Chapter II

Literature Review

Starch is a polymeric carbohydrate composed of anhydroglucose units. It is extracted in granular form from plant tissues. Starch granules are deposited in the seeds of cereal grains (maize, wheat, sorghum, rice and glutinous rice), tubers (potato), roots (tapioca, sweet potato, arrowroot) and the pith of the sago palm, as a reserve food supply for period of dormancy, germination and growth. The microscopic study reveals that starch is composed of tiny, white granules, ranging in size from about 1 to 100 micrometer in diameter. Sized shapes of the granules are related to the botanical sources of the starch. After cellulose, starch is the next most abundant compound synthesized by plant cells (Chaley, 1982).

Starch is a carbohydrate, composed of carbon, hydrogen and oxygen atoms in the ratio 6:10:5 $(C:H:O)_n$ glucose, consisting of anhydroglucose units. Glucose units are linked to one another with glucoside bond (Figure 1). Most starches are a mixture of amylose and amylopectin, each having a wide range of molecular sizes. Starches from the different origins have different amylose to amylopectin ratios (Rutenberg, 1980).

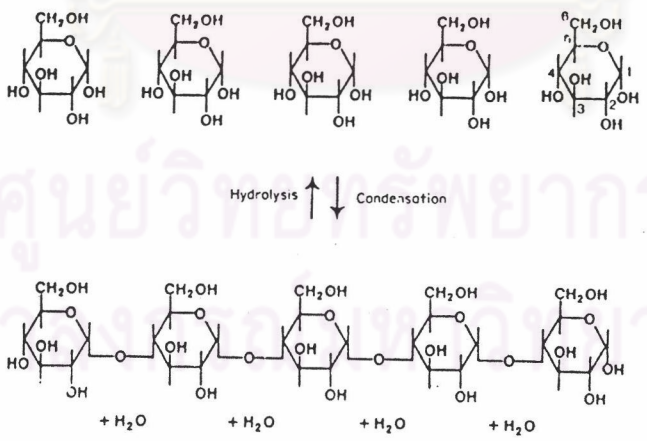


Figure 1 Starch considered as condensation polymer of glucose (Rutenberg, 1980)

Note numbering of carbon atom in glucose and the spatial relationship of hydroxyl groups with respect to six membered ring containing 5 carbon atom and 1 oxygen atom.

Starch is a homopolymer of D-glucose in which 96% or more of the D-glucose units are linked alpha-D-(1→4) and 4% or less are linked alpha-D-(1→6). Furthermore, all D-glucose units which are linked alpha-D-(1→6) are also linked alpha-D-(1→4). This evidence is interpreted to mean that starch is primarily a branches polymer consisting of D-glucose units linked alpha-D-(1→4) with branches at the C-6 position on an average of once every 26 or more D-glucose units. This evidence further supports the concept that starch may be either a homologous branched polymer or a heterogeneous mixture of branched and linear polymers. The unbranched component of starch is called amylose and the branched component is called amylopectin (Chalmers, 1968).

Amylose

Amylose is essentially a linear polymer in which the anhydroglucose units are predominantly linked through alpha–D-(1→4) glucosidic bonds. It is illustrated in Figure 2. The anhydroglucose unit contains one primary and two secondary hydroxyls as well as an aldehydic reducing ends of the form of an inner hemiacetal. This is called the reducing ends of the molecule. The opposite end, or nonreducing ends, contains an anhydroglucose unit containing one primary hydroxyl and three secondary hydroxyls. The other anhydroglucose units contain one primary and two secondary hydroxyls.

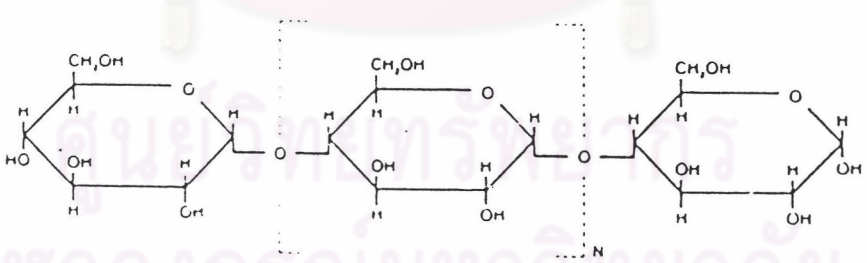


Figure 2 Linear-chain structure of amylose molecules (Foster, 1965)

The abundance of hydroxyls imparts hydrophilic properties to the polymer, giving it an affinity for moisture and dispersibility in water. However, because of their linearity, mobility, and hydroxyl groups, amylose polymers have a tendency to orient themselves in a parallel fashion and approach each other closely enough to

permit hydrogen bonding between hydroxyls on adjacent polymers. As a result, the affinity of the polymer for water is reduced and the sol becomes opaque.

Amylose molecules also have an affinity for iodine. The complex of amylose with iodine produces a deep blue color which is used to identify amylose-containing starches.

