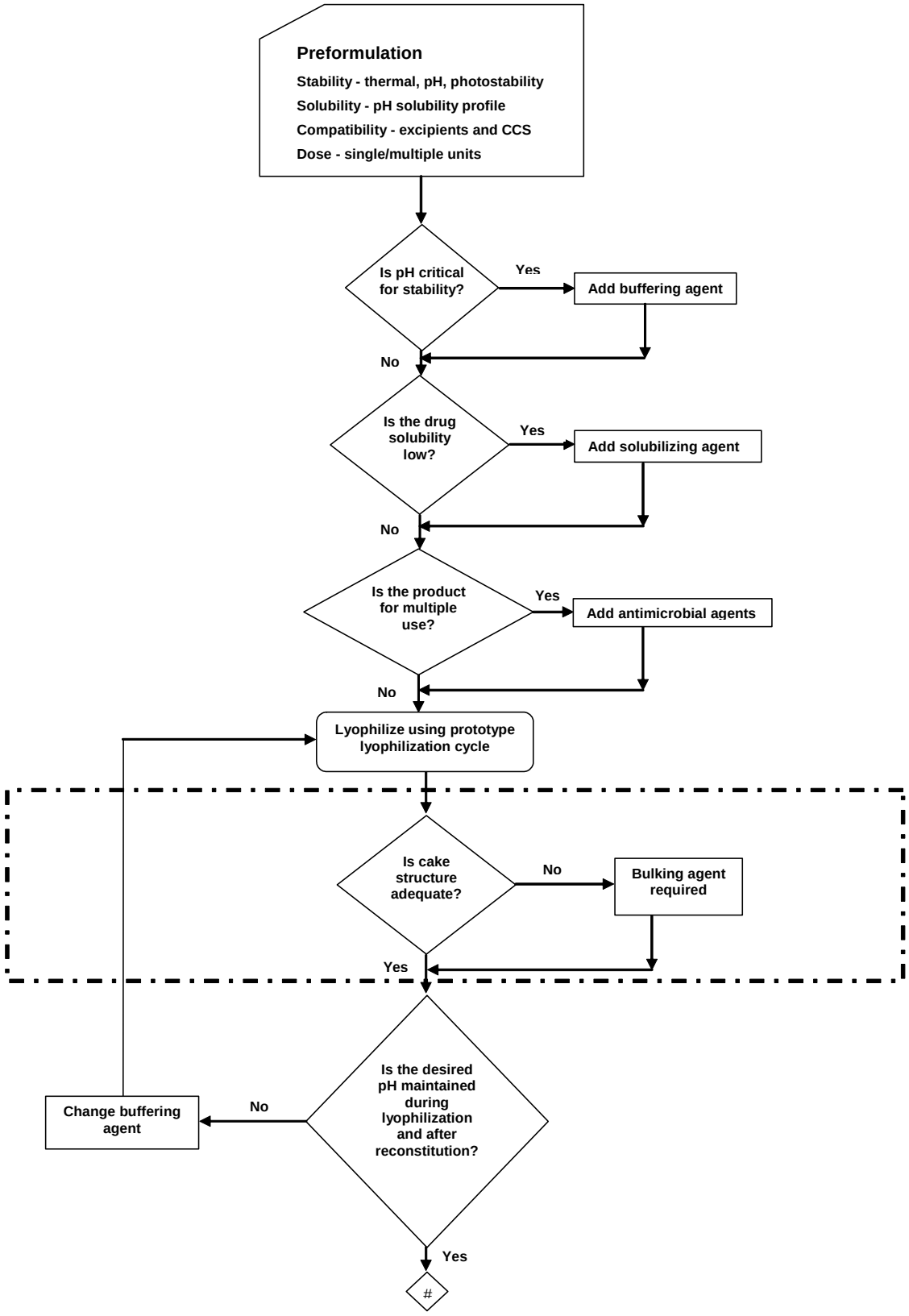
