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Review

Practical aspects of lyophilization using non-aqueous co-solvent systems

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Abstract

Non-aqueous co-solvent systems have been evaluated for their potential use in the freeze-drying of pharmaceutical products. The advantages of using these non-aqueous solvent systems include: increased drug wetting or solubility, increased sublimation rates, increased pre-dried bulk solution or dried product stability, decreased reconstitution time, and enhancement of sterility assurance of the pre-dried bulk solution. Conversely, the potential disadvantages and issues which must be evaluated include: the proper safe handling and storage of flammable and/or explosive solvents, the special facilities or equipment which may be required, the control of residual solvent levels, the toxicity of the remaining solvent, qualification of an appropriate GMP purity, the overall cost benefit to use of the solvent, and the potential increased regulatory scrutiny. The co-solvent system that has been most extensively evaluated was the tert-butanol/water combination. The tert-butanol possesses a high vapor pressure, freezes completely in most commercial freeze-dryers, readily sublimes during primary drying, can increase sublimation rates, and has low toxicity. This co-solvent system has been used in the manufacture of a marketed injectable pharmaceutical product. When using this solvent system, both formulation and process control required optimization to maximize drying rates and to minimize residual solvent levels at the end of drying. Other co-solvent systems which do not freeze completely in commercial freeze-dryers were more difficult to use and often resulted in unacceptable freeze-dried cakes. Their use appears limited to levels of not more than 10%. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Non-aqueous; Solvent; Freeze-drying; tert-Butanol

1. Introduction

Freeze drying of pharmaceutical solutions to produce an elegant stable powder has been a standard practice employed to manufacture many marketed pharmaceutical injectable products. The overwhelming majority of these products are lyophilized from simple aqueous solutions. Water is typically the only solvent of significant quantity that is present which must be removed from the solution via the freeze-drying process. However, it is not unusual for small quantities of organic solvents to be present in either the active pharmaceutical ingredient or one of the excipients. These low levels of organic solvent are commonly found because they may be carried through as part of the manufacture of these individual components since the ingredient may be precipitated, crystallized, or spray dried from organic solvents. Therefore, most freeze-dried products may be dried from solutions which contain low levels of organic solvents. However, in addition to freeze-drying solutions with organic solvent impurities present, there may be instances where freeze-drying from organic solvents or mixtures of water and organic solvent may offer the formulation scientist advantages over simply drying from an aqueous solution. An example of at least one pharmaceutical product on the market which has utilized an organic co-solvent system during freeze-drying is CAVERJECT® Sterile Powder (Teagarden et al., 1998a,b). This particular product has been successfully manufactured by freeze-drying from a 20% v/v tert-butanol/water co-solvent system.

There are many reasons why it may be beneficial to both product quality and process optimization to select a lyophilization process which employs a strictly organic or organic/water co-solvent system. A list of some of these potential advantages includes: increases rate of sublimation and decreases drying time, increases chemical stability of the pre-dried bulk solution, increases chemical stability of the dried product, facilitates manufacture of bulk solution by increasing drug wettability and solubility in solution, improves reconstitution characteristics (e.g. decreases reconstitution time), and enhances sterility assurance for pre-dried bulk solution. However, the development sci-

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entist must be aware that use of these organic/water co-solvent systems can cause a multitude of problems. A list of some of these potential disadvantages includes: toxicity concerns, operator safety concerns due to high degree of flammability or explosion potential, lack of compendia grades or monographs, may require special manufacturing facilities/equipment or storage areas, possess difficult handling properties, requires high purity solvent with known impurities at low levels, must reach acceptable residual solvent in final product, high cost to use, potential for splash/spattering of product in vial neck, and lack of regulatory familiarity. One should remember that successful sterile formulations should always employ an understanding of the fundamental interrelationships between the formulation, the process, and the package. The knowledge gained from the interrelationships enables optimization of the formulation which can be processed and packaged at a production scale. These same principles still apply to the use of organic solvents in freeze-drying. The advantages and disadvantages of their use must be carefully weighed before they are chosen to be used in the manufacture of a pharmaceutical product, especially one that is an injectable dosage form.

A list of some of the solvents which have been tested in freeze-drying studies is provided in Table 1. Included in this table is a summary of some of the critical physical/chemical properties for each of these solvents. Several pharmaceutical products or drugs in various stages of formulation and/or clinical development have been manufactured via a process which required freeze-drying from organic co-solvent systems. These types of solvent systems were chosen for many of the reasons described above. Table 2 contains a list of examples of a few drug preparations which have been evaluated.

