Chapter I

Introduction

Rationale

Famotidine (3-[[[2-[(aminoiminomethyl) amino]-4-thiazolyl] methyl] -N-(aminosulfonyl) propionamide) is a relatively new and potent histamine-2 receptor antagonist (Compoli and Clissold,1986). It is structurally related to cimetidine and ranitidine, but differs principally in having a nucleus which is a thiazole rather than an imidazole (cimetidine) or a furan (ranitidine) ring (Fig. 1).

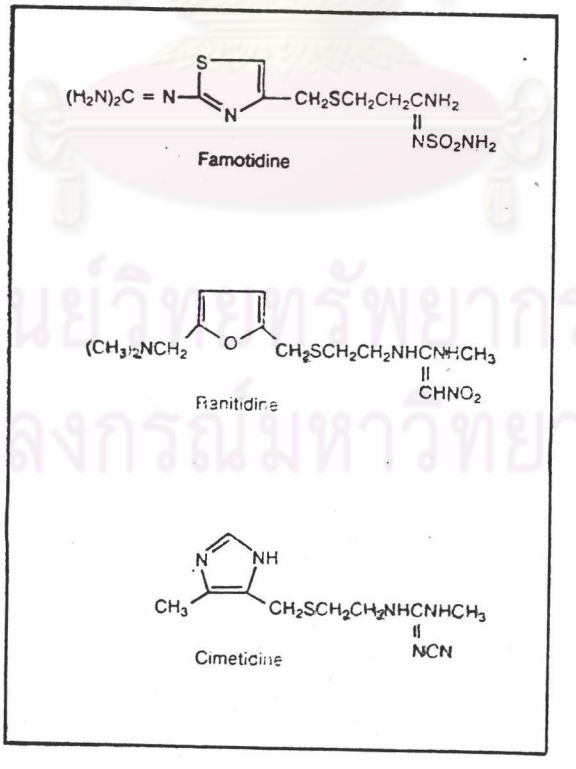


Figure 1 Structural formula of famotidine, ranitidine and cimetidine.

Famotidine has been found to be effective for acute treatment of duodenal ulcer, maintenance therapy in duodenal ulcer and treatment of pathological hypersecretory conditions like Zollinger Ellison syndrome. The drug is available as tablets of 20 and 40 mg and as vials for injection containing 20 mg famotidine.

The drug is incompletely absorbed, its oral bioavailability being 37-45 % (Campoli and Clissold, 1986; Kromer and Klotz, 1987). This has been partly attributed to its low water solubility (Vincek, 1988) and its susceptibility to acid-catalyzed hydrolysis in the acidic environment of the stomach. It was found to lose about 34 % of the original concentration in 1 hour and about 88 % in 3 hours (Suleiman et al., 1989).

Famotidine has an aqueous solubility of 0.1 % w / v at 20 ° C (Vincek et al., 1985). It is possible to change the physicochemical properties of such poorly soluble drug in order to improve the solubility and dissolution rate. This is, therefore considerably important in the manufacture of this tablet product. Since it was claimed that the products which produced high drug concentration at the site of absorption could exibit higher bioavailability.

While in the production of famotidine injection, there are also some factors to be concerned with. Since the drug has low water solubility and low stability in acidic solution, it is therefore suitable to produce as solid dispersion powder via lyophilized technique. This dosage form is not only make the product stable but also very benefitcial in giving rapid dissolution of the drug when adding the vehicle before injected into the body.

Mohammad et al. (1990) prepared an inclusion complex between famotidine and β-cyclodextrin by mixing the two components in a millimolar ratio in distilled water and heating under reflux for one hour followed by stirring at room temperature for 5 days. Phase solubility studies revealed the formation of a 1:1 complex of the A_L type with a rate constant of 74.96 M^{-1} . The formation of the complex in the solid state was confirmed by infrared spectroscopy and differential scanning calorimetry. The inclusion complex was shown by x-ray powder diffraction to be significantly less crystalline than any of the pure components. More significantly, the dissolution rate of the complex from constant surface area discs was determined to be about twice and six times higher than that of the physical mixture and the pure drug, respectively.

One of the suggested techniques for increasing dissolution rate of relatively insoluble drug is " solid dispersion " . The exact physical nature of these compositions is not clear, but it is believed that the insoluble drug is dispersed molecularly in the matrix of the (soluble) inert carriers. Upon exposure to the dissolution medium, the carrier dissolves or disperses rapidly and the finely dispersed particles are then released with optimum properties for dissolution. These reports (Chiou and Riegelman, 1971; Bloch and Speiser,1987; Ford, 1986) attributed the observed effects principally to a decrease in particle-size.

Mummareni and Vasavada (1990) prepared famotidine-xylitol solid glass dispersions by fusion method and investigated the aqueous solubility and dissolution rate. Solubility of famotidine from

solid glass dispersions and physical mixtures containing varing proportions of famotidine and xylitol at 37 ± 0.5 ° C was found to be higher than that of famotidine alone in water. Dissolution studies on glass dispersions with famotidine : xylitol ratios of 1:1, 1:10 and 1:20 in water at 37 ± 0.5 ° C revealed a marked increase in the dissolution rate of famotidine. The solid dispersions obtained by differential scanning calorimetry showed no evidence of chemical interaction between famotidine and xylitol. The phase diagram of the dispersion system by the capillary tube method suggested the formation of a eutectic mixture of famotidine and xylitol at a drug : carrier ratio approaching 1:40.

The advantages of solid dispersion systems in solubility and dissolution rate enhancement have been well documented. A number of publications have shown that carriers such as urea, polyethylene-glycol can increase the solubility and dissolution rate of various drugs (Chiou and Riegelman, 1970; Mayersohn and Gibaldi, 1966; Ford and Rubinstein, 1977). However, most of these systems have shown to be of limited application to manufacturing due to their poor processing properties resulting from the use of hydrophilic compounds such as polyethylene glycol and polyvinylpyrrolidone, which impart wax-like stickiness to the system (Khan, 1981). Therefore, if appropriate carriers can be found, this pharmaceutical technique and its underlying principles will be essential in increasing therapeutic efficacy of many drugs.

In addition to dissolution and absorption enhancement, the solid dispersion technique may have numerous pharmaceutical

applications which remain to be further explored. It is possible that such a technique can be used to stabilized unstable drug (Thakar, Hirsch and Page, 1977).

On the basis of explanation mentioned earlier , the solid dispersion technique should be used to develop the quality of famotidine products. The studies have been divided into two parts.

1. the application of the fusion method of the solid dispersions to increase the solubility and dissolution rate of famotidine which will improve the absorption of the drug from tablets that eventually may increase the bioavailability.

2. the utilization of lyophilized technique in the production of famotidine powder for injection in order to increase the solubility and maintain stability of the drug that could be applied in parenteral formulation.