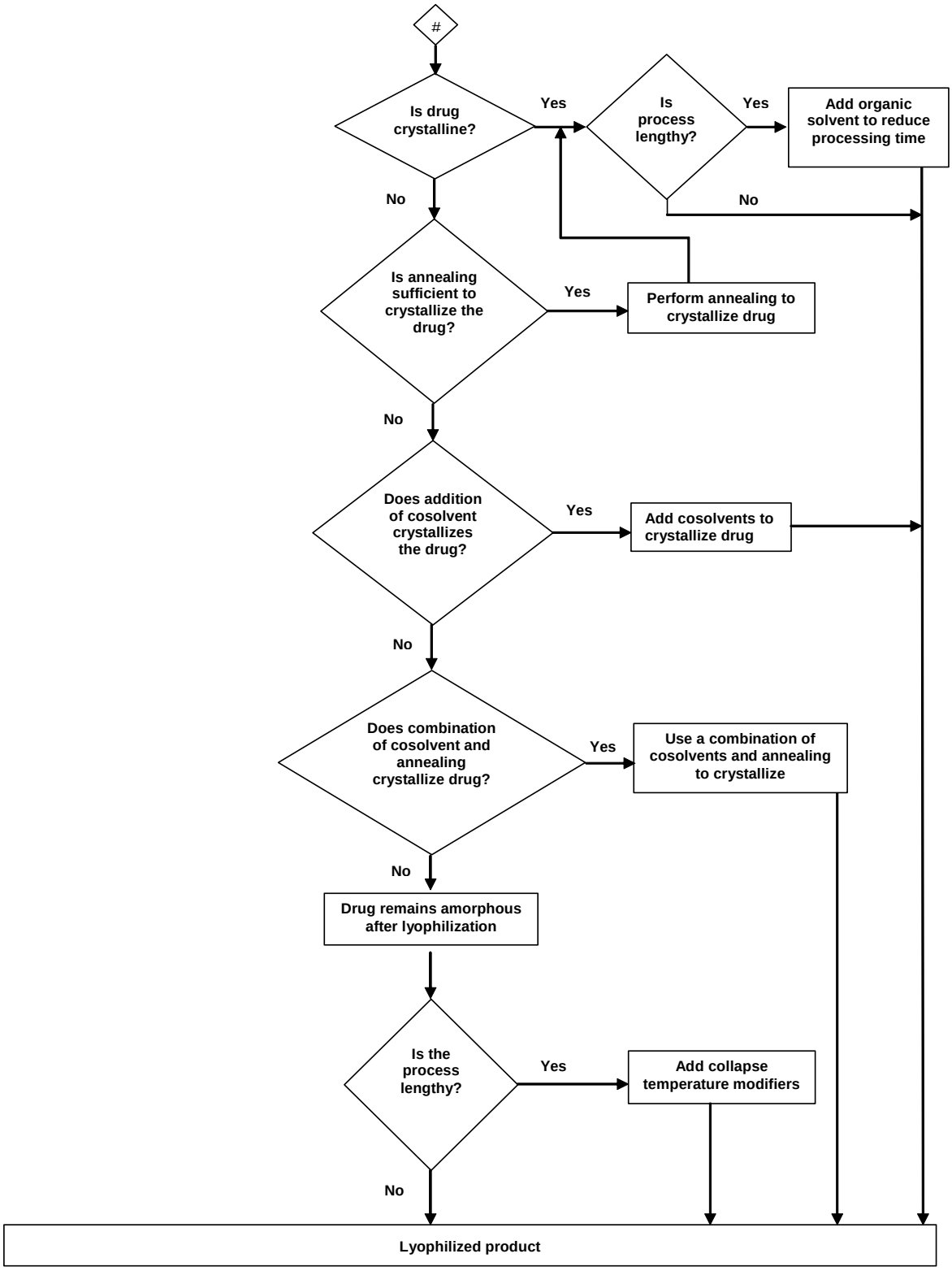


