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Review Article

FREEZE DRIED INJECTABLE DRUG PRODUCT DEVELOPMENT: SELECTION OF NON FUNCTIONAL ADDITIVES

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ABSTRACT

Excipients used in injectable formulation during pharmaceutical product development are the integral part to achieve desired product critical quality attributes. This review deals with understanding of the physicochemical properties of excipients used in injectable formulation development drug products. However, in spite of proper excipients selection, judicious use during formulation manufacturing process based on their critical properties is also important to avoid negative effects such as loss of drug solubility, activity and stability. This review article deals with proper selection of excipients in injectable drug products which gives stability (physical and chemical), high critical temperature, good bulking properties avoiding melt back and collapse with improved dried product appearance. Article also emphasized on appropriate selection of excipients for injectable dosage forms and linking their physiochemical properties with optimum manufacturing method with suitable case studies. This review will highlight various excipient related issues optimizing product performance with documented references and practical approaches based on scientific justification. The reader will gain better understanding of excipients complexity during stability studies and resolving problems with practical approach.

Keywords:, Injections, Product development, Freeze dried products, Excipients.

INTRODUCTION

Excipients are traditionally referred to as inactive or inert ingredients to distinguish them from active pharmaceutical ingredients. Excipients may not be as inert as the term *inactive* suggests. Due to safety issues, several countries have restrictions on the type or amount of excipient that can be included in the formulation of injectable drug product. For example, in Japan, the U. S., and the E. U., amino mercuric chloride or thiomersal use is prohibited, despite the presence of these excipients in products in other regions [1].

As defined in Ph. Eur. and the British Pharmacopeia (BP),"Injectable preparations are sterile preparations intended for administration by injection, infusion, or implantation into the human or animal body." In the present article, only sterile preparations for administration by injection or infusion into the human body will be surveyed[2,3]. Injectable products require a unique formulation strategy. The formulated product must be sterile, pyrogen-free, and, in the case of solution, free of particulate matter. No coloring agent may be added solely for the purpose of coloring the injectable preparation. The formulation should preferably be isotonic, and depending on the route of administration, certain excipients are not allowed. The injected drug by-passes natural defense barriers; hence, for any given drug, the risk of an adverse event may be greater or the effects difficult to reverse if administered as an injection rather than a non-parenteral route. For this reason ultra high purity grades of excipients are available for parenteral administration. Sterility requirements demand that an excipient is able to withstand terminal sterilization or aseptic processing. These factors limit the choice of excipients available.

Excipients which are already present in marketed formulations and accepted by the Food and Drug Administration (FDA) as safe, increases the assurance to a formulator that these excipients will probably be safe for a new drug product. But, this does not give complete assurance when combined with other excipients or drug molecules as this may lead to unwanted potentiation or synergistic toxic effects. However, regulatory bodies may favorably view an excipient previously approved in an injectable dosage form, and will require less safety data. A new additive in a formulated product always requires additional studies, adding to the cost and timeline of product development. Importantly, inclusion of an excipient in the GRAS (Generally Recognized as Safe) list or pharmacopoeia does not mean that the excipient has been deemed safe by the food and drug administration for use in parenteral products.

Excipients are typically the major components in a drug product. Many formulations contain only a small percentage of the active drug molecules. Pharmaceutical excipients or additives are compounds added to the finished drug products to serve a specific function. They are added to increase bulk, aid manufacturing, improve stability, enhance drug delivery and targeting, and modify drug safety or pharmacokinetic profile. Ingredients that are used during drug product manufacturing but may not be present in the finished drug product are also considered excipients (examples include water for lyophilized products, and inert gases in the head space of containers)[4]. In recent years the "functionality" of excipients in a dosage form (similar to pharmacological activity of an active pharmaceutical ingredient or drug substance) has been recognized by United State Pharmacopoeia and European Pharmacopoeia. Many excipient monographs do not address this aspect of excipient functionality or its control.

Excipient used in freeze dried products

Bulking agents and cryoprotectants

Bulking agents forms 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 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%[5]. In such cases, a bulking agent is added to the formulation matrix. The structure of the lyophilized 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[6].

Cryo-protection is defined as the stabilization and prevention of the degradation of a molecule both during freeze-drying and afterwards, during storage. Among disaccharides, sucrose and trehalose appear to be the most commonly used. In comparison to sucrose, trehalose seems to be a preferable cryoprotectant, because it has a less hygroscopicity, very low chemical reactivity and finally, higher glass transition temperature (Tg')[7,8,9]. Critical temperature is the temperature above which the freeze-dried product loses macroscopic structure and collapses during freeze drying.