Amylopectin

Amylopectin is a highly branched polymer containing, in addition to anhydroglocose units linked together as in amylose through alpha-D-(1→4) glucosidic bonds, periodic branches at the C-6 position. These branches are linked to the C-6 by alpha-D-(1→6) glucosidic bonds. Each branch contains about 20 to 30 anhydroglucose units. The structure of amylopectin molecule is illustrated in Figure 3.

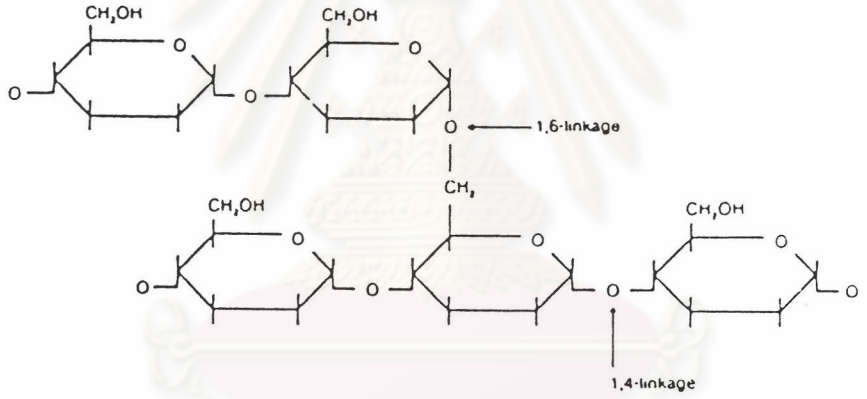


Figure 3 Structure of amylopectin branching points (Foster, 1965)

The large size and branched nature of amylopectin reduce the mobility of the polymers and interfere with any tendency for them to become oriented closely enough to permit significant levels of hydrogen binding. As a result, aqueous sols of amylopectin are characterized by clarity and stability as measured by resistance to gelling on aging.

Starch could be obtained from different plan sources. The range of amylose content in different plant species was varied. This variation contributes to the different properties with respect to the uses as additives in dosage forms. Table 1 is a partial list of starches, their plant sources, and their amylose contents.

Table 1 Types of starches and their sources and amylose contents (Hullinger, 1960)

Types of Starch	Source	Amylose (%)
Corn or Maize	Mature grain of *Zea mays*	22-28
Wheat	Mature grain of *Triticum aestivum*	17-27
Barley	Mature grain of *Hordeum vulgare*	24-27
Potato	Tuber of *Solanum tuberosum*	23
Oat	Mature grain of *Avena sativa*	23-24
Rice	Mature grain of *Oryza sativa*	16-17
Glutinous Rice	Mature grain of *Oryza sativa* (*var. glutinous*)	0-5
Tapioca or Cassava	Tuber of *Manihot esculenta*	17-22

Schwartz and Zelinskie (1978) studied the binding properties of the corn starch fractions amylose and amylopectin and concluded that the binding properties of starches were due to amylopectin fraction while the amylose fraction was responsible for the disintegrant properties. With the exception of the waxy starches that consist only of amylopectin, it has been found that nearly all starches contain 20-30% of the linear component, amylose.

Modified Starches and Starch Derivatives

There exists in the literature some difference of opinion as to the definitions of modified starch and starch derivatives. The I.S.O. (International Organization for Standardization) Technical Committee 93 distinguishes only between native starch and modified starch, the latter being defined as native starch treated in such a way as to modified one or more of its original physical or chemical properties. The term 'modified starch' thus includes: pregelatinised starch, oxidized starch, thin boiling starch, dextrin, dialdehyde starch, starch ester, starch ether, ect. (Bij, 1976)

Modified starches were developed to overcome and expand the usefulness for a myriad of industrial applications. These modifications are commonly called conversions involve treatment of granule starch by chemical and/or physical means to cause rupturing of some or all the starch molecules, thus weakening the granules, decreasing their capacity to swell on pasting or cooking in water, and decreasing the size of molecules. As a result, the viscosity of the product from the starch on heating in water is reduced, thus permitting the converted starch to be dispersed at higher concentration than unmodified starch (Wurzburg, 1986)

Principal Reasons for Starch Modification. (Bemiller, 1965)

- To modify cooking characteristics
- To decrease retrogradation
- To decrease gelling tendencies of pastes
- To increase freeze-thaw stability of pastes
- To decrease paste and/or gel syneresis
- To improve paste and/or gel clarity and sheen
- To improve and/or gel texture
- To improve film formation
- To improve adhesion
- To add hydrophobic groups (for emulsion stabilizations)

Practiced Starch Modification (Roberts, 1967)

1. Chemical Modification

1.1 Derivatization

- Monostarch substitution (etherification and esterification; includes polymer grafting)
- Crosslinking (via starch esterification)

1.2 Acid-thinning/ hydrolytic depolymerization
(both acid-and enzyme-catalyzed)

1.3 Dextrinization (depolymerizations and tranglycosylatons).

1.4 Oxidations (bleaching and depolymerizations)

1.5 Hydrolysis (to maltodextrin, glucose syrups, glucose, etc.)

2. Physical modification

2.1 Gelatinization (to produce pregelatinized starch)

2.2 Preparations of cold-water-swelling starch

3. Genetic modification

3.1 Waxy maize starch

3.2 High-amylose maize starch.

The Definition of the Degree of Substitution (DS)

Degree of substitution defined by Bij (1976) as follows : the degree of substitution is a measure of the substitution of any anhydroglucose unit without regard to the molecular size of the substituent. In fact it gives the number of hydroxyl groups in an anhydroglucose unit that has been substituted.