Figure 6 Decision tree for selection of excipients for lyophilization of small molecules (continued next page)



CCS – Container closure system

Figure 6 Continued from previous page

molecules, leading to a relative decrease in the amount of water available in the micro-environment of drug.

The bulking agent may also crystallize in different polymorphic forms during lyophilization, which could also have serious implications on physical stability of drug product. Liao *et al.* reported that mannitol crystallizes as a mixture of δ-mannitol and mannitol hemihydrate during annealing. Release of water from hemihydrate form during shipping and storage conditions might cause degradation of moisture sensitive drug product (23).

The nature of lyophilized cake also depends on the ratio of drug and bulking agent, showing an increased crystallization with an increase in amount of bulking agent. Korey and Schwartz studied the lyophilization of active constituents such as atropine sulfate, sodium cefoxitin, cefazolin sodium and procainamide hydrochloride. They showed that crystallization was induced by excipients such as glycine, alanine, serine, methionine, urea, and niacinamide, whereas lyophilization with some excipients such as mannitol and lactose gave an amorphous product. The degree of crystallization increased with an increase in the mole fraction of the excipient. For example, when the mole fraction of glycine in the solution was 0.41, the lyophilized cake of cefazolin sodium was amorphous. As the mole fraction was increased to 0.80, complete crystallization of cefazolin sodium was observed. It was hypothesized that (i) the active constituent and excipient forms a molecular complex that crystallizes during the freezing process and/or (ii) the excipient crystallizes during the freezing process and serves as a seed crystal for the active constituent and/or (iii) the active constituent and excipient formed a eutectic during the freezing process (24).

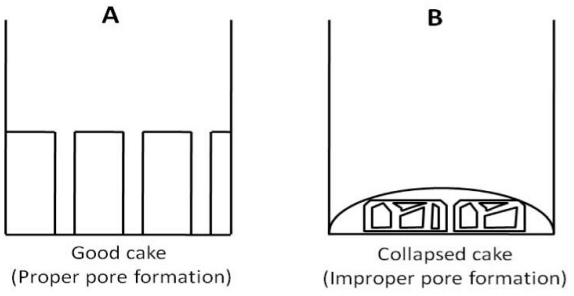


Figure 7 Good cake structure ensures proper pore formation which provides the channels for vapor removal (A) whereas in case of cake collapse, a poor structure is obtained, leading to increased resistance in removal of vapor (B).

Buffering agent

Control of pH is critical to avoid degradation of drug during processing, storage and reconstitution, thereby necessitating addition of buffering agent in the lyophilized formulation. The choice of buffer depends on the pH stability profile of active ingredient as drug needs to be reconstituted and stored for some time before it could be administered to the patient. For this purpose, the pH of maximum stability of drug should be known and maintained. Selection of a suitable buffer and its concentration is important for sensitive molecules. For example in aspartame lyophilizates, the presence of 0.1M phosphate buffer caused the half life of the material to decrease from 921 days in unbuffered material to 98 days; increasing the buffer concentration further causes a reduction to 77 days (25).

The buffering agent should have a high collapse temperature, be non-volatile and have a high glass transition temperature (T_g) (26). A high collapse temperature would facilitate a faster primary drying, and its non-volatile nature would prevent pH drift, that might be detrimental to the product stability. Additionally, a high glass transition temperature (T_g) would ensure stability during storage. In this context, acetate buffer is not used due to its volatile nature, as it can be partially lost during lyophilization (27). Crystallization of buffer components can also lead to a drastic shift in pH, resulting in degradation of the active component. Sodium and potassium phosphate salts are not often used in the lyophilization, since these crystallize during cooling and in

frozen solution, which leads to a decrease in pH of about 4 units (28). Shalave *et al.* studied citrate, succinate and tartrate buffer for their crystallization behavior and its effect on pH of the formulation. Citrate buffer was found to be the most preferred as it remained amorphous, with the shift in pH being minimal, in comparison to succinate and tartrate, which crystallized during lyophilization (29).

