Excipients used in lyophilization of small molecules

Ankit Baheti[a], Lokesh Kumar[b], Arvind K. Bansal[b*]

[a] Department of Pharmaceutical Technology (Formulations) National Institute of Pharmaceutical Education and Research (NIPER) Sector 67, S.A.S. Nagar, Punjab-160062, India

[b] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER) Sector 67, S.A.S. Nagar, Punjab-160062, India

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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

* Corresponding author: Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER) Sector 67, S.A.S. Nagar, Punjab-160062, India, Tel: +91-172-2214682, Fax: +91-172-2214692, e-mail: akbansal@niper.ac.in

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.

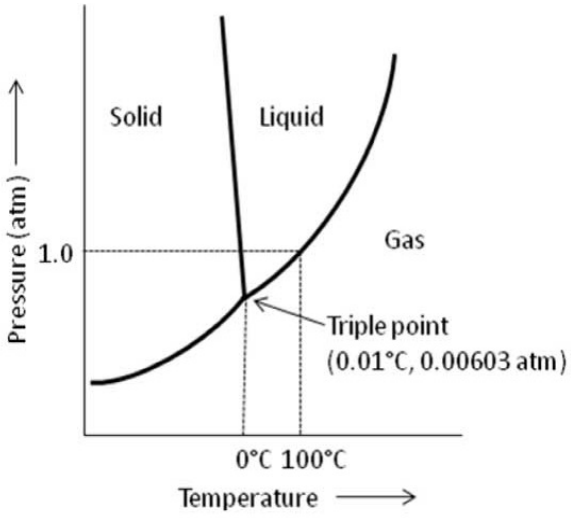

Figure 1 Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm. Lyophilization is carried out below the triple point to enable conversion of ice into vapor, without entering the liquid phase (known as sublimation).

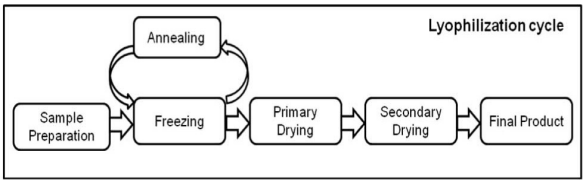

Figure 2. Steps involved in lyophilization from sample preparation to final product formation. Annealing is an optional step, occasionally used to crystallize the formulation component(s).

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

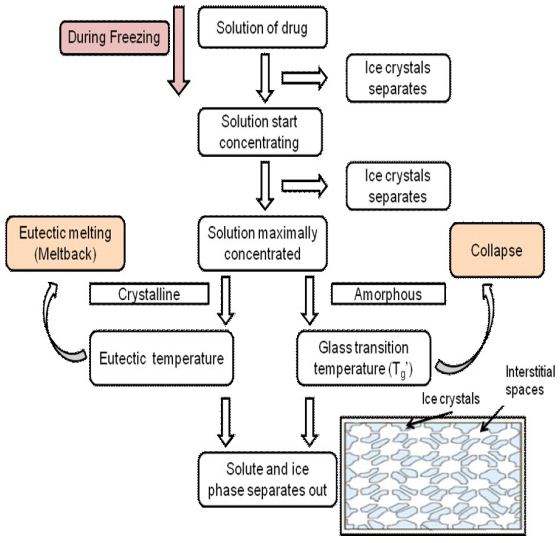

Figure 3 Flowchart showing the concept of eutectic temperature and T_g, and their importance during primary drying

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

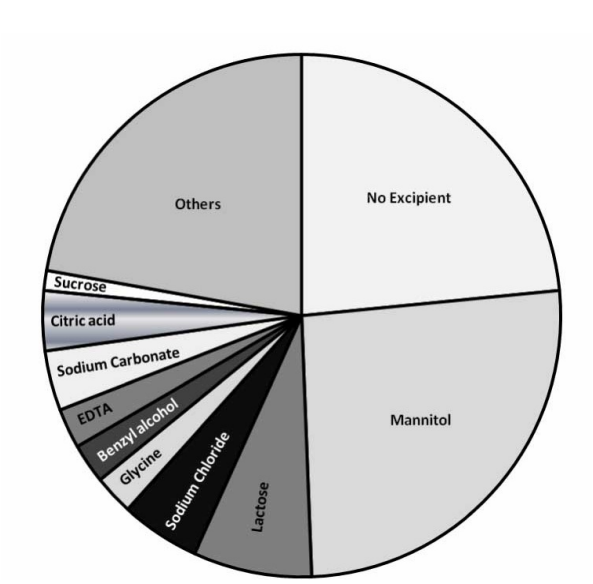

Figure 4 Distribution of commonly used excipients in marketed lyophilized formulations of small molecules. About 67% of marketed lyophilized formulations of small molecules contain excipients.

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)

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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

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