The objectives of the present study were to:

a) prepare solid dispersions by a fusion and solvent method with various inert carriers.

b) compare some physicochemical properties of the prepared solid dispersions with pure drug, pure carrier and physical mixture.

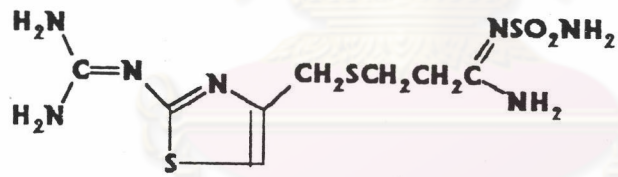
c) obtain famotidine solid dispersions which possess good tableting characteristics that can be practically applied to

manufacture and compare their dissolution rate with those of commercial tablet products.

d) select appropriate solid dispersion system from the lyophilization process and investigate the stability of the products both in the solution and solid state that can be formulated as injection.

Famotidine

1. Formular (United States Pharmacopeia Convention, 1990)



Empirical formula = $C_8H_{15}N_7O_2S_3$; Molecular weight = 337.43

2. Characteristics

Famotidine occures as a white to pale yellow , odorless, nonhygroscopic crystalline powder having a moderately bitter taste (Pepcid Prescribing Information, 1990).

3. Solubility

Famotidine is soluble in dimethylformamide, freely soluble in glacial acetic, slightly soluble in methanol, and very slightly soluble in water (Pepcid Prescribing Information, 1990).

4. Stability

Famotidine is very stable in the presence of heat. It should be preserved in well-closed, light-resistant containers.(Bullock and et. al., 1989; Jay, Fanikos and Souney, 1989; Montoro and et. al., 1989; Underberg, Koomen and Beijnen, 1988; Shea and Souney, 1990).

The tablets have an expiration date of 30 months following the date of manufacture when stored at the temperature of 40 ° C or less. (Pepcid Production Information for American Hospital Formulary Service, 1986 Oct.).

Famotidine injection should be refrigerated at 2-8 ° C. The powder form has an expiration date of 24 months following the date of manufacture when stored at 40 ° C or less. When diluted with most commonly used IV solutions (e.g., 0.9 % sodium chloride injection, 5 or 10 % dextrose injection, Lactated Ringer's, 5 % sodium bicarbonate) , famotidine solutions containing approximately 0.2-4 mg/ml are stable for 48 hours at room temperature (Pepcid Prescribing Information; 1989).

5. Pharmacology

After oral administration, famotidine in a range of 5 to 40 mg, inhibites basal and stimulated gastric acid secretion in a dose related manner. Peak antisecretory activity is reached within one to three hours, and inhibition lasts 10 to 20 hours. Intravenous administration of famotidine produces a similar antisecretory profile, with these doses being approximately twice as potent as the oral doses.

Controlled pharmacodynamic studies have revealed that famotidine does not affect physiologic functions other than those associated with gastric acid secretion. Famotidine has not been observed to affect other gastrointestinal functions or the functioning of the cardiovascular, endocrine, and renal systems.

Famotidine 40 mg twice a day does not alter gastric emptying time and exocrine pancreatic function. Blood pressure, heart rate, and electrocardiographic patterns remain unchanged after oral or intravenous dose 40 and 20 mg, respectively. Levels of serum prolactin do not rise after intravenous administration of famotidine 20 mg. During four weeks of treatment with famotidine 40 mg daily, levels of prolactin, testostorone, dehydroepiandrosterone, follicle stimulating hormone (FSH), luteinzing hormone (LH), and other circulating hormones remained unchanged (Campoli and Clissold, 1986).

6. Pharmacokinetics

After oral administration, dose related peak plasma concentrations are achieved within 1 to 3.5 hours. Mean peak plasma concentrations are between 50 and 60 µg/l after a 20 mg oral dose. A plasma concentration of 13 µg/l was required for 50 % inhibition of tetragastrin-stimulated gastric acid secretion in healthy volunteers. Plasma concentration is maintained at this level for 12 hours after a 40 mg oral dose. Bioavailability has been reported to be 37 to 45 % and is not dose dependent.

The apparent volume of distribution of famotidine is 1.1 to 1.4 l/kg, but the tissue distribution of famotidine has not been reported. Protien binding is relatively low (15 to 22 %).

Famotidine is excreted in the urine, by both glomerular filtration and tubular secretion, and in the feces. From 25 to 30 % of orally administration doses are recovered unchanged in the urine. The elimination half-life of orally administered famotidine is between 2.5 and 4 hours in patients with normal renal function, but increase to approximately 12 hours in patients with a creatinine clearance of less than 30 ml/min (Campoli and Clissold, 1986; Krans and et al, 1990).

7. Use

Famotidine is recommended for the following uses : (1) the short term treatment of active duodenal ulcer and acute benign gastric ulcer; (2) the prevention of relapses in duodenal ulcer

disease;(3) the treatment of patients with pathologic hypersecretory conditions, such as Zollinger Ellison syndrome and (4) the treatment of both symtomatic and erosive GERD (Campoli and Clissold, 1986; Pepcid Prescribing Information,1990).

8. Side Effect

Cumulated Japanese and Europian side effect data from short term controlled and open studies of the treatment of peptic ulceration, and patients maintained on treatment for longer periods (up to 10 weeks), reveal that famotidine 20 mg twice daily or 40 mg at bed time is well tolerated, side effects having been reported in only 3 to 7 % of patients, overally. The most frequently reported side effects are headache, dizziness, constipation and dirrhoea (Campoli and Clissold, 1986).

9. Dosage and Administration

The recommended oral dose of famotidine for acute duodenal or gastric ulcer is one 40 mg tablet daily at bed time for 4 to 8 weeks. However, the duration of treament may be shortened if endoscopy reveals that the ulcer has healed. For the prevention of ulcer recurrence, maintenance therapy with 20 mg daily at bedtime is recommended. Patients with Zollinger Ellison syndrome should be started on a dose of 20 mg 6 hourly and dosage adjusted to individual patient needs; doses of up to 480 mg daily have been used for up to one year.

Dosage may be decreased in patients with impaired renal function (Campoli and Clissold, 1986).

10. Preparations in Thailand (TIMS 1994)

Famotidine preparations in Thailand are described in trade name, distributor, dosage form, dose per dosage form in Table 1.

Table 1 Famotidine products in Thailand.