The degree of chemical modification may be expressed in several ways. A DS of 1.0 indicates an average of one substituent for each anhydroglucose unit; since the D-glucopyranosyl monomers contain, on average, three free hydroxyl groups, the maximum possible DS is 3.0 (Shah, Sheth and Jarowski, 1981)

Starch Ether (Bij, 1976)

Starch ethers are as a rule prepared by reaction of sodium starch with etherifying agents such as epoxides, chlorohydrins, halogen carbonic acids, etc. The principal starch ethers at present are the alkyl and hydroxyalkyl starches and the carboxymethyl starches, while the interest in cationic starch ethers is growing. The starch ethers can be divided into three groups according to their properties :

(a) Non-ionic ethers, obtained by reacting starch with epoxides or chlorohydrins, alkyl- or aralkylhalogens and sometimes with unsaturated compounds as acetylene (⟶ vinylstarch).

(b) Anionic starch ethers, from starch and monochloro acetic acid, propane sultone, etc.

(c) Cationic starches, from starch with nitrogen compounds as diethyl-aminoethylchloroethane, epoxypropane amines, ethylene-imine, etc.

Sodium Carboxymethyl Starch

Sodium carboxymethyl starch is an anionic starch ether derivative usually obtained as the sodium salt. It is prepared by the reaction of starch with monochloroacetic acid or sodium chloroacetate in the presence of aqueous sodium hydroxide according to the equation (Paschall, 1967).

$$St\text{-}OH + NaOH + ClCH_2COONa \longrightarrow St\text{-}O\text{-}CH_2COONa + NaCl + H_2O$$

or

$$St\text{-}OH + 2\ NaOH + ClCH_2COOH \longrightarrow St\text{-}O\text{-}CH_2COONa + NaCl + 2H_2O$$

Several procedures employing these alkalizing agents have been reported (Wu, Gan, and Chen, 1993; Zhang et al., 1993) and patented (Filbert, 1952). Low degree of substitution (DS) carboxymethyl starch, up to about 0.1, is not cold water soluble. Hence, they may be obtained by conducting the reaction in aqueous alkaline slurry containing added salts such as sodium sulfate. Higher DS product, up to 1.0 has been obtained in a water-miscible solvent. The solvents which are widely used are isopropanol, methanol and ethanol. (Roberts, 1965; Hofreiter, 1987)

Mechanism of Reaction

The reaction mechanism of carboxymethylation is a bimolecular displacement (Substitution nucleophilic bimolecular; S_N2). This means the formation of an intermediate complex. Therefore, the etherification of starch with sodium chloroacetate in aqueous sodium hydroxide must take place according to these equations. Typically, the starch is first converted into an alkaline starch called a starchate nucleophile, followed by a reaction with a nucleophilic agent.

$$St - O - H \longrightarrow St - O^- + H_2O$$

$$\uparrow$$

$$: OH$$

$$St - O^- + ClCH2COONa \longrightarrow St - O^- - CH_2COONa^+ - Cl^-$$

$$\downarrow$$

$$St - O - CH_2COONa + Cl^-$$

Carboxymethylation of amylose occurs preferentially at the secondary hydroxyl groups. It seems reasonable to propose that as carboxymethyl groups are introduced into the amylose molecule, electrostatic repulsion between the groups disrupts the 3D conformation of the polymer chain; and the secondary hydroxyl groups at C-3 no longer hydrogen bonded, become available etherification. (Roberts, 1965; Radley, 1968; Hofreiter, 1987).

Applications of Sodium Carboxymethyl Starches

Commercial utility of sodium carboxymethyl starch, if economically produced, is the textile and paper manufacturing industry. Its use in textile finishing and dyeing has been demonstrated; and because of its resistance to biological degradation, it has been considered as a warp size that would not contribute to the biological oxygen demand of the waste stream from a desizing operation. Other major industrial applications proposed for sodium carboxymethyl starch are as soil suspending agents in detergents and as flocculants. One of the oldest miscellaneous uses for sodium carboxymethyl starch is as an indicator in iodometric titrations due to its superiority over starch. Other uses include soil conditioner, component of latex-based paint and of a paint remover, binder for kaolin castings, replacement of gum arabic in lithography, component of photographic film emulsion, and additive to impart free-flowing characteristics to granular explosives. For many uses, a high viscosity characteristic is generally preferred (Filbert, 1952; Chalmers, 1968)