Additional uses for the technique of freeze-drying from organic co-solvent systems, other than in the manufacture of pharmaceuticals, include the preparation of biological specimens or the preparative isolation of lecithin. The biological specimens can be prepared by lyophilization from organic co-solvent systems in order to improve specimen preservation for scanning electron microscopy examinations (Inoue and Osatake, 1988; Akahori et al., 1988; Hojo, 1996). tert-Butanol appears to be the major organic solvent selected for this use. The surface structure of the specimen remains intact when employing rapid freezing followed by freeze-drying from an appropriate organic solvent such as tert-butanol (Herman and Müller, 1997). Lecithin can be prepared in a solvent-free form via lyophilization from cyclohexane (Radin, 1978).

2. Facilitating manufacture of bulk solution

The first step in the manufacture of almost all freeze-dried products is the formation of a solution of the ingredients to be dried. Typically these solutions are sterile filtered, aseptically filled into containers, and freeze-dried. Some hydrophobic ingredients (e.g. the bulk drug or

Table 1
Properties of organic solvents evaluated in freeze-drying

Solvent	Solubility in water (%[a])	Vapor pressure (mmHg at 20 °C)	Freezing point (°C)	Boiling point (°C)	Flammability			
					Flash point (°F/°C)	Autoignition temperature (°F/°C)	Lower flamm. limit (in air vol.%)	Upper flamm. limit (in air vol.%)
tert-Butanol	100	26.8	24.0	82	52/11	892/478	2.4	8.0
Ethanol	100	41.0	−114	78.5	62/16	793/423	3.3	19
n-Propanol	100	14.5	−127	97.1	59/15	760/404	2.1	13.5
n-Butanol	7.7	5.6	−90	117.5	95/35	689/365	1.4	11.2
Isopropanol	100	31.0	−89.5	81	53.6/12	750/398	2.5	12
Ethyl acetate	8.7	64.7	−84	77.1	24/−4	800/426	2.2	11.5
Dimethyl carbonate	9.5	72	2	90	65/18	–	4.2	12.9
Acetonitrile	100	69.8	−48	80.1	45/8	975/524	4.4	16.0
Dichloromethane	1.3	343.9	−97	40	None	1033/556	14	22
Methyl ethyl ketone	27.0	76.2	−87	79.6	26/−3	885/474	1.7	10.1
Methyl isobutyl ketone	2.0	5.1	−80	117	56/13	860/460	1.2	8
Acetone	100	160.5	−94	56.2	1/−17	1000/538	2.6	12.8
1-Pentanol	2.7	1.8	−78	138	120/49	572/300	1.2	10
Methyl acetate	25	148.7	−98	57	15/−9	935/502	3.1	16
Methanol	100	87.9	−98	65	52/11	835/446	6.0	36
Carbon tetrachloride	0.08	78.9	−23	76	None	None	None	None
Dimethyl sulfoxide	100	0.5	18.4	189	188/87	572/300	3.5	42
Hexafluoroacetone	100	5.0[b]	−129	−26	None	None	None	None
Chlorobutanol	0.8	–	97	167	>212/>100	–	–	–
Dimethyl sulfone	100	–	107	248	290/143	–	–	–
Acetic acid	100	11.6	16.2	118.5	103/39	960/516	6.6	19.3
Cyclohexane	0.008	66.4	6.5	81	−1/−18	500/260	1	9

[a] 100% = miscible.
[b] 25 °C.

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Table 2
Examples of drug preparations freeze-dried from co-solvents

Drug	Co-solvent system	Reference
Alprostadil (CAVERJECT® S.Po.)	20% v/v tert-butanol/water	Teagarden et al., 1998a
Aplidine	40% v/v tert-butanol/water	Nuijen et al., 2000
Amoxicillin sodium	20% v/v tert-butanol/water	Tico Grau et al., 1988
Gentamicin sulfate	tert-Butanol/water	Baldi et al., 1994
N-Cyclodexyl-N-methyl-4-(2-oxo-1,2,3,5-tetrahydroimidazo-[2,1-b] quinazolin-7-yl)oxybutyramide with ascorbic acid	50% v/v tert-butanol/water	Benjamin and Visor, 1989
Cyclohexane-1,2-diamine Pt(II) complex	tert-Butanol	Tanno et al., 1990
Annamycin	tert-Butanol/dimethyl sulfoxide/water	Zou et al., 1999
Cephalothin sodium	5% w/w isopropyl alcohol/water	Koyama et al., 1988
Cephalothin sodium	4% ethanol, 4% methanol or 4% acetone/water	Cise and Roy, 1981
Prednisolone acetate/polyglycolic acid	Carbon tetrachloride/hexafluoroacetone sesquihydrate	DeLuca et al., 1989a
Gabexate mesylate	Ethanol/water	Kamijo et al., 1987
Piraubidin hydrochloride	Ethanol/water	Kaneko et al., 1993
Progesterone, coronene, fluasterone, phenytoin	Chlorobutanol hemihydrate/Dimethyl sulfone	Tesconi et al., 1999
Fructose-1,6-diphosphate	tert-Butanol/water	Sullivan and Marangos, 1998
Poly(lactide-co-glycolide)	Acetic acid	Meredith et al., 1996
Dioleoylphosphatidylcholine and dioleoylphophatidylglycerol	Cyclohexane	Felgner and Eppstein, 1991
Vecuroniumbromide	Acetonitrile	Jansen, 1997
Bovine pancreatic trypsin inhibitor	Dimethyl sulfoxide/1% water	Desai and Klibanov, 1995