Therefore, the excipients which provide higher critical temperature are preferred for lyophilization.

Mannitol

It is the most commonly and widely used excipient in the lyophilized products. Mannitol has a very high eutectic melting temperature (-1.4°C) after crystallization and is processed well in lyophilization. Crystallization of the bulking agent, however, might adversely affect the physical stability of the product in certain instances, for which, an amorphous bulking agent is preferred[10].

Lactose

It is a good bulking agent but is a reducing sugar and may undergo Maillard reaction with proteins leading to instability of the formulation[11]. The critical temperature of 1% lactose is -32°C.

Sucrose

It is having similar collapse temperature i. e -31°C (2%) as of lactose but it is not a reducing sugar and does not undergo Maillard reaction[12]. Sucrose has a higher density as compared to lactose which can cause slight collapse during drying.

Polyethylene glycol (PEG)

It provides good cake structure and increases viscosity of water[13]. The 2% solution of PEG has a critical temperature of -22°C. Apart from lyophilization it is also used as a co-solvent and viscosity modifier in parenteral including ophthalmic.

Polyvinyl pyrollidone (PVP)

The low-molecular grades, Povidone K 12 and K 17 are used as solubilizing agents, dispersants and crystallization inhibitors, particularly for injectables. This application is used in particular for antibiotics in solution or in lyophilized form. Povidones with higher K-values may not be administered parenterally as, due to their high molecular weights, they cannot be excreted by the kidneys and hence accumulate within the body.

The povidone grades K12 and K 17 are used as solubilizers in parenteral applications. In addition Polyvinyl pyrollidone also provides cryo-protection to the product. The C-grades are supplied with low endotoxin levels ("pyrogen-free"). Bovine Serum Albumin (critical temperature of 0.5% solution of BSA is -9°C), Dextran (critical temperature of 2.0% solution of Dextran mw 9500 daltons is -12°C) due to its longer chain polymer of glucose gives higher viscosity and higher critical temperature. In addition other excipients which can be used for lyophilization are listed in below table along with their critical process temperatures.

Buffering agents

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. The buffering agent should have a high collapse temperature, be non-volatile and have a high glass transition temperature (Tg)[24].

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 (Tg) 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[25]. 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. 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. Tris buffer is known to release formaldehyde in peptide formulations stored at 70°C. "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. Commonly used buffers in the parenteral formulations are Acetate, Citrate, Tartrate, Phosphate, Triethanolamine (TRIS).

Table 1: Critical process temperatures of various excipients used in Lyophilization[14-23]

Excipient	Tg'0C	Tc0C
Tonicity Modifier		
Dextrose	-44	-
Collapse Temperature Modifier		
Gelatin	-9	-8
Hydroxy ethyl starch	-	-5
Dextran	-10	-9,-10
Bulking Agent		
Sucrose	-32	-32
Lactose	-28	-31
Trehalose	-29	-34
Mannitol	-35	-
Sorbitol	-46	-45
Glucose	-43	-43
Raffinose	-27	-26
Glycine	-62	-
Histidine	-33	-
Buffering Agent		
Sodium hydroxide	-	-
Sodium citrate	-41	-
Sodium phosphate	-45	-

Tonicity adjusting agents

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 tonicity agent is dextrose, while others, such as glycerol and sodium chloride are less commonly used. Other commonly used tonicity adjusting agents are: Glycerin and Mannitol.

Preservatives

Antioxidants, Antimicrobial and Chelating agents. The antioxidants are used to prevent/minimize the oxidation reaction of the drug or excipients over the shelf life of the product whereas antimicrobial agents are used to prevent the growth of micro-organisms in the drug product. The most commonly used antioxidants in the sterile formulations are Ascorbic acid, Acetylcysteine, Sulfurous acid salts (bisulfite, metabisulfite), Monothioglyercol etc. The commonly used antimicrobial agents are Phenol, Meta-cresol, Benzyl alcohol, Parabens (methyl, propyl, butyl), Benzalkonium chloride, Chlorobutanol, Thimerosal, Phenylmercuric salts (acetate, borate, nitrate) etc. In addition to the antioxidant and antimicrobial a chelating agent can be defined as a substance whose molecules can form several bonds to a single metal ion. Against the general understanding several single dose preparations contain preservatives due to inheritance.