Modified Starch as Pharmaceutical Excipients

Direct Compression Filler

The compression characteristics of modified rice starch (Primotab® ET) was evaluated by Bos et al. (1992). It was found to have excellent flow and binding properties. In contrast with other starch based filled-binders the binding properties stay sufficient after mixing with a hydrophobic lubricant. In tablet formulation, it can be used as unique filler-binder, which may be of benefit when drugs like primary amines are tabletted. Modified rice starch can also be combined with other filler-binders such as alpha-lactose monohydrate 100 mesh or anhydrous bata-lactose. Combinations with microcrystalline cellulose should be avoided because of the poor flowability of the blends and the slow disintegration of the tablets.

The compressibility of various modified starches was evaluated in comparison with commercially available modified starches. The results showed that modified rice starch derived by physico-chemical treatments exhibited the high degree of compressibility and allowed rapid disintegration (Siriyos Timaroon and Poj Kulvanich, 1992; Wallop Weecharangsan, 1995).

Binder

Crosslinked starches have been evaluated on their potential use as binding agent in several studies. The pregelatinized starch and pregelatinized-crosslinked starches showed promising use as a binding agent in the conventional wet granulation process (Visavarungroj et al., 1990a) and high shear wet granulation process (Visavarungroj et al., 1990b)

Disintegrant

Starch and starch derivatives are frequently used in tablets as disintegrant at concentration of 3-15% w/w. The mechanism of action of disintegrants in tablet has mainly been attributed to the swelling of the particles when they are brought into contact with water. A comparative evaluation of the properties of some tablet disintegrants, including those of starches and derivatives, has been reported that sodium carboxymethyl starches provided a very short disintegrating time and were the most efficient disintegrants (Wade and Weller, 1994; Thavisak Teruya, 1995).

Visavarungroj and Remon (1990) investigated modified waxy-corn starch as a disintegrating agent. The crosslinked–only waxy-corn starches showed the same disintegrating properties as potato starch. They revealed the lowest granule swelling power and a poor rate and amount of water uptake. The pregelatinized-crosslinked waxy-corn starches showed better disintegrating properties than the crosslinked only starches. The tablets containing pregelatinized starch revealed considerable variation and longer disintegration time than those formulated with pregelatinized-crosslinked starches.

Baie et. al. (1995) reported that tapioca starch was chemically modified with different degree of crosslinking and carboxymethylation to produce sodium starch glycolate. The effect of these structural modifications on the swelling properties and disintegration time of directly compressed aspirin tablets using the modified starch were investigated. The results indicated that suitable degree of crosslinking and

carboxymethylation, the sodium starch glycolate produced from tapioca starch had similar swelling properties to commercially available modified starch, but a better disintegration time.

Suspending Agent

The ideal characteristics of a suspending agent for extemporaneous dispensing have been defined and survey conducted to select non-liquid substances which meet these characteristic. In the BPC suspension mixture which currently contains tragacanth, the inclusion of pregelatinised starches (Instant Clearjel, Snowflakes), sodium starch glycolate (Primojel), a mixture of alginated salts (Alginated YZ), or aluminium magnesium silicate (Veegum) produced systems which, in most instances, demonstrated suspension and redispersibility properties better than those systems made with tragacanth or compound tragacanth powder (Farley and Lund, 1976). However, Smith and McIntosh (1976) were to suggestion those resulted. They found that the addition of electrolytes led to an immediate and marked decrease in viscosity of 2% of Primojel. For example, the addition of 0.5% of sodium chloride caused a loss of about 80% of original viscosity.

Ornanong Suwannapakul (1996) prepared sodium carboxymethyl starch in three different degrees of substitution (DS) based on Filbert's method (Filbert, 1952). Four domestically available starches, including tapioca starch, rice starch, glutinous rice starch and corn starch were chemically modified and evaluated for their properties as suspending agent. Suspending property of modified starches was evaluated in terms of viscosity, ease of redispersion and sedimentation volume. As a result, suitable modified starches being selected in ibuprofen suspensions were modified glutinous rice starch (MGS), modified rice starch (MRS) and modified tapioca starch (MTS) with degree of substitutions of 0.16, 0.26 and 0.38, respectively.

Reconstitutable Oral Suspensions (Ofner, Schnaare and Schwartz, 1996)

Introduction

Although conventional oral suspension can be administered immediately, there is an important category of suspensions that requires mixing prior to administration. These suspension are commercial dry mixtures that require the addition of water at the time of dispensing and have a titles designated in the United States Pharmacopoeia (USP) of the form "... for Oral Suspension."

The reconstituted suspension is the formulation of choice when drug stability is a major concern. After reconstitution, these suspensions have a short but acceptable shelf life if stored at refrigerator temperatures.