excipients) may be difficult to wet and get into solution or may require large amounts of water to adequately solubilize. The use of organic co-solvents can greatly facilitate the wetting of the hydrophobic substance, decrease the time to achieve a solution or uniform dispersion, and decrease the amount of solvent which needs to be removed during the drying process. All of these attributes can potentially have a positive effect on the consistency and ease of product manufacture. Several examples of this increased drug solubility in the presence of organic co-solvents targeted for lyophilization include: (1) alprostadil formulated in a tert-butanol/water solution (Teagarden et al., 1998a) and (2) cardiotonic phosphodiesterase inhibitors complexed with vitamins formulated in a tert-butanol/water solution, however, other alcohols such as ethanol, n-propanol, or isopropyl alcohol are also claimed to provide further increases in solubility (Benjamin and Visor, 1989) and (3) aplidine formulated in tert-butanol/water solution (Nuijen et al., 2000). The actual tert-butanol concentration (i.e. 40% v/v in water) selected for aplidine produced a greater than 40-fold increase in solubility compared to that in pure water.

3. Stabilization of bulk solution

A major challenge in developing a sterile injectable product can be its instability in solution. Most freeze-dried products are developed as this dosage form in order to circumvent poor stability. The manufacture of a freeze-dried product necessitates that the product is usually first manufactured as a solution, filtered to sterilize, aseptically filled, and finally lyophilized to remove the solvents. All of these unit operations require that the product be held in the solution state for a defined period of time. However, as the product is held in the solution phase it can experience various levels of degradation which are dependent on the kinetics of the degradation mechanism. The presence of the various levels of organic solvent can have a profound effect on the chemical stability. Those drug candidates which are very labile in aqueous solutions may require the added stability to achieve an acceptable level of degradation during manufacture. Early efforts to freeze-dry an anti-neoplastic agent (1,3-bis(2-chloroethyl)-1-nitrosourea) from an ethanol/water solution were initiated because of the rapid degradation in aqueous solution and improved solution stability in ethanol/water solutions (Flamberg et al., 1970). Unfortunately freeze-drying this product in the ethanol/water co-solvent system proved to be unsuccessful due to potency losses and unacceptable clarity. Flamberg et al. suggested that an alternative process to freeze-drying solvent systems containing ethanol would be to use low temperature vacuum drying. However, alprostadil has been successfully freeze-dried from a tert-butanol/water solution. The first-order degradation rate constant of alprostadil in 20% v/v tert-butanol/water ($k=0.0011$ day^{-1} at 25 °C) was significantly reduced compared to water buffered at the same pH value ($k=$

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0.0041 day^{-1} at 25 °C). These data are consistent with the claims of extraordinary stability of prostaglandins in tert-butanol (Monkhouse, 1975). This decreased degradation rate enables the manufacturing unit operations to be performed at ambient conditions without requiring cooling of the solution during manufacture. Additionally, it adds flexibility in scheduling these various operations because the solution degradation is now minimized.