Trade Name	Distributor	Dosage form	Dose/Dosage form
1. Fadine	Biolab	Tablet	20 mg
2. Famoc	Berlin	Tablet	20 and 40 mg
3. Fanitin	Eurodrug	Tablet	20 and 40 mg
4. Pepcidine	Merck Sharp & Dohme	Tablet Injection	20 and 40 mg 20 mg (lyophilized)
5. Pepfamin	Siam Pharmaceutical	Tablet	20 and 40 mg
6. Peptoci	Pharmasent lab	Tablet	40 mg
7. Pepzan	Douglus	Tablet	20 and 40 mg
8. Ulceran	Medochemie	Tablet	20 and 40 mg
9. Ulcofan	Codal Synto	Tablet	20 and 40 mg

Literature Review

1. Historical Background of Solid Dispersion

It has been observed that the rate of absorption of many poorly water-soluble drugs from the gastrointestinal tract is limited by the rate of dissolution of the drug substance (Florence and Attwood, 1981). Enhancement of dissolution properties generally improves the rate and extent of gastrointestinal absorption and bioavailability of such drug (Chiou and Riegelman, 1970; Wiseman , McIlhenny and Bettis,1975; Stupak and Bates,1972).

Many efforts have been made for increasing dissolution characteristics of poorly-water-soluble or insoluble drugs. These include the use of soluble salts, polymorphs, hydrates or solvates, molecular complexes; adsorption onto an inert water soluble compound (Monkhouse and Lach, 1972; Khan ,1981; Wiseman, McIlhenny and Bettis, 1975); the use of drug derivatives (which is a chemical approach (Sinkula and Yalkowsky, 1975) and reduction of particle size (Ammar, Kassem and Salama, 1980). Among these techniques, reduction of particle size remains the most accepted method for increasing drug dissolution. Particle size reduction is usually achieved by :

1. Conventional trituration, grinding and other methods such as ball milling and fluid energy micronization (Chiou and Riegelman , 1971) .

2. Controlled precipitation by change of solvents or temperature on application of ultrasonic waves and spray drying (Chiou and Riegelman, 1971; Hem, Skauen and Beal, 1967; Sheikh, Price and Gerraughty, 1966).

3. Administration of liquid solutions from which, upon dilution with gastric fluids, the dissolved drug may precipitate in very fine particles.

4. Administration of water - soluble salts of poorly soluble compounds from which the parent, neutral forms may precipitate in ultrafine form in GI fluids (Chiou and Riegelman, 1971) .

However, easily and directly the reduction of particle size can be accomplished by the four method mentioned aboved, the resultant fine particles may not produce the expected faster dissolution and absorption. This is primarily due to the possible aggregation and agglomeration of the fine particles and air adsorption caused by their increased surface energy and the subsequent stronger Van Der Waal's attraction between nonpolar molecules , or their poor wettability in water. Moreover, drug with plastic properties are difficult to subdivide by method 1-4. Controlled precipitation by change of solvent by method 2 is not frequently employed in commercial market due to such reasons as selection of a nontoxic solvent , limitation to low dose , and high costs of production. The water-souble salts of many pooly soluble acidic or basic drugs have been widely used clinically in solid dosage forms and have been shown frequently to produce better adsorption

than their parent compounds. Nevertheless, the sodium and potassium salts may react with atmospheric carborn dioxide and water to precipitate out poorly soluble parent compounds. This occurs especially on the outer layer of a dosage form and thereby retards the rate of dissolution and absorption. In addition, the alkalinity of some substances may cause epigastric distress following administration (Chiou and Riegelman, 1971; Ford, 1986; Bloch and Speiser, 1987).

A unique approach of solid dispersion to reduced particle size and increase dissolution and absorption rates was first introduced by Sekiguchi and Obi (1961) . They proposed the formation of a eutectic mixture or molecular dispersion of a poorly soluble drug such as sulfathiazole with a solid matrix of physiologically inert , easily water-soluble carrier such as urea. Upon exposure to aqueous fluids , the carrier dissolveds rapidly and finely dispersed active drug was expected to be released with optimum properties for dissolution. Since then , extensive studies and excellent work were made and modifications of this technique have been suggested (Anastasiaduo et al., 1983; Attia and Habib , 1985; Babar and Jarowski , 1983).

2. Definition

The term " solid dispersion " refers to " the dispersed system of one or more active ingredients in an inert carrier or matrix at solid state prepared by melting (fusion), solvent, or melting-solvent method " . Since the dissolution rate of a component from a surface

Is affected by the second component in a multiple-component mixture , the selection of the carrier has an ultimate influence on the dissolution characteristics of the dispersed drug. Therefore, a water-soluble carrier results in a fast release of the drug from the matrix, and a poorly water soluble carrier leads to a slower release of the drug from the matrix. Since this techique is commonly used for increasing dissolution, this review paper emphasizes on fast-release solid dispersion. However, some of the principles may also be applied to slow-release solid dispersion system (Chiou and Riegelman, 1971; Bloch and Speiser , 1987; Ford, 1986).

The solid dispersion may also be called under a variety of names, including solid state dispersion , solid solution , eutectics , fast release solid dispersion and coprecipitates . The term " coprecipitates " has also been frequently used to refer to those preparations obtained by the solvent methods such as reserpine-PVP coprecipitates (Geneidi, Ali and Salama , 1978; Miralles, Mcginty and Martin , 1982; Reddy, Khahl, and Gouda, 1976; Simonelli, Mehta, and Higuchi, 1969; Stupak, and Bates, 1972).

3. Methods of Preparation of Solid Dispersions

3.1 Melting Method

The melting or fusion method was first proposed by Sekiguchi and Obi and was subsequently employed with some modification by many investigations (Ford and Elliott, 1985; Jachowicz, 1987; Jain and Parikh, 1986; Ravis and Chen, 1981). A schematic diagram of preparation steps is shown in Figure 2 .