Characteristics of Suspensions for Reconstitution

The most common reason for formulation of suspensions for reconstitution is inadequate chemical stability of the drug in aqueous vehicle. In this case, dissolution or even suspension of the drug results in a very short shelf-life. For example, reconstituted suspensions of penicillin have a maximum shelf-life of 14 days. The manufactured dry mixture, however, has a shelf-life of at least 2 years.

Another reason for formulating suspensions for reconstitution is to avoid the physical stability problem often encountered in conventional suspensions. These problems include possible increased drug solubility due to pH change from chemical degradation, incompatibility of gradients, viscosity changes, conversion of polymorphic form crystal growth and caking.

Suspensions for reconstitution reduce the weight of the final product because the aqueous vehicle is absent and, consequently, transportation expenses may be reduced.

The dry mixture may be shipped without regard to seasonal temperatures because its physical stability is less susceptible to temperature extremes as compared with conventional suspensions.

Suspensions for reconstitution require special considerations. Many antibiotic are formulated for reconstitution and are intended for a pediatric patient population.

In addition, acceptable properties must be maintained before, during, and after reconstitution. Finally, the formulator must realize that the last step in the preparation of the product will be conducted beyond the control of manufacturer.

Table 2 lists the required characteristics of suspensions for reconstitution. During manufacture, the dry blend, or mixture, must not segregate into a nonuniform mixture because errors in dosage may be result. Appropriated ingredients that disperse quickly much be employed. After reconstitution the high viscosity caused by refrigerated storage temperatures should not obstruct dose administration by the patient. The formulator must be aware that a final pediatric product must be acceptable to children who are ill and often uncooperative with administration of mechanism of medication. If the patient will not swallow the medication, the product is useless.

Table 2 Required characteristics of suspensions for reconstitution (Ofner et al, 1996)

1. The powder blend must be a uniform mixture of the appropriate concentration of each ingredient.
2. During reconstitution the powder blend must dispersed quickly and completely in the aqueous vehicle.
3. The reconstituted suspension must be easily redispersed and poured by the patient of provide an accurate and uniform dose.
4. The final product must have an acceptable appearance, odor, and taste.

Commonly Used Ingredients

There are usually fewer ingredients in suspensions for reconstitution than in conventional suspensions. The criteria for selecting ingredient are based both on suitability for reconstitution and on the physical type of powder mixture desired. General guidelines can aid the formulator in the selection of ingredient, but every formulations of a different drugs are unique, and the extract ingredient must be determined by the formulator .

A. General consideration

Table 3 lists the functional excipients employed in suspensions for reconstitution. The ingredients are separated into categories of frequent used. Not all formulators agree on the necessary excipients. Although it is list here as an infrequent excipients, some formulators believe that an anticaking agent is mandatory. Other formulators question the necessity of dry. Some dry mixtures are prepared by granulation, and the ingredients used in this process are listed in Table 3 .

Table 3 Frequent and infrequent excipients used in suspensions for reconstitutions (Ofner et al, 1996)

Frequent	Inferquent
Suspending agent	Anticaking agent
Wetting agent	Flocculating agent
Sweetener	Solid diluent
Preservative	Antifoaming agent
Flavor	Granule binder
Buffer	Granule disintegrant
Color	Antioxidant
	Lubricant

B. Drug

There are 30 monographs of oral suspensions for reconstitution in the United States Pharmacopeia, 23rd edition (USP 23). These is approximately one-half the number of monographs of conventional oral suspensions (Ofner et al, 1996).

C. Suspending Agent

Suspending agents should be easily dispersed by vigorous hand shaking during reconstitution. This rules out several common suspending agents because many suspending agents required hydration, elevated temperatures, or high shear mixing for adequate dispersion. Some suspending agents that are not recommended include agar, carbomer, and methylcellulose. Although both methylcellulose and

aluminum magnesium silicate are not recommended, they have been used successfully in a cephalexin and erythromycin ethylsuccinate formulation, respectively.

Table 4 list suspending agents recommended for use in suspensions for reconstitution. The ionic charges of the agents are included for purposes of avoiding chemical incompatibility with other ingredients.

The required concentrations for rapid dispersion during reconstitution must be determined for each suspending agent.

Table 4 Suspending agent suitable for using in suspensions for reconstitution (Ofner et al, 1996)

Agent	Ionic charge
Acacia	-
Carboxymethylcellulose sodium	-
Iota carageenan	-
Microcrystalline cellulose with carboxymethylcellulose sodium	-
Povidone	0
Propylene glycol alginate	-
Silicon dioxide, colloidal	0
Sodium starch glycolate	-
Tragacanth	-
Xanthan gum	-

D. Sweetener

The sweetener is significant component of suspensions for reconstitution. Drugs frequently have a bitter taste and suspending agents, particularly clays, may have a blend taste. Sweeteners can mask the unfavorable taste and enhance patient acceptance in the pediatric population that uses this product. Any increased viscosity as a result of the sweeteners aids suspension of the drug particles.