The formulation of trecetilide fumarate, a sterile injectable in clinical development for treatment of arrhythmias, also involved freeze-drying from a tert-butanol/water mixture (Baker, 1998). Kinetic analysis showed solution degradation occurred by a process of defluorination through SN_1 substitution and E_1 elimination, both proceeding through the same carbonium ion intermediate. Since factors such as ionic strength, buffer type, solution pH, and drug and buffer concentrations did not significantly affect degradation rate, destabilization of the fluoride leaving group was one of the few methods left to control this reaction. Use of tertiary butyl alcohol as a co-solvent slowed solution state degradation by a factor of approximately 4–5. This significantly increased the probability of being able to scale up the manufacturing process while maintaining tight control of the level of degradation. The rate constant (k) for drug degradation was decreased substantially as the tert-butanol content was increased. The work required to separate two charges to infinite distance is related to the function $(1-1/Er)$ where Er is the relative permittivity of the medium. The linear relationship observed between log k and this function (Fig. 1) indicates that the decreased relative permittivity of the solvent system (i.e. the increased work required to remove the fluoride group) was the major effect for the improved solution state stability of trecetilide fumarate. The decreased ability of tert-butanol (relative to water) to solvate and stabilize the two ions appeared to be less of a factor. The use of tert-butanol allowed formulation and filling on a production scale over a 24-h period for this compound. The resulting freeze-dried product was predicted to have an acceptable shelf-life of at least 2 years at ambient temperature. This was a dramatic improvement compared to a frozen aqueous solution which had to be stored at −80 °C and required use within 3 h of thawing and admixture preparation. This type of effect would be expected to be observed for many other drug products which are degraded in the presence of water.

4. Impact on the freeze-drying process

4.1. Effect on freezing

The first stage of freeze-drying involves freezing the solution to remove solvent (typically water) from the drug and excipients through the formation of ice. The resulting semi-frozen system is cooled further to transform all components into a frozen state. A selected time/temperature profile is achieved by placing the solution, which is commonly held in glass vials or syringes, onto cooled shelves. Suspended impurities in the solution or imperfections in the walls of the container initiate heterogeneous nucleation during freezing. This event almost always involves supercooling where upon crystallization occurs below the equilibrium freezing point of the solution. Consequently, when freezing does occur, crystal growth tends to be rapid and results in a complex mixture of

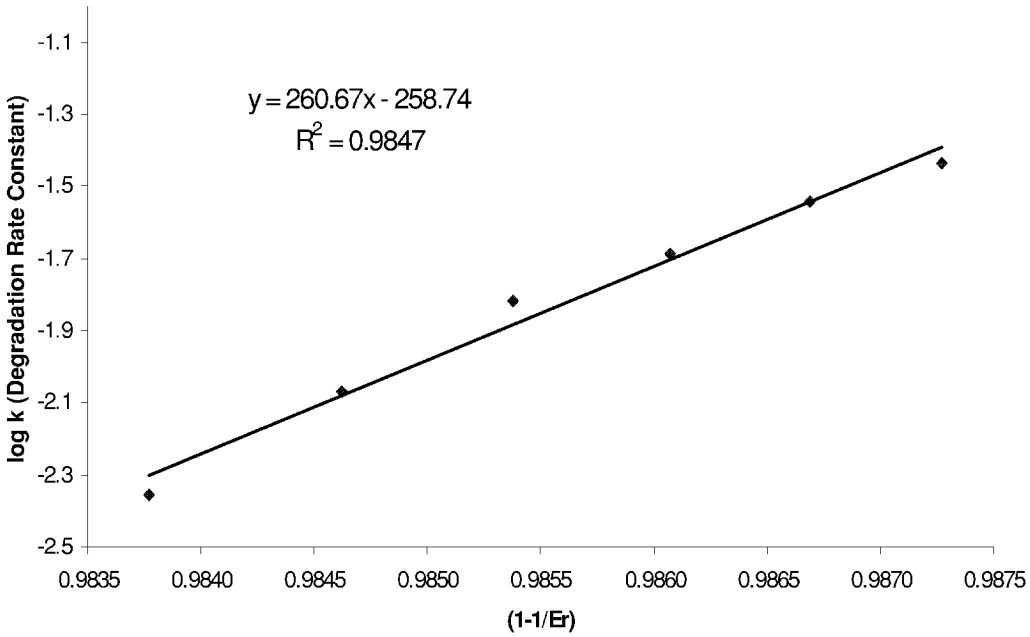

Fig. 1. Effect of relative permittivity (Er) on the solution degradation rate constant for trecetilide fumarate, a compound undergoing SN_1 substitution and E_1 elimination through the same carbonium ion intermediate.