"pH memory" is a term used to denote the relationship between pH-activity and pH-stability profiles, in the solution and dried state respectively, as the pH of the solution before drying has an impact on the rate of chemical reactivity in the resulting amorphous material (30-33). Guo *et al.* found that depending on the initial pH of an aqueous solution of quinapril hydrochloride, lyophilization produced a mixture of quinapril hydrochloride and the neutralized form. Increase in pH led to a proportionate increase in the amount of neutralized form. Since neutralized form is less stable than hydrochloride salt, the stability of the lyophilized sample decreased with an increase in the pH of solution used for lyophilization (34). In certain cases, however, pH stability of a drug in lyophilized form may show an unusual behaviour in comparison to the pH-stability in solution state. For example, moexipril is most stable at a pH of 4.5 in solution. However, when moexipril was lyophilized using a solution of pH range of 2-11, it was found to be most unstable at a pH of 5.1. This was attributed to the altered mechanism of reactivity in absence of water in lyophilized product, compared to the aqueous solution (35). A similar effect was shown for the ionization of sulfonephthalein in trehalose-citrate systems, where protonation of indicators were higher in the lyophilized sample than in solution at a particular pH (36).

Collapse temperature modifiers

Lyophilization of amorphous material requires the primary drying temperature to be kept below the collapse temperature of the formulation. However, some excipients (37) in amorphous state have a very low collapse temperature, thus increasing the duration of primary drying significantly. In such case, collapse temperature modifiers are utilized, which shift the overall collapse temperature higher (owing to their high individual collapse temperatures), with a consequent reduction in the primary drying cycle, without compromising the product quality (25). Commonly used collapse temperature modifiers are dextran, Ficoll®, gelatin and hydroxyethylstarch (38). Their collapse temperatures (along with other critical process temperatures for different excipient classes) are summarized in Table 2. However, it must be noted that their use in lyophilized formulations is not very frequent.

Table 2 Critcal process temperatures of various excipients used in lyophilization

Excipient	T_g'	T_c	References
BULKING AGENT			
Sucrose	-32, -35	-34,-32	(39-41)
Lactose	-28	-31, -32	(39, 40, 42)
Trehalose	-27, -29	-29.5, -34	(39, 41, 42)
Mannitol	-35, -28		(39, 42)
Sorbitol	-46	-45	(40, 42, 43)
Glucose	-43	-40, -41.5 -43	(40, 41, 44)
Raffinose	-27	-26	(43)
Glycine	-62		(45)
Histidine	-33		(39)
PVP (K40)	-20	-23	(40, 42, 43)
BUFFERING AGENT			
Sodium citrate	-41		(39)
Sodium phosphate	-45		(39)
Sodium hydroxide			
Meglumine			
Tris base	-51		(39)
Tris HCl	-65		(39)
Tris acetate	-54		(39)
TONICITY MODIFIER			
Sodium chloride			
Dextrose	-44		(42)
COLLAPSE TEMPERATURE MODIFIER			
Dextran	-10	-9, -10	
Ficoll	-19	-19.5, -20	
Gelatin	-9	-8	
Hydroxyethyl starch		-5	(38)

Miscellaneous Excipients

Tonicity modifiers

Parenteral formulations should be isotonic with human plasma so as to avoid damage to the tissues. However, not all drugs at their recommended dosage are isotonic with blood, thus requiring the addition of a tonicity adjusting agent to the formulation. The most commonly used tonacity agent is dextrose, while others, such as glycerol and sodium chloride are less commonly used. The addition of tonicity modifiers to the lyophilization mixture, however, can complicate the formulation development, since they may lower the collapse temperature of the entire formulation owing to their very low col- lapse temperatures, thus increasing the primary drying time significantly. An alternative approach is to add the tonicity modifier to the reconstitution diluent rather than the freeze dried product. Deviations from isotonicity may be acceptable when the injection volumes are small and the infusion rate is slow (46).