Sucrose can perform both above functions of sweetener and suspending agent and, serve as a diluent in the dry mixture. In addition, sucrose can be milled to increase its surface area and be used as carrier of liquid excipients such as volatile oils. Other sweeteners include mannitol, dextrose, aspartame, and sodium saccharin. Aspartame has fair acid stability but poor heat stability. Saccharin is restricted by the Food and Drug Administration because of its carcinogenic potential.

E. Other Ingredients

The ingredients described in this section include buffers, preservatives, flavors, and colors. Flocculating agents are not commonly used in suspensions for reconstitution because these products are usually redispersed frequently enough to prevent caking.

1. Buffers are used to maintain the optimum pH for all ingredients. Suspension pH is often adjusted to ensure that the drug remains insoluble. The polymeric suspending agents, however, have the greatest viscosity at the pH of the system for the shelf-life of the product. Certain preservatives, such as sodium benzoate, are most effective at low pH values in which the molecule is un-ionized. Sodium citrate is a common buffer used in suspensions for reconstitution.
2. Flavors also enhance patient acceptability of product. They are very important in the products that are intended for pediatric patients. Both natural and artificial flavors are used. Additional flavors used include raspberry, pineapple, and bubble gum. In some cases, refrigeration after reconstitution is required for the stability of the flavoring agent rather than for the stability of the drug.
3. Colorants are intended to provide a more aesthetic appearance to the final suspension. As relatively large cations or anions, these agents may be chemically incompatible with other ingredients.
4. Anticaking agents, such as amorphous silica gel, have several functions in suspensions for reconstitution. A common problem in dry mixtures is poor powder flow and caking. This is often caused by powder agglomeration due to moisture uptake. As a desiccant, these agents remove moisture from the dry mixture to facilitate good powder flow and prevent caking. In addition, screen and insulate static charge conditions, and are chemically inert.

Preparation of Dry Mixture

A. Powder Blends

Powder blends, sometimes called powder mixtures, are prepared by mixture the ingredients of the dry mixture in powder form. Ingredients present in small quantities may require a two-stage mixing operation. Such ingredients can be mixed with a portion of a major ingredient to aid in their dispersion.

B. Granulated Products

All ingredients in granulated products are processed by granulation. Wet granulation is the usual process and the granulating fluid is water or an aqueous binder solution. There are two methods of incorporating the drug. The drug can be dry-blended with the other ingredients or it can be dissolved or suspended in the granulating fluid.

C. Combination Product

Powdered and granulated ingredients can be combined to overcome some disadvantages of granulated products. Less energy and equipment for granulation may be required if the majority of the diluent can be added after granulation. Also, heat-sensitive ingredients, such as flavors, can be added after drying of the granulation to avoid exposure to elevated temperatures.

The end result of preparing the dry mixture, regardless of the type, is to achieve physical uniformity. This ensures uniform potency during processing, bulk storage, and packaging. Table 5 summarizes the advantages and disadvantages of the above three types of dry mixtures used in suspensions for reconstitution.

Table 5 Advantage and disadvantage of types of dry mixtures in suspensions for reconstitution (Ofner et al, 1996)

Type	Advantage	Disadvantage
Powder blend products	Economy; low incidence of instability	Mixing and segregation problems ; Losses of drug
Granulated products	Appearance; flow characteristics; less segregation; less dust	Cost; effects of heat and granulating fluids on drug and excipients
Combination of powder and granulation	Reduced cost; use of heat sensitive ingredients	Ensuring nonsegregating mix of granular and non granular ingredients

D. Processing the Dry Mixture

The following guidelines have been recommended for processing the dry mixture:

1. Use efficient mixing. Evaluate processing performance of batches on pilot scale-up equipment, not laboratory scale equipment.
2. Determine an adequate duration of mixing time.
3. Avoid accumulation of heat and moisture during mixing.
4. Limit temperature/humidity variations. A general rule is 70 °C at ≤ 40% relative humidity.
5. The finished batch should be protected from moisture. Store in lined containers with silica desiccant bags.
6. Sample for batch uniformity. Test of the top, middle, and bottom levels of dry mixture.

Stability

Chemical stability is usually of more concern in suspensions for reconstitution than in conventional suspensions because the drug has poor stability in the presence of water. While there are usually only 14 days after reconstitution for separation problems to occur, physical stability before and after reconstitution is still a concern.

A. Chemical Stability

Drugs that degrade by hydrolysis, such as the penicillins, are common candidates for reconstitution. Drugs highly susceptible to oxidation or photolysis in solution may also be considered. In this case, degradation in the dry mixture would be limited to the surface of the solid particles and the interior would generally be protected from degradation.

Chemical stability should be determined in both the dry mixture and reconstituted suspension. Both should be examined not only at controlled room temperature but also at temperatures of potential exposure such as during shipment or storage of the products. Reconstituted antibiotic suspensions have been described as having a shelf life of 14 days at refrigerator temperatures in which the drug concentration does not fall be low 90% of the original concentration.

