



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 0.611 kPa 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.

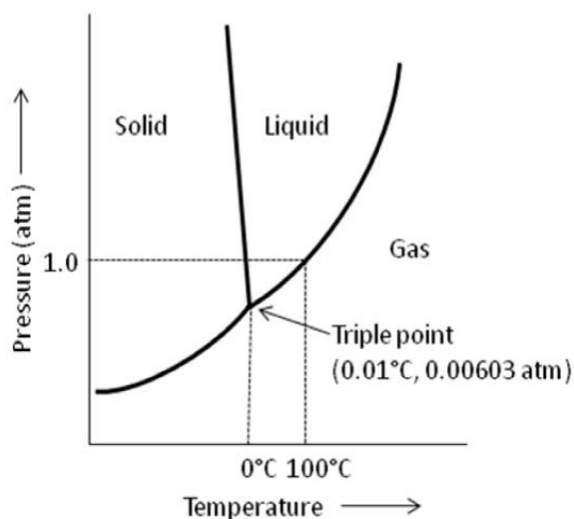


Figure 1 Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm. Lyophilization is carried out below the triple point to enable conversion of ice into vapor, without entering the liquid phase (known as sublimation).

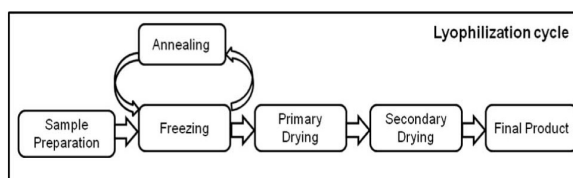


Figure 2. Steps involved in lyophilization from sample preparation to final product formation. Annealing is an optional step, occasionally used to crystallize the formulation component(s).

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

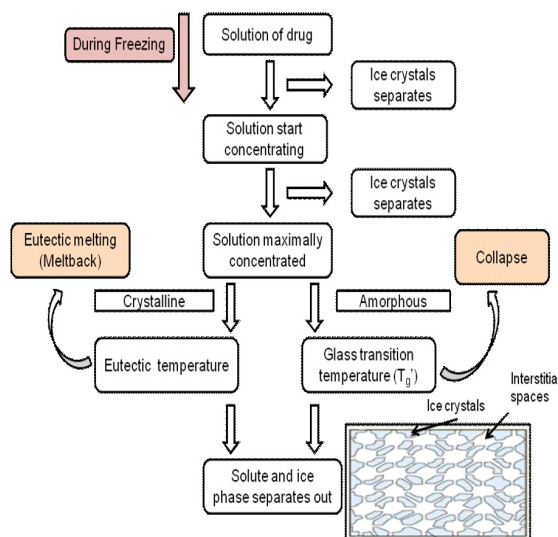


Figure 3 Flowchart showing the concept of eutectic temperature and T_g , and their importance during primary drying

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

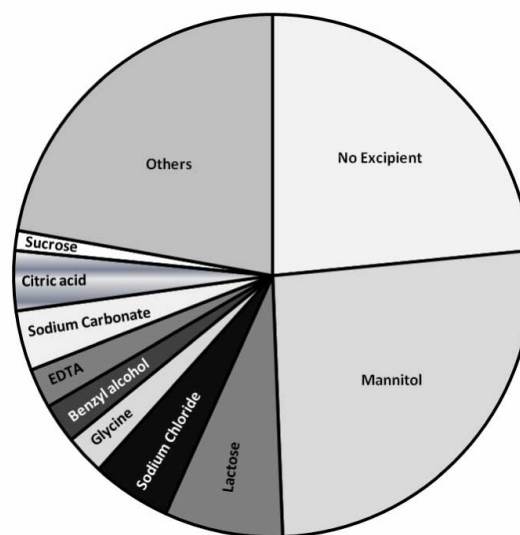


Figure 4 Distribution of commonly used excipients in marketed lyophilized formulations of small molecules. About 67% of marketed lyophilized formulations of small molecules contain excipients.

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 | Ethylol® (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 | Antifungal | Hydrogenated soyaphosphatidylcholine Disteroylphosphatidyl 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® (Pharmacia 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 | Flofan® (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 replacement | 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 | Mannitol | 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) |
| Thiopental 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 | Tyagcil® (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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