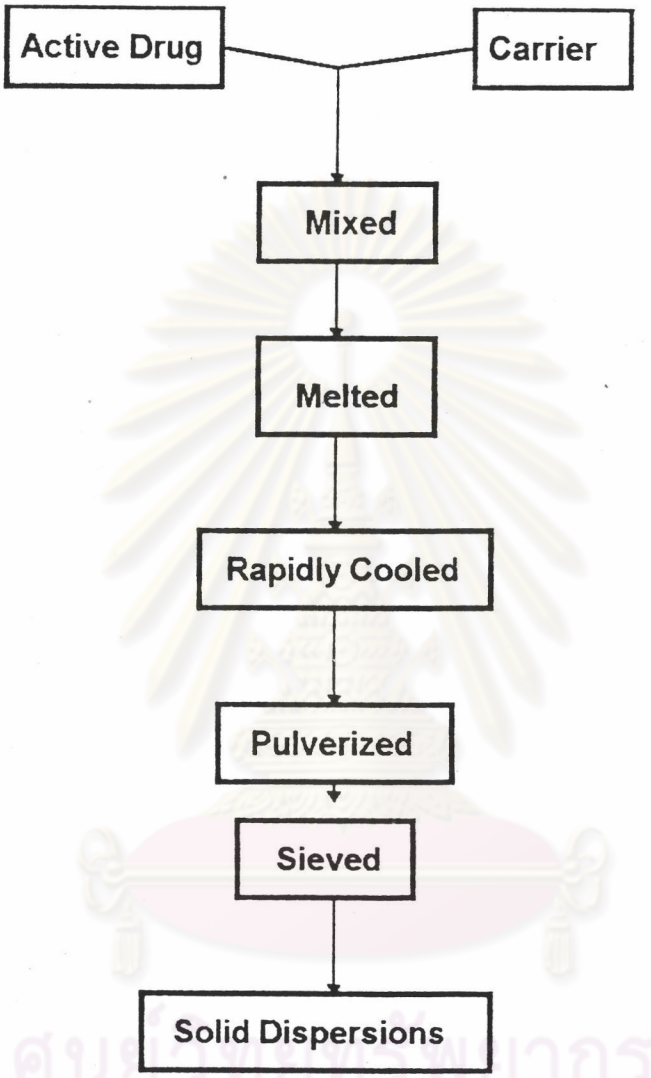


Figure 2 A schematic diagram for preparing solid dispersions by melting method.

The advantage of melting method are:

1. Its simplicity and economy.

2. A supersaturation of a solute or drug in a system can often be obtained by quenching the melt rapidly from a high temperature.

3. A much finer dispersion of crystallites was obtained for systems of simple eutectic mixtures if such quenching techniques were used.

The disadvantages are:

1. Many substances, either drugs or carriers may decompose or evaporates during the fusion process at high temperature.

2. Only low melting carriers can be used.

3. The resulting product from melting method is always difficult to be pulverized.

3.2 Solvent Method

This method was initially used by Tachibana and Nakamura (Tachibana and Nakamura, Quoted in Ford, 1986). After that it was widely used in the preparation of many solid dispersion systems, such as chlorpropamide-PVP (Deshpande and Agrawal, 1984), frusemide - PVP (Doherty and York, 1987), nalidixic acid - Myrj ® (Elgindy, Shalaby, and Elkhalek, 1983), corticosteroid-PEG (Khalil , Elfattah , and Mortada , 1984), prednisolone - gelatin (Kimura et al., 1990).

Solid dispersions prepared by solvent removal processes were termed by Bates (1969) as " coprecipitates " and this term has been misconstructed by many other wokers. They should, more correctly, be designated as " coevaporates ", a term that has been recently adopted (Sekikawa et al., 1983). A schematic diagram of this method is shown in Figure 3 .

The advantages of the solvent method are:

1. Thermal decomposition of drugs or carriers can be prevented because of the low temperature required for the evaporation of organic solvents.
2. High melting carriers can be used.

The disadvantages are:

1. The higher cost of preparation.
2. The difficulty in completely removing liquid solvent which may affect the chemical stability of the drug.
3. The difficulty in selecting a common volatile sovent.
4. The difficulty of reproducing crystal forms.
5. A supersaturation of the solute in the solid system cannot be attained except in system showing highly viscous properties.
6. Solvent flammability and toxicity.

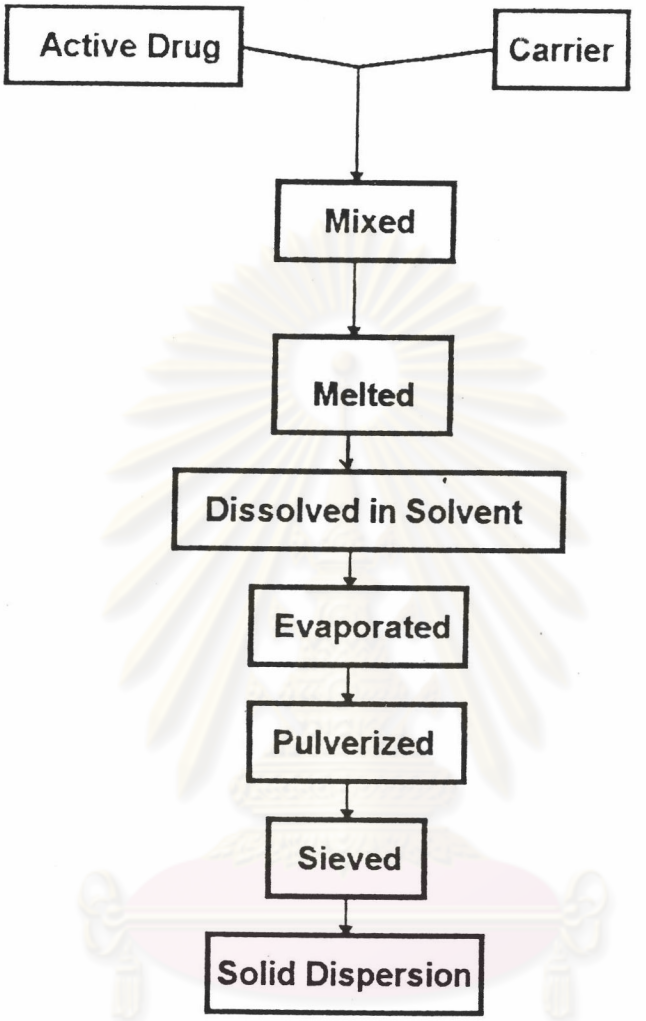


Figure 3 A shematic diagram for preparing solid dispersions by solvent method.

By the solvent method, organic solvent can be eliminated by three possible techiques.

1. simple evaporating
2. freeze drying (lyophilization)
3. spray drying

3.3 Melting-Solvent Method

This method is derived from melting method by Chiou and Smith (1971). It was shown recently that 5-10% (w/w) of liquid compounds could be incorporated into PEG 6000 without sigificant loss of its solid property. Hence, it is possible to prepare solid dispersions by first dissolving a drug in a suitable liquid solvent and then incorporating the solution directly into the melt of PEG, obtained below 70°C, without removing the liquid solvent. A schematic diagram of this method is shown in Figure 4. The feasibility of this method was demonstrated on spironolactone-PEG 6000 and griseofulvin-PEG 6000 systems (Chiou and Riegelman, 1971).

The advantages of both melting and solvent methods, from a practical standpoint, are only limited to drugs with a low therapeutic dose, e.g, below 50 mg. (Chiou and Riegelman, 1971).