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crystalline, amorphous and metastable materials. The impact of the presence of organic solvents on the various phases of freeze-drying has been discussed in detail (Seager et al., 1978, 1985; Seager, 1978, 1979a,b). Not surprisingly, the type and concentration of the organic solvent present affects the freezing characteristics of the solution prior to initiation of drying. The resulting frozen or semi-frozen solution significantly impacts the crystal habit of the ice, the drying rates, the collapse temperatures, the appearance of the dried cake, the surface area of the dried cake, and reconstitution properties, etc. The choice of solvent can also affect the degree of crystallinity of the drug. It has been demonstrated that incorporation of isopropyl alcohol readily results in highly crystalline cefazolin sodium (Koyama et al., 1988). Scaling up this process required incorporation of a heat treatment step to insure complete crystallization of the drug. Conversely, use of co-solvents can sometimes have deleterious effects during freezing. The use of volatile organic solvents has been reported to result in drug precipitation in the latter parts of freezing due to solvent evaporation. This can lead to an increase in drug concentration above its saturation level (Seager, 1979b). Care should be taken to select excipient concentrations such as buffer salts so that they do not exceed their saturation solubility. This is particularly important for phosphate buffers since they have very low solubility products with certain cations such as aluminum, calcium or iron (Hasegawa et al., 1982a,b,c, 1983). As a result, salt precipitation can produce a haze upon reconstitution. This problem can be exacerbated in co-solvent systems due to the decreased solubility and higher association constants for such systems.

The size and shape of the ice crystals has been found to vary with different organic solvents. The presence of high melting point solvents such as tert-butanol results in solvent crystallizing between the ice matrix as the temperature is decreased. The presence of the tert-butanol altered the crystal habit of the ice as it formed. The size of the ice crystals (i.e. large vs. fine) changed depending on the quantity of tert-butanol present in the system. Thermal analysis studies (via Differential Scanning Calorimetry and freeze-dry microscopy) have been used to evaluate the various stable and metastable states which form for tert-butanol/water systems during freezing (Kasraian and DeLuca, 1995a). The DSC warming thermograms for the tert-butanol/water mixtures are illustrated in Fig. 2. The authors were able to apply various annealing techniques to eliminate the metastable states and were able to construct the true phase diagram (Fig. 3). Although this phase diagram agreed well with other tert-butanol/water phase diagrams reported in the literature (Ott et al., 1979; Woznyj and Lüdemann, 1985), it was claimed that the slight differences could be explained by the presence of metastable events which thermal treatments eliminated. These data suggested that tert-butanol levels in the range of 3–19% caused the ice to form needle-shaped crystals. As these large needle-shaped crystals sublimed they created a more porous, less resistant matrix, which facilitates drying. Other solvents such as dimethyl carbonate also appear to freeze under production freeze-dryer conditions producing eutectic solids, solid organic solvent mixed with ice, drug, and excipients. However, most of the organic solvents investigated (Seager et al., 1985) such as methanol, ethanol, n-propanol, n-butanol, acetonitrile, methyl ethyl ketone, dichloromethane, and methyl isobutyl ketone do not freeze in typical commercial freeze-dryers but remain as liquid residues within the frozen matrix. Solutions containing 8% ethyl acetate, 10% dimethyl carbonate, or 10% n-butanol appeared to dry rapidly. Solutions containing 10% ethanol, 10% n-propanol, or 10% methanol appeared to dry slowly. Solutions containing up to 20% ethanol experienced collapsed cakes and were near impossible to dry (Seager et al., 1985). Some of the more hydrophilic solvents such as ethanol and methanol retained significant amounts of associated water which only partially froze as the temperature decreased. In those systems which completely froze (e.g. tert-butanol) the ice and frozen solvent grew upwards until reaching the solid surface and formed a eutectic skin. Hydrophilic solvents which retained large volumes of water formed thick liquid skins containing ice whereas less hydrophilic solvents containing less water formed thinner skins with less ice. The samples being dried need to be protected from radiant heat in order to prevent temperature fluctuations and non-uniform drying. This is especially important since the heat of sublimation is significantly lower for the organic co-solvents compared to ice (Willemer, 1975). It should also be noted that the time between filling the co-solvent solution and the freezing of this solution should be carefully controlled. The volatility of the organic portion of the solution can be such that a significant portion of the organic solvent can be lost due to evaporation. One should be aware of the potential for a reflux type phenomenon when using highly volatile solvents such as tert-butanol. This situation can happen when the evaporating tert-butanol condenses near the top of the vial and forms a stream of solvent returning to the solution. The dissolved substances in the solution can diffuse in this stream. After freeze-drying has been completed, the vial can contain spots of powder near the neck of the vial. The presence of dried powder near the neck of a vial is not desired because of both a poor appearance and the possibility of negatively impacting the seal with the rubber closure. This problem can be decreased by shortening the time period between the filling and the freezing of the solution.

4.2. Acceleration of sublimation rate

The freeze-drying process is a unit operation which typically involves a long and expensive process. Improvements in the rate of mass transfer of solvent through the partially dried cake layer will increase the rate of sublima-

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