B. Physical Stability

Tests of physical stability should evaluate both the dry mixture and reconstituted suspension. Common evaluations on reconstituted suspensions include measurements of sedimentation volume and the ease of redispersion.

Sedimentation volume is obtained by measuring the height of settled drug particles in undisturbed bottles at intervals of time and expressing this height as a fraction of the initial height. Graduated cylinders are not currently used because the sedimentation height in small-diameter containers, such as 100-mL cylinders, can differ from that in wider bottle. A high fraction or sedimentation volume indicates good suspending ability. The worst case is the formulation of compact sediment of drug particles that can not be redispersed. This cake would have a small sedimentation volume.

Ease of redispersion is a qualitative evaluation. It can be expressed in terms of easy, moderate, difficult, or caked. Numeric scales have also been used. One evaluation used the number of inversions required to redisperse a 3-day-old suspension as the measurement of redispersibility.

Evaluation

A. Evaluation of Dry Powder

The main physical concerns in this type of product are appearance, organoleptic properties, and powder dispersibility. There are several reasons a powder may change dissolution time as a function of storage time. The most common reasons are (a) cohesion, (b) crystal growth, and (c) moisture sorption, which cause a lumping up of powders (Carstensen, 2000).

1. Cohesional Force

Cohesional force is the force between two particles, and cohesion in general is the stress (force per cm^2 of surface) that a particle experiences due to the surrounding particles. Problem due to cohesion are particularly predominant when a powder is fine, and great fineness of a powder is often required for dissolution reason. Cohesional forces are inversely proportional to square of distance between the particles, so that in storage, where vibration, for instance, may consolidate the powder bed, these forces become large, and the powder "caked up." This may give rise to problem in reconstitution (Carstensen, 2000).

2. Dispersibility (Reconstitution Time)

Dispersibility or reconstitution time is the ability of powder to get wetted without formation of dry lumps in water. (Jaya and Das, 2004).The procedure of dry powder after added with water was determined. The number of time interval used for completing dispersed was measured. The mixture was shaken at time interval of 30 seconds. At each time interval, the mixture was rapidly shaken for 5 times. The number of time interval required for complete dispersion was determined as reconstitution time. If the sample was not completely dispersed after shaking vigorously, the mixture was described as "lump". The reconstitution time determination was done in triplicate. Good dry syrup formulation had less of the number of time interval for completed dispersion.

B. Evaluation of Reconstituted Suspensions

There are many dimensions of a suspension dosage form that are indicative of its quality, acceptance, and performance. General appearance, color, odor, pH, density, viscosity, sedimentation volume, ease of redispersibility, particle size, dissolution, zeta potential, microbial integrity, labeled potency, and human safety are all important and useful parameters that provide guidance in evaluation procedures for suspensions. The following parameters are commonly evaluated when a suspension is formulated.

1. Sedimentation Volume

In most pharmaceutical suspensions the particles are too large to remain permanently suspended in water as a consequence of Brownian movement. These suspensions will therefore sediment and the rate of sedimentation is related to the particle radius, the density of both the particle and the liquid, and the viscosity of the medium. These relationships are described by Stokes' law (Carstensen, 2000) shown in the following equation;

$$\nu = \frac{2r2(\Delta\rho)}{9\eta} g$$

where ν = sedimentation rate (cm/s)

r = particle radius (cm)

$\Delta\rho$ = density difference between internal phase and external phase (g/cm^3)

g = acceleration due to gravity (cm/s^2)

η = viscosity of continuous phase.(1 poise = 10^{-1}Pa s).

Experimentally, sedimentation volume can be determined from the ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension as the suspension settles in a cylinder under standard conditions and the equilibrium has been reached. The larger this value, the greater is the degree of flocculation of the particles and the better is the suspendability (Martin, 1961). The cylindrical graduate method is a simple, inexpensive, and standard-size tool for determining the sedimentation volume of a suspension. The method is done by

making periodic measurement of sedimentation height without disturbing the system. Normally, the interpretation of result is achieved by plotting a graph between the Hu/Ho ratios and time. The different formulations can be compared by observing the lines, the better formulations produce lines that are more horizontal and/or fewer stops (Patel et. al., 1986).

2. Ease of Redispersibility

Redispersibility is one of the major considerations in assessing the acceptability of a suspension. It is defined as the ability to resuspend the settled particles with a minimum amount of shaking after a suspension was allowed to settle for some times (Nash, 1988). The determination was encountered by rotating the tubes containing the dispersions in a vertical plane about the midpoint of the tube, either by hand or by a rotating machine (Rawlins and Kayes, 1983). The number of revolutions required to obtain dispersion was recorded and was termed the redispersibility value.

3. Rheological Measurements and Viscosity

Rheological behavior of suspensions can be used to describe quantitatively the flow behavior of material for the purpose of quality control, to determine the settling behavior, the arrangement of the vehicle and particle structural features, to quantitative the effect of time, temperature, ingredients, and processing parameters on a formulation, as well as to understand the overall characteristics and the fundamental nature of a product system for purposes of comparison (Carstensen, 2000). In particular, a suspension that possesses high thixotropic value is the most preferable.