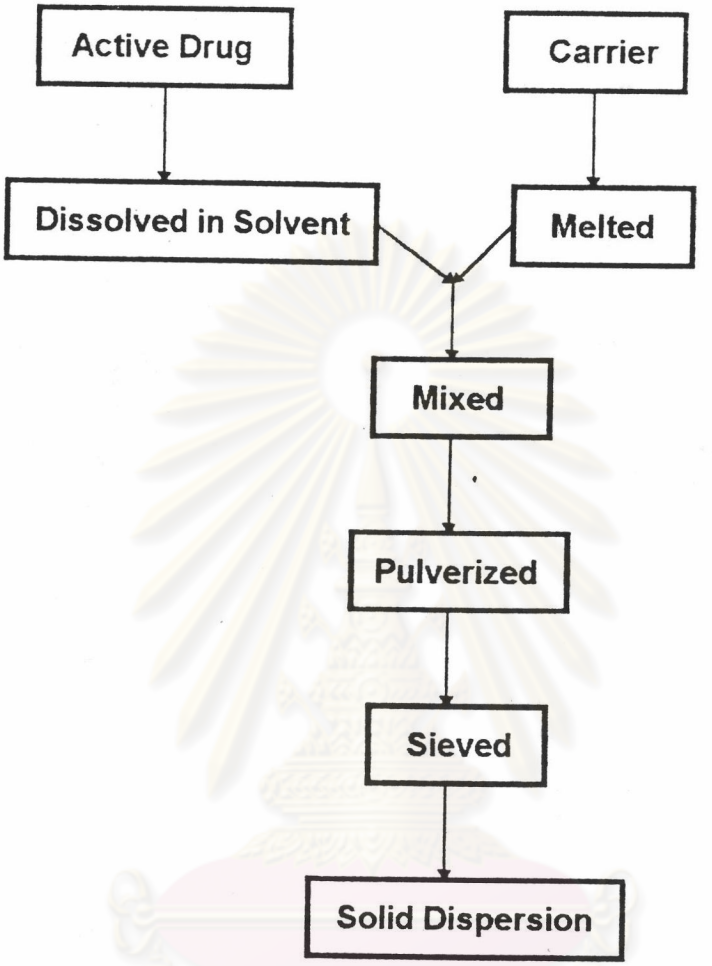


Figure 4 A schematic diagram for preparing solid dispersions by melting-solvent method.

4. Method for Determination of Types of Solid Dispersion Systems

Many methods are available for contributing information regarding the physical nature of a solid dispersion system. In many instances, a combination of two or more methods is required to study its complete picture (Chio and Riegelman, 1971; Mcginity, 1978).

4.1 Method for Establishment of Phase Diagrams

These methods concern thermal analysis. From phase diagram, the type of system can be obtained.

4.1.1 Thermomicroscopic Method - By this method , polarized microscopy with a hot stage is used. The thaw and melting points of binary systems are determined by visual observation and various forms of phase diagrams can be constructed (Chiou and Riegelman, 1971; Ford and Rubinstein, 1978).

4.1.2 Thaw-Melt Method - In this method, a sample of solidified mixtures in a capillary melting-point tube is heated gradually and the thaw point is determined. (The thaw point is the temperature on crossing a solidus line). Such method was modified and utilized by using a stirring divice in capillary tube for more accurate results, the stirring facilitates the attainment of a homogenous system (Chiou and Riegelman, 1971).

4.1.3 Cooling-Curve Method - In this method, the physical mixtures of various compositions are heated until a homogeneous

melt is obtained. The temperature of the mixture is then recorded as a function of time. From a series of temperature-time curves, the phase diagram can be established (Chiou and Riegelman, 1971).

4.1.4 Differential Thermal Analysis and Differential Scanning Calorimetry - These are effective thermal methods for studying a pure compound or a mixture. Differential effects, associated with physical or chemical changes, are automatically recorded as a function of temperature or time as the substance is heated at a uniform rate. Melting and decomposition can be setected.

These techniques are especially valuable in detecting the presence of a small amount of eutectic in the mixture, because its melting at the eutectic temperature can be sensitively detected (Chiou, 1971; Chiou and Niazi, 1971; Chiou and Riegelman, 1971; Ford, 1978; Ford and Rubinsten, 1977).

4.2 Methods for Determining Physical Nature of Solid Dispersions

4.2.1 Microscopic Method - Microscopy has been used to study morphology and polymorphism of solid dispersions. The high resolution of an electron microscope was used to study the dispersed particle of hydrocortisone in PEG dispersions. Photoplan microscope was also used to study aging of indomethacin-PEG 6000 solid dispersion (Ford and Rubinstein , 1979; Kaur, Grant, and Eaves, 1980).

4.2.2 Spectroscopic Method - IR spectroscopy is usually used to study polymorphic phenomena or complex formation in solid dispersion systems. For example, the utilization of IR spectrum could indicate the complexation between PVP and cholic acid in the coprecipitate (Chiou and Riegelman, 1971; Geneidi, Adel, and Shehata, 1980).

4.2.3 X-Ray Diffraction Method - In this method, the intensity of the X-ray diffraction (or reflection) from a sample is measured as a function of diffraction angles.

The diffraction method is a very important and efficient tool in studying the physical nature of solid dispersions. It is also valuable in detecting compound or complex formation since its spectra or lattice parameters are markedly different from those of pure components. It has been used to study quantitatively the concentration of a crystalline component in the mixture. This method was used to study various dispersion systems (Chiou, 1971; Chiou and Niazi, 1971; Chiou and Riegelman, 1971; Christian and Oreilly, 1986; Ravis and Chen, 1981; Takayama, Nambu and Nagai, 1982).

Lyophilization

Lyophilization frequently term " freeze drying " is a drying process where the solvent normally water, is first frozen and then removed by sublimation in a vacuum environment. In the pharmaceutical industrial, the aqueous solution is filled into suitable container, generally vials, which are loaded onto temperature-

controlled shelves installed in a large drying chamber (see the process and model of basic industrial lyophilizer in Figure 5 and 6, respectively).

In the lyophilization process , water is removed from a frozen solution by sublimation to leave a dry porous solid that is easy to redissolve. The sublimation of the frozen solution is occured at the pressure and temperature below the tripple point (see Figure 7). A product is freezed dried if the aqueous solution does not have sufficient stability for marketing, and if the product cannot be crystallized in bulk. Compared to spray drying , freeze drying is a low temperature process, and is normally regarded as being less destructive to the product, particularly for protiens. In addition, control of sterility and foreign particulate matter is relatively straight forward. Thus, although inherently expensive from the view points of plant cost and energy consumption, freeze drying is often the processing method of choice for the production of a parenteral product. The impact of high costs of freeze drying is often the processing method of choice for quality and the realization that raw material costs frequently dwarf the freeze drying costs. Moreover, an understanding of the freeze drying process coupled with process optimization studies can significantly reduce the duration , and therefore costs of the precess as well as ensure control of product quality (James, C. and Boyle, N, 1992).

Example of some typical materials which are dried by freeze drying : (James M. Flink and Henning Knudsen, 1983).