Antimicrobial agents

Antimicrobial agents are added to multi-dose formulations to prevent microbial growth during its shelf life. Benzyl alcohol and a mixture of ethyl- and methyl- parabens are commonly used. Additionally, phenol and m-cresol are utilized in lyophilization. At very low levels (i.e., ≤0.05% w/w in solution), antimicrobial agents generally do not alter the collapse temperature of the formulation. Compatibility of antimicrobial agent with other ingredients in the formulation needs to be checked, when to the lyophilized cake (8). However, since the antimicrobial agent is not needed during the lyophilization process antimicrobial agents are often typically included in the diluent for reconstitution (27).

Solubilizing agent

Surfactants

Surfactants may be added at low levels (e.g., ~0.05% w/w aqueous solution) to aid reconstitution if the drug does not show good wetting behaviour. Surfactants are added to low dose products to minimize losses due to surface adsorption. Their addition in large quantity is not recommended due to the low glass transition temperature of commonly used non-ionic surfactants (e.g. Polysorbate 80) (47). Halikala *et al.* showed the effect of surfactant on the crystallization behavior of mannitol. It was observed that with increase in concentration of polysorbate 80, the crystallinity of mannitol increased and at concentration of 0.01% and above, increased amount of δ–mannitol was formed (48). They can be included in the diluting medium so as to keep the lyophilized formulation simple.

Co-solvents

Water is the most commonly used solvent for lyophilization. However, organic solvents are sometimes used to increase the primary drying rate by increasing the sublimation rates, improve product stability, decrease reconstitution time by improving drug wettability or solubility, and also enhance the sterility assurance of the sample solution (49). Since lyophilization works on the principle of vapor pressure differential, it is necessary for excipients to have low vapor pressure, to minimize their loss during the lyophilization process. However, co-solvents are added due to their high vapor pressure to facilitate faster removal from the product during drying process and thus speeding up the lyophilization process. It has been reported that tert-butyl alcohol has been used to speed up the lyophilization process (50). The rate of degradation of active constituent in the presence of water can be reduced by addition of non-aqueous solvents (51, 52). In some cases, solvent may play no role in stabilization

of the active constituent but improve the aesthetic property of the final product (50).

The use of co-solvents, however, requires proper handling and storage, consideration of the level of residual solvent and its potential toxicity. The most commonly used solvent is a tertbutanol/water combination. Tertbutanol modifies the crystal habit of ice and promotes its sublimation, thus reducing the duration of primary drying (53, 54). Telang *et al.* studied the effect of solvents on the crystallization of cefalothin sodium. The lyophilized cake formed with ethanol and isopropanol were found to collapse, while tert-butyl alcohol gave a good cake structure. Increased degree of supersaturation and nucleation during freezing, followed by annealing due to co-solvent, led to a faster crystallization rate (55). Ethanol, although being the most generally used solvent in the laboratory, is less preferred as a co-solvent, as a shelf temperature of about -120°C is required to attain frozen state, thus requiring a tremendous rise in energy consumption. In contrast, tert-butyl alcohol does not require additional cooling (50).

Complexing agent

Complexation is sometimes used to improve the solubility of drug in the solvent especially water. Kagkadis *et al.* used hydroxypropyl-β-cyclodextrin (HP-β-CD) complex of ibuprofen to increase the solubility of lyophilized product (56). The addition of complexing agent may, however, lead to a reduction in the critical. temperature of the formulation, thus complicating the formulation development.

LEGAL STATUS OF EXCIPIENTS

Excipients added to the lyophilized cake should have regulatory acceptance, as they are intended for parenteral administration. The list of approved excipients with their maximum limit for lyophilization of small molecules, as reported in the IIG (Inactive Ingredients Guide) is listed in Table 3 (37).