Solubilizing agents

The agents which help in dissolving or increase the drug solubility into the formulation are known as solubilizing agents, the solubilising agents can be broadly classified into surfactants and co-solvents. The surfactants increase the dissolution by reducing the surface tension of the drug substances whereas, co-solvents are deined as a solvent that in conjunction with another solvent can dissolve a solute. Few examples of surfactants are Polyoxyethylene sorbitan monooleate (Tween 80), Sorbitan monooleate Polyoxyethylene sorbitan monolaurate (Tween 20), Lecithin, Polyoxyethylene– polyoxypropylene copolymers (Pluronics). Examples of co-solvents are Propylene glycol, Glycerin, Ethanol, Polyethylene glycol (300 and 400), Sorbitol, Dimethylacetamide and Cremophor EL etc.

Complexing and dispersing agents

Complexation is sometimes used to improve the solubility of drug in the solvent especially water. Cyclodextrins have emerged as very effective additive compounds for solubilizing hydrophobic drugs. In the parenteral dosage form, modified cyclodextrins, such as hydroxypropyl-b-cyclodextrin and sulfobutylether- b –cyclodextrin have been reported to solubilize and stabilize many injectable drugs, including dexamethasone, estradiol, interleukin-2, and other proteins and peptides without apparent compatibility problems.

Buffering agents

Buffers are added to a formulation to adjust and stabilize pH and optimize drug solubility and stability, for parenteral preparations, it is desirable that the product pH be close to physiologic pH. Selection of a buffer concentration (which contributes to the ionic strength of the formulation) and a buffer species is important. For example, citrate buffers in the range of 5–15 mM are typically used in formulations but increasing the buffer concentration to 50 mM will result in excessive pain on sub-cutaneous injection and toxic effects due to chelation of calcium in the blood. Buffers and chemicals used for pH adjustment and maintenance of the drug product pH range, phosphate, citrate, and acetate are the most common buffers used in parenteral products. Citrates are common buffers that serve a dual role as chelating agent. Lactate and tartrate are occasionally used as buffer systems. Acetates are good buffers at low pH, but they are not frequently used for lyophilization because of the potential sublimation of acetates.

Wetting agents

Various nonionic surfactants and non-aqueous solvents like glycerin, alcohol and propylene glycol are types of wetting agents commonly used in injectable suspensions. Wetting agents reduce the contact angle between the surface of the particle & the wetting liquid to obtain maximum wetting eficiency; surfactants with hydrophilic lipophilic balance (HLB) value in the range of 7 to 9 should be selected. The usual concentration of surfactants varies from 0.05% to 0.5% depending on the solid contents of the suspension. Care should be taken in terms of the amount used; excessive amounts may cause foaming or caking or provide an undesirable taste/odor to the product.

Surfactants (wetting agent)

Lecithin, Polysorbate 20, Polysorbate 80, Pluronic F-68, Sorbitan trioleate (span 85) are used, as surfactants in injectable suspensions for e. g. in the preparation of a non-aqueous suspension of Cefazolin sodium in peanut oil, addition of Polysorbate-80 at concentration greater than 0.17% resulted in deflocculated suspension which was difficult to redisperse.

Microscopic examination revealed extensive agglomeration and crystal growth of cefazolin sodium in the presence of polysorbate 80.

Solvent system

Solvent systems used in freeze dried formulations are classified as either aqueous or non-aqueous vehicles. Choice of a typical solvent system depends on solubility, stability & desired release characteristics of the drug. Non-aqueous vehicles include both water miscible and water immiscible vehicles.

• Water for injection is generally the preferred solvent system. However, non-aqueous water miscible agents are used as co-solvents with water for injection to promote the solubility and stability in parenteral preparation. Examples of water miscible non-aqueous vehicles include tert butyl alcohol, ethanol, acetone, glycerin, propylene glycol and n–lactamide.

• The use of water miscible co-solvents can lead to undesirable side effect for e. g. intramuscular injection of propylene glycol water, ethyl alcohol-water & polyethylene glycol (PEG) 400 water mixtures was found to cause muscle damage as measured by in vitro release of creatinine kinase from isolated rat skeletal muscle.

Tonicity agents

Isotonicity of the parenteral formulations for subcutaneous or intramuscular administration is desired to prevent pain; irritation and tissue damage at the site of administration, the aqueous solution of tonicity agents used in parenteral preprations include dextrose various electrolytes.