- Microorganisms (bacteria, yeast) and Virus
- Vaccines , antitoxins and blood fraction

- Enzymes (for analytical systems and / or reactions in general)
- Tissues and bone materials for transplants
- Biological reagents and standards
- Food materials (beverages, fruit, vegetables, meat, fish)
- Pharmaceutical preparations
- Most types of sensitive agents in chemical / biochemical laboratories (including free radicals and physiological media)
- Tissues and cells for electron microscopy

The freeze drying process may be divided into three stages : (N.A. Williams and G.P. Polli, 1984)

1. Freezing
2. Primary Drying
3. Secondary Drying

The three stages can be explained in details as follow :

1. <u>Freezing</u>

In general, the freezing of an aqueous binary solution , consisting of a solute in water , may be considered to occur as follows : assuming the solubility of the solute is high enough so that the former is not deposited on cooling, ice crystals first firm at the temperature usually below 0 ° C. As the ice crystals form and grow throughout the system , the remaining solution , referred to as " interstitial fluid " , becomes more concentrated in the solute. If the solute forms a true eutectic with water e.g., sodium chloride, a eutectic phase consisting of finely divided crystals of the solute and ice crystallizes out and the whole system becomes solidified.

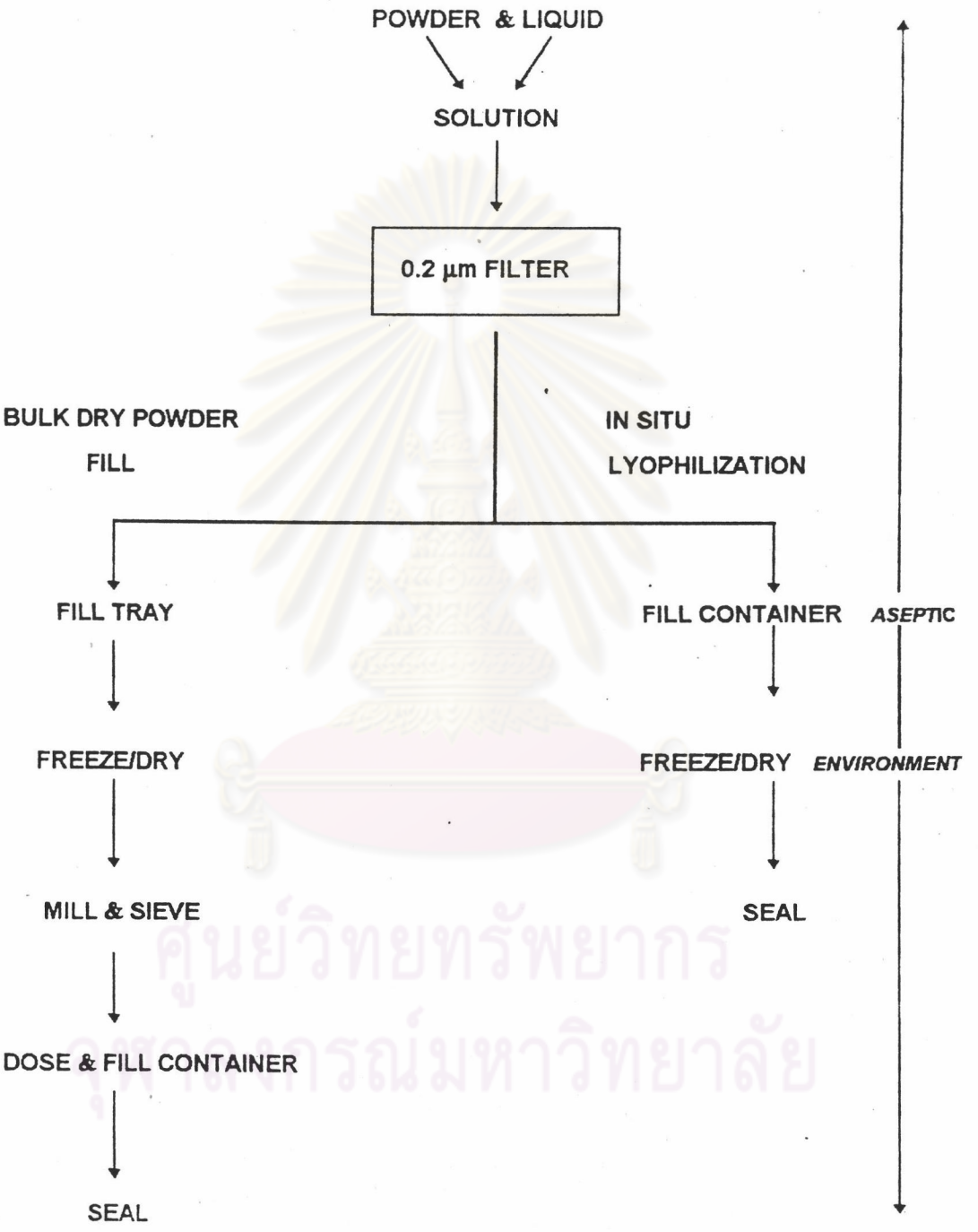


Figure 5 A schematic diagram for preparing dry powder by lyophilization process.

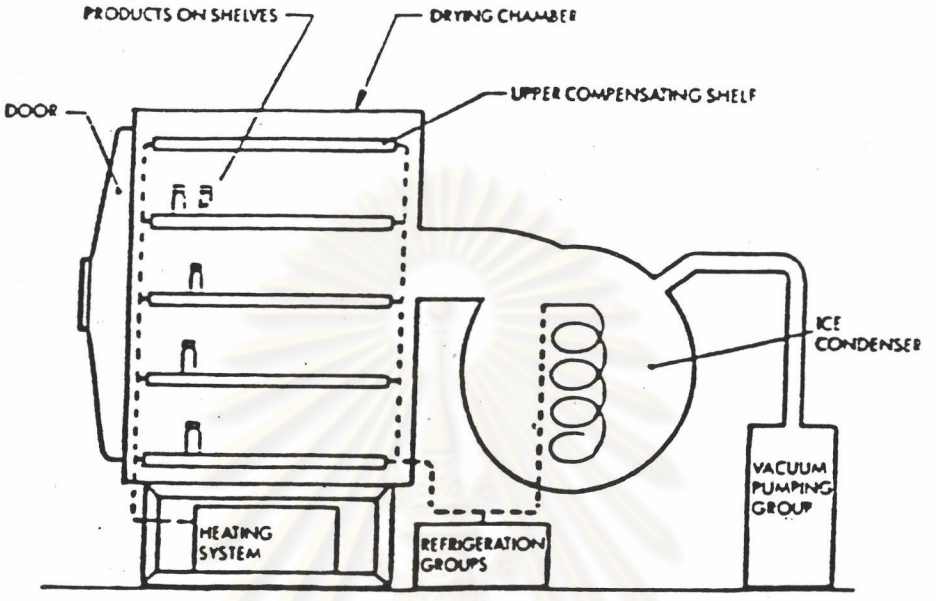


Figure 6 Basic industrial freeze dryer.