Table 3 List of excipients used in 'Powder, for injection solution, lyophilized' with the maximum allowable limits as per IIG (37)

Inactive ingredient	Route of administration	Maximum potency
Mannitol	Intravenous	60.00%
Mannitol	IV(infusion)	93.75%
Mannitol[a]	IV(infusion)	7.50%
Mannitol	Intramuscular	8.50%
Mannitol[a]	Intramuscular	3.60%
Anhydrous lactose	Intracavitary	4.75%
Anhydrous lactose	Intravenous	28.75%
Lactose	Intracavitary	19.38%
Lactose	Intramuscular	1.00%
Lactose	Intravenous	1.00%
Lactose monohydrate	IM - IV – SC	10.70%
Lactose monohydrate	Intracavitary	4.54%
Lactose monohydrate	Intravenous	69.00%
Lactose monohydrate	IV(infusion)	90.00%
Lactose, hydrous	Intramuscular	1.05%
Sucrose	Intramuscular	6.84%
Sucrose[b]	Intravenous	90.00%
Sucrose	Intravenous	7.78%
Sucrose	IV(infusion)	5.40%
Sucrose	Subcutaneous	6.84%
Sucrose[a]	Subcutaneous	4.10%
Glycine	Intramuscular	2.76%
Glycine	Intravenous	25.00%
Glycine	Subcutaneous	2.76%
Dextran 40	Intravenous	30.00%
Anhydrous citric acid	Intravenous	42.18%
Citric acid	Intravenous	7.69%
Citric acid monohydrate	Intravenous	41.36%
Sodium citrate	Intracavitary	5.3e-03%
Sodium citrate	Intramuscular	0.64%
Sodium citrate	Intravenous	16.35%
Sodium citrate	Subcutaneous	0.64%
Trisodium citrate dihydrate	Intravenous	23.52%
Sodium phosphate dihydrate	Subcutaneous	0.13%
Sodium phosphate, dibasic	Subcutaneous	0.29%
Sodium phosphate, dibasic anhydrous	Intramuscular	0.03%
Sodium phosphate, dibasic anhydrous	Intravenous	1.50%
Sodium phosphate, dibasic anhydrous	Subcutaneous	0.03%
Sodium phosphate, dibasic, dihydrate	Subcutaneous	0.11%
Sodium phosphate, dibasic, heptahydrate	Intramuscular	4.80%
Sodium phosphate, dibasic, heptahydrate[a]	Intramuscular	0.37%
Sodium phosphate, dibasic, heptahydrate	Subcutaneous	0.20%
Sodium phosphate, monobasic	Subcutaneous	0.02%

Table 3 *continued*

Inactive ingredient	Route of administration	Maximum potency
Sodium phosphate, monobasic anhydrous	Intramuscular	1.20%
Sodium phosphate, monobasic anhydrous	Subcutaneous	0.11%
Sodium phosphate, monobasic, dihydrate	Subcutaneous	0.04%
Sodium phosphate, monobasic, monohydrate[a]	Intramuscular	0.14%
Sodium phosphate, monobasic, monohydrate	Intravenous	0.05%
Sodium acetate anhydrous	Subcutaneous	0.01%
Sodium hydroxide	Intravenous	19.27%
Sodium hydroxide	IV(infusion)	1.56%
Hydrochloric acid	Intravenous	16.00%
Hydrochloric acid[c]	IV(infusion)	0.83%
Benzyl alcohol	Intracavitary	0.84%
Sodium chloride	IM – IV	18.00%
Sodium chloride[a]	Intramuscular	0.24%
Sodium chloride	Intravenous	45.00%

[a] with additives; [b] lyophilized powder for liposomal suspension; [c] powder, for injection suspension, lyophilized

CONCLUSION

Lyophilization is a commonly used technique for formulation development of small molecules which are unstable in aqueous medium and/or are thermolabile in nature. Lyophilization of drug alone, however, presents certain formulation development challenges, which may be overcome by incorporation of excipients (e.g. bulking agents, buffering agents, tonicifying agent, wetting agent and cosolvents, preservatives and collapse temperature modifiers) in the formulation. A need-based approach should be employed for proper selection of excipients in the formulation for lyophilization, so as to keep the formulation simple for easier processing, while simultaneously maintaining an optimal functionality.

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