Preservatives

Antimicrobial agents are required for parenteral products that are intended for multiple dosing, in order to protect the product from accidental microbial contamination during clinical usage & maintain sterility. Some typical preservative used in parenteral formulations and their commonly used concentrations are as follows.

Benzyl alcohol (0.9% to 1.5%)

Methylparaben (0.18%to0.2%)

Propylparaben (0.02%)

Benzalkonium chloride (0.01% to 0.02%)

Thiomersal (0.001% to 0.01%)

Benzalkonium chloride is used in ophthalmic dosage forms and not in injectable dosage forms.

Propyl and methyl parabens are referred to chemically as propyl and methyl esters of p-hydroxy benzoic acids. Because of the inherent chemically reactive nature of preservatives, stability and compatibility problems need to be evaluated for their usage in the final formulation.

Criteria for the selection of excipient

The following key points should be considered in selecting an excipient for parenteral products:

1. Compatibility of excipient with drug and the packaging system.

2. Influence of excipient on the overall quality, stability, and effectiveness of drug product.

3. Compatibility of excipient with the manufacturing process, for example, preservatives may be adsorbed by rubber tubes or filters, acetate buffers will be lost during lyophilization process, etc.

4. The amount or percentage of excipients that can be added to the drug product.

5. Route of administration. The USP, Ph. Eur BP do not allow preservatives to be present in injections intended to come in contact with brain tissues or CSF. Thus intracisternal, epidural, and intradural injections should be preservative free. Also, it is preferred that a drug product to be administered via intravenous (iv) route be free of particulate matter. However, if the size of the particle is well controlled, like in fat emulsion or colloidal albumin or amphotericin B dispersion, it can be administered by iv infusion.

6. Dose volume. All LVPs and those SVPs where the single dose injection volume can be greater than 15 ml are required by the EP/BP to be preservative free (unless justified). The USP recommends that special care be observed in the choice and the use of added substances in preparations for injections that are administered in volumes exceeding 5 ml.

7. Whether the product is intended for single or multiple dose use. According to USP, single dose injections should be free of preservative. The FDA takes the position that even though a single dose injection may have to be aseptically processed, the manufacturer should not use a preservative to prevent microbial growth. European agencies have taken a more lenient attitude on this subject.

8. The length or duration of time that the drug product will be used once the multidose injection is opened.

Regulatory perspective

Based on available safety testing information, the International Pharmaceutical Excipients Council (IPEC) has classiied excipients into following classes:

1. New chemical excipients: These excipients require a full safety evaluation program. It is estimated that the cost of safety studies for a new chemical excipient is about $35 million over 4 –5 years. E. U. directive 75/318/EEC states that new chemical excipients will be treated in the same way as new actives. In the U. S. a new excipient requires a Drug Master File (DMF) to be filed with the FDA. Similarly, in Europe a dossier needs to be established. Both the DMF and dossier contain relevant safety information. IPEC Europe has issued a guideline (Compilation of Excipient Master Files Guidelines) providing guidance to excipient producers on constructing a dossier to support MAA (Marketing Authorization Application) while maintaining confidentiality of the data.

2. Existing chemical excipient—first use in man: This class implies that animal safety data exists and that the excipient may have been used in some other route of administration (e. g., from oral to parenteral), new dosage form, higher dose, etc. may require additional safety information.

3. New modifications or combinations of existing excipients: These excipients indicate a physical interaction *not* a chemical reaction. No safety evaluation is necessary in this case.

The excipient included in the GRAS list does not mean that the excipient can be used in Injectables; the excipients which are meant for IV/IM/SC/IP etc need to be mentioned in GRAS list and then only the excipient qualifies to be used for parenteral dosage form.

The United States and Europe require all excipients to be declared, along with their quantity, on the label (what is put on the immediate container) if the product is an injectable preparation. In Japan, only the excipient names are required in the labeling (information that is included with the product, such as a package insert); E. U. Article 54(c) requires that all excipients must be declared on the labeling if the medicinal product is an injectable, a topical, or an eye preparation The European guide for the label and package leaflet also lists excipients with special issues and are addressed in Annex 31. A package leaflet must include a list of information on those excipients, knowledge of which is important for the safe and effective use of the medicinal product. Similarly, 21 CFR 201.22 requires prescription drugs containing sulfites to be labeled with a warning statement about possible hypersensitivity. According to the Notes for Guidance on Pharmaceutical