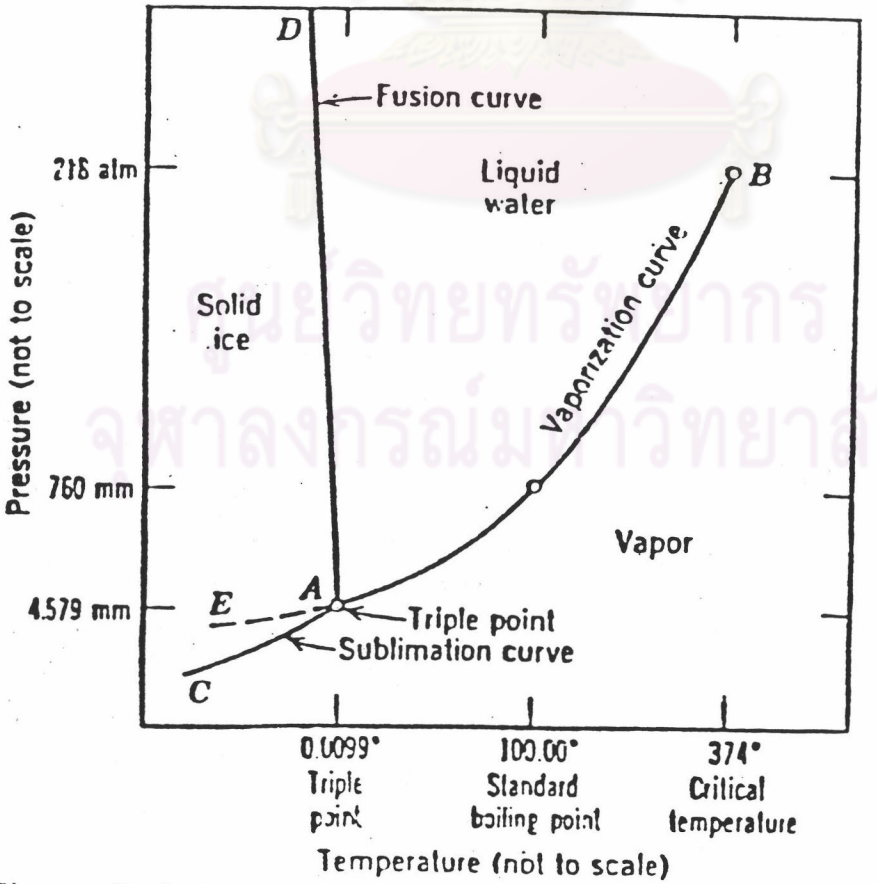


Figure 7 A temperature-pressure diagram of pure water.

Critical Temperature

1. The temperature of complete solidification (Tcs) is the highest temperature at which any liquid state ceases to be present in the preduct during cooling. It is a state that must be achieved if a solution is to be considered freeze-dried in the strict sense.

2. The temperature of incipient melting (Tim). It is often referred to as the eutectic temperature, only equivalent to the eutectic temperature in compounds like sodium chloride, which form true eutectic mixtures in water.

2. Primary Drying

As described earlier , the primary drying stage involves sublimation of ice from frozen product. In this process :

(I) Heat is transferred from the shelf to the frozen solution through the tray (if there is any) and the vial, and conducted to the sublimation front (see Fig. 9).

(ii) The ice sublimes and the water vapor formed passes through the dried portion of the product to the surface.

(iii) The water vapor is transferred from the surface of the product through the chamber to the condenser.

(iv) The water vapor condenses on the condenser.

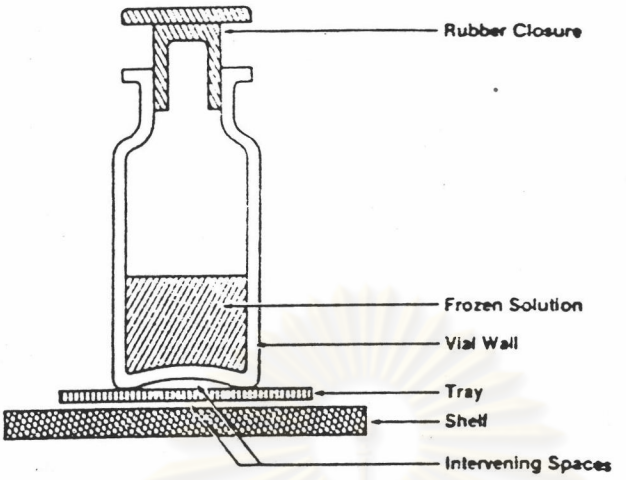


Figure 8 Diagram showing barriers to the transfer of heat from a shelf to a frozen solution in a vial.

2. Secondary Drying

Secondary Drying involves the removal of observed water from the product. This is the water which did not separate out as ice during the freezing, and so did not sublimate off. It is usually present in enough quantities to cause rapid decomposition of the product , if it is stored at room temperature as it is.

The residual moisture content desired for a product usually determines the length of time devoted to secondary drying. For pharmaceuticals, it appears that moisture contents of 1 % and less are the most desirable. Since it is impossible to achieve zero percent moisture content in the dryer, a limit somewhere below 1 % must be selected.

To accomplish the desorption of the water , the product temperature is usually raised and the chamber pressure

further reduced. Although the secondary drying stage generally occures after primary drying , some desorption of water may accompany the sublimation of ice.

The temperature to which the product must be raised during secondary drying must be considered carefully. For example, exposure of heat-labile substances, e.g., antibiotics, to high temperatures for a long period may cause significant decomposition. Sometimes, a choice has to be made between exposure to a high temperature for a short period or to a longer temperature for a longer period.

During secondary drying, the product temperature generally rises gradually and finally equals the shelf temperature. If there is no precise method of determining the point at which the moisture content is low enough for the run to be terminated, the dryer is allowed to run for several hours more before termination. On the one hand, this may result in an unnecessary waste of time and energy if the product dries quickly. On the other hand, if the desorption of moisture occures slowly, it may result in higher than desired moisture contents because of premature termination of the cycle.

To help in determination much more precisely the " end point " of secondary drying, several methods have been developed. These include :

(i) Windmil Device (Couriel, B., 1977)

(ii) Trapped Pressure Ratio (Mellor,J.D., 1978)

(iii) Sample Thief Probe (LeFloc'h, L.)

(iv) Residual Gas Analytical System (Jennings, T.A., 1980)

Windmill Device

The windmill device has been described by Couriel. Sensitive windmills mounted on a tray are placed in certain locations above the vials being freeze-dried, so that as water vapor flows out of the vials , it spins the windmills stop spinning and the product is considered dry. This method reportedly produces consistent moisture contents. Since successful use of the windmill device depends on proper observation, it needs to viewed from the outside. In Couriel's report, there was no indication of how sensitive the device is to low partial pressure of water on the sample surface.