Development (CHMP/ICH/167068/04), the choice of excipients, their grade, compatibility, concentration, and function should be described in the P2 section of the Common Technical Document. It is necessary to justify inclusion of all ingredients in the drug product and describe their intended function. A specification of ±10% at the end of shelf-life is acceptable except for antioxidants and preservatives where performance data from PET or stability data may justify broader limits. Bioburden and endotoxin limits of excipients used in the manufacture of sterile medical products shall be stated. Individual testing of excipients may be omitted. If an excipient is present in Ph. Eur or other major pharmacopoeia, the monograph specifications are usually acceptable in the registration file. For excipients not described in any pharmacopoeia, specifications should include physical characterization, identification tests, purity test, assay, and impurity tests. A certification must be included to confirm that excipients are of non-animal (specifically non-ruminate) origin. If this is not the case, a regulatory agency will require documentation to demonstrate freedom from viral and transmissible spongiform encephalopathies (TSE) and Bovine Serum Encephalopathy (BSE) risks. Currently, there are concerns regarding TSE via animal derived excipients such as gelatin. TSEs are caused by prions that are extremely resistant to heat and normal sterilization processes. TSEs have a very long incubation time with no cure. In the current regulatory environment, if given a choice, it is beneficial to select non-animal-derived excipients. Concerns about bovine serum albumin or human serum albumin (HSA) because of possible derivation from virus-contaminated blood remain. European Commission directive EMEA/410/01/rev2 requires manufacturers to provide a "Certificate of Suitability" or the underlying "scientific information" to attest that their pharmaceuticals are free of TSEs. Below are the few points which are to be considered in selecting an excipients and its supplier for parenteral products.

CONCLUSION

Several new excipients, such as cyclodextrins, are being evaluated to improve solubility or stability of parenteral drugs. Currently, there are two FDA-approved parenteral products that utilize alpha and gamma cyclodextrins. Beta-cyclodextrin is unsuitable for parenteral administration because it causes necrosis of the proximal kidney tubules upon intravenous and subcutaneous administration. Chitosan,b-1,4-linked glucosamine, a naturally occurring, biodegradable, nontoxic polycationic biopolymer, is being investigated for its potential as across-linked microsphere matrix to deliver Antineoplastic drugs. Biodegradable polymeric materials (polylactic acid, polyglycolic acid, and other poly-alpha-hydroxy acids) have been used as medical devices and as biodegradable sutures since the 1960s. Currently, the FDA has approved for marketing only devices made from homopolymers or co-polymers of glycolide, lactide, caprolactone, p-dioxanone, and trimethylene carbonate. Such bio-polymers are inding increased application as a matrix to deliver parenteral drugs for prolonged delivery.

Polyanhydrides degrade primarily by surface erosion and possess excellent in vivo compatibility. In 1996 the FDA approved a polyanhydride-based drug implantable delivery system to the brain for the chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BiCNU).

Several phospholipid-based excipients are inding increased application as solubilizing agents, emulsifying agents, or as components of liposomal formulations. The phospholipids occur naturally and are biocompatible and biodegradable, for example, eg. phosphatidylcholine, soybean phosphatidylcholine, hydrogenated soybean phosphatidylcholine (HSPC), dimyristoyl phosphatidylcholine (DMPC), distearoyl phosphatidylcholine (DSPC), 1,2 dioleoyl-sn-glycero-3-phosphocholine (DOPC), distearoy l phosphoethanolamine

(DSPE), L-alphadimyristoylphosphati-dylglycerol (DMPG), 1,2-dipalmitoyl-snglycero- 3-phosho-rac-(1-glycerol). Poloxamer or pluronic are block copolymers comprised of polyoxyethylene and polyoxypropylene segments. They exhibit reverse thermal gelation and are being tried as solubilizing, emulsifying, and stabilizing agents. Sucrose acetate isobutyrate (SAIB) a high viscosity liquid system converts into free lowing liquid when mixed with 10–15%

ethanol. Upon subcutaneous or intramuscular injection, the matrix rapidly converts to a water-insoluble semi-solid capable of delivering proteins and small molecules for a prolonged period. SAIB is biocompatible, and it biodegrades to natural metabolites. Several other biodegradable, biocompatible, injectable polymers are being investigated for drug delivery systems. They include polyvinyl alcohol, block copolymer of PLA-PEG, polycyanoacrylate,

polyanhydrides, cellulose, alginate, collagen, modified Human Serum Alubumin, albumin, starches, dextrans, hyaluronic acid and its derivatives and hydroxyapatite.

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