Trapped Pressure Ratio

This method works on the principle that the lower the moisture content of the product being dried, the lower the partial pressure of water in the chamber. Therefore, at the beginning of secondary drying, the partial pressure of water in the chamber will be high and will gradually decrease to an equilibrium value at the end of the cycle. The apparatus described by Mellor consists of a copper U-tube conncted to a Pirani vacuum gauge at one end, while the other end opens into the drying chamber by a connection which can be closed or opened by a valve. The end connected to the chamber has a tube inside the chamber that can be directed close to the product.

To make a measurement, the valve is opened and the total pressure (water vapor and air) is read on the gauge. Then the valve is closed, the water vapor is condensed out by means of a Dewar flask placed under the U-tube and the pressure again read. The trapped pressure ratio (TPR) is defined as the pressure, after the water is condensed out divided by the pressure before condensation. Typically, TPR starts out being less than 0.1 , increases and then levels out as it approaches 1 , i.e., as the partial pressure in the chamber decreases. To relate TPR measurements to actual moisture content, samples should be taken out and analyzed for moisture and the values correlated with TPR values. A calibration curve of TPR versus moisture content may then be drawn from a few points. The apparatus is said to be able to determine TPR values in the range 0.5 to 5 % with an accuracy of 0.5 % .

Sample Thief

The sample thief probe is now a common optional feature of many laboratory-size freeze-dryers designed for freeze-drying liquids on vials. It allows an operator to select a vial, stopper it, and retrieve it from the drying chamber, during any stage of the lyophilization process without altering the conditions in the chamber. Thus, vials can be taken out and analyzed for moisture content (often by the Karl Fischer Method), weight loss, or any property desired without having to stop the cycle. This facilitates the determination of the end point of secondary drying by following the moisture content or weight loss (of preweighed vials) with time.

Residual Gas Analytical System

The Residual Gas Analytical System (RGAS) is a mass spectrometer specially disigned for analysis of the vapor and gaseous contents of the freeze-dryer chamber. To obtain the mass spectra of the chamber contents, a valve connecting the chamber to the RGAS is adjusted to allow the vapor/gas in only at a specified low pressure. The fragmentation patterns of the gases and vapors are then obtained.

The components of the chamber are identified by their mass to charge ratio m/e, e.g., for water a peak appears at an m/e of 17 and 18. To be able to use the RGAS to determine the end of secondary drying , a pressure gauge must be connected to the chamber. Assuming the gauge is able to measure total pressure accurately, the partial pressure of water in the chamber may be calculated as follows : the peak heights of all the gases (e.g., N_2,O_2, Ar, CO_2) and water are summed up. The ratio of the peak height of water to the total is then equated to the ratio of the partial pressure of water to the total pressure. Since the total pressure is known, the partial pressure of water can be calculated.

Formulation Consideration

1. Selection of Optimum Fill Volume

For a given dose , a decision must be made regarding the concentration (and therefore the fill volume) to be used. In general,

the primary drying time increases as the concentration decreases and the fill volume increases. Basically, three factors are involved :

(a) a lower fill volume means less ice to sublime , and therefore a shorter drying time results.

(b) a lower volume means a smaller cake depth which, if the corresponding higher concentration did not increase the product resistance at a given dried product thickness, and therefore a shorter drying time would result.

(c) a lower fill volume means a higher concentration which, in general, increases the dried product resistance at a given thickness. This effect increases the primary drying time.

2. Excipient Selection

For freeze- dried products , formulation and process are interrelated. Properties of the formulation, in particular the collapse temperature, have a significant impact on design of the freeze-drying process, and excipient selection may, in large part, determination the process economics. In addition to the active component (the drug), other components may be added for specific purposes. Bulking agents, such as mannitol or glycine, are added to enhance appearance as well as to prevent product " blow out ". With formulations very low in total solids (about 1 %) , the streaming water vapor can disrupt cake structure and carry some or all of the dried material out of the container with the water vapor stream, thus giving product " blow out ". Buffers are frequently added for pH control, and salts (e.g., NaCl) may be added to yield an isotonic solution. Use

of NaCl in the formulation may lower the collapse temperature of the system, if NaCl fails to crystallize ,or give a relatively low eutectic temperature (-21 ° C) , if NaCl does crystallize . To avoid these problems, tonicity is best addressed in formulation of the diluent provided to reconstitute the solid.In special cases, exipients may be used to increase solubility and stability. For example , tissue plasminogen activator does not have sufficient solubility for clinical use In a pH range compatible with stability (~ pH 7.4) , but when arginine is incorporated into the formulation , the solubility is acceptable at pH 7.4 . In addition, solution stability is enhanced in the presence of arginine (Pearlman. R., and Nguyen T. H, 1989).

Excipients may also be added to increase collapse temperature. Usually, the collapse temperature of the mixture is intermediate between that of the individual components, but collapse temperatures of candidate formulations must be measured. They generally cannot be predicted with useful accuracy. Collapse temperature can be modified by dextran (-10 ° C) , ficoll (-20 ° C) , gelatin (-8 ° C) , human serum albumin (-9.5 ° C) ,and hydroxyethyl starch (> -5 ° C) . It should be emphasized that the composition of the amorphous phase formed during freezing determines the collapse temperature. Therefore, organic solvent impurities, buffers and other salts, which do not crystallize, also affect (usually decrease) the collapse temperature. Particularly with protiens, an excipient (a so called lyoprotectant) is frequently added to enhance stability during the freeze drying process as well as increase the stability of the freeze-dried solid during storage. Commonly , sugars

(e.g., trehalose, sucrose) and protiens (e.g., human serum albumin, bovine serum albumin) are used as lyoprotectants. Unfortunately , of the many materials that have been shown to stabilize protiens during or after freeze drying, only a few (mannitol, glycine, arginine, lactose) have a " generally accepted " history of use in formulating products for parenteral therapy. A " nontraditional " excipient may cause toxicology or market-acceptability concerns. Several other potential excipients (human serum albumin, dextran, hydroxyetyl-starch) have themselves been used in parenteral therapy and therefore should not pose serious problems of toxicity.

3. Buffer and Changes in pH on Freezing

The pH of a given buffer system depends on the ratio of the molar concentrations of the acid and base components of the buffer system. As long as all buffer components remain in solution, temperature changes do not greatly affect the pH. However, during the freezing process, buffer components may crystallize, resulting in large pH shifts. The differences between the cooling and warming curves reflect supersaturation during cooling. The large decrease in pH as the temperature decrease is due to crystallization of the buffer component,