3.1 Rheology and Application in Pharmaceutical Products

Rheology is the science which deals with deformation of body under the influence of stress. Bodies in this context can be solids, liquid, or gases. Ideal solids deform elastically. The energy of deformation is fully recovered when the stresses are removed. Ideal fluids such as liquids and gases deform irreversibly-they flow. The end of deformation is dissipated into the fluids in the form of heat, and it cannot be recovered just by releasing stresses. The real bodies we encounter are neither ideal solid nor ideal fluids. Rheology is involved in the mixing and flow of materials,

removal prior to use, and packaging into containers. Its applications in pharmacy are wide ranging including liquids, semi-solids, gel, solid deformation, coating, and packaging materials.

In suspension formulation, the rheology of the suspending agent usually dominates the overall properties of the suspension. A study showed that an ideal suspending agent should be shear thinning, should have a high viscosity at low shear for shelf stability and a low viscosity at high shear for easy dispensing (Deem, 1988).

3.2 The Basic Law of Viscosity

To measure the viscosity of liquids requires first the definition of the parameter which is involved in the flow. Then one has to find suitable test conditions which allow the measurement of flow properties objectively and reproducibly.

Issac Newton was the first to find the basic law of viscometer describing the behavior of an ideal liquid (Martin, 1993);

$$\text{Viscosity } (\eta) = \frac{\textit{shear stress (T)}}{\textit{shear rate (D)}}$$

Shear stress

An internal force (F) applied to an area (A) being the interface between the upper plate and liquid underneath leads to a flow in the liquid layer. The velocity of flow that could be maintained for a given force will be controlled by the internal resistance of liquid, i.e. by its viscosity.

The SI unit "Pascal" (Pa) has replaced the former unit "dyne/cm^2" which had previously been used for stresses especially in scientific literature (Martin, 1993). One pascal equals 10 dyne/cm^2. Therefore, the cge units for poise are dyne/cm^2 and 1 cp being equal to 0.01 poises.

$$T = \frac{F(force)}{A(area)} = \frac{N(Newton)}{m^2} = P(Pascal)$$

Shear rate

The shear stress (T) causes the liquid to flow in a special pattern. A maximum flow speed V_{max} will be found at the upper boundary. The speed drops across the gap size down to $V_{min} = 0$ at the lower boundary contacting the stationary plate. Laminar flow means that minute thin liquid layers slide on top of each other, similar to cards in a deck-of-cards. One laminar layer is then displaced with respect

to the adjacent ones by a fraction of the total displacement encountered in the liquid between both plates.

In its general form the shear rate (D) is defined by a differential (Briceno, 2000);

$$D = \frac{dv}{dy}$$

when v is velocity (m/s) and y is distance (m). therefore, the unit of shear rate is (Martin, 1993);

$$D = \frac{m/s}{m} = \frac{1}{s} = s^{-1}.$$

3.3 Flow and Viscosity Curves

The correlation between shear stress and shear rate defining the flow behavior of a liquid is graphically displayed in a diagram of T on the ordinate and D on the abscissa. This diagram is called the "Flow Curve". The most simple type of a flow curve is shown below. (Figure 4) The viscosity is assumed to be constant and independent of D.

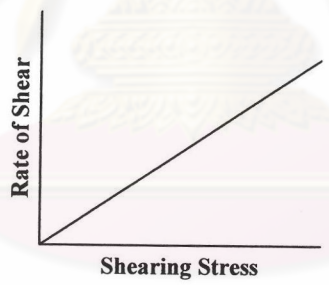


Figure 4 Flow curve of a Newtonian liquid (Briceno, 2000)

Another diagram is very common: v is plotted versus D. This diagram is called the "Viscosity Curve". (Figure 5)

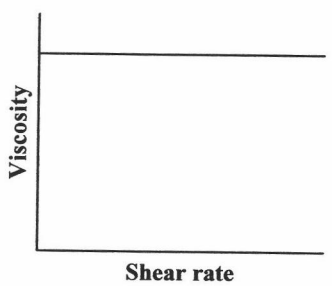


Figure 5 Viscosity curve of a Newtonian liquid (Briceno, 2000)

Viscosity measurement was lead always first to the flow curve. Its results can then be rearranged mathematically to allow plotting the corresponding viscosity curve. The different types of flow curves gave their counterparts in types of viscosity curves.

3.4 Flow Characteristics of Fluids (Deem, 1988)

Newtonian Flow

Newton recognized that the rate of flow of a liquid is proportional to the stress (F) applied, the constant of proportionality is called the (dynamic) viscosity (η). The kinematics viscosity is the dynamic viscosity divided by the density of the liquid. Some examples of typical Newtonian liquid include water, mineral oil, bitumen, and molasses.

The viscosity of a dilute solution (η) is always greater than of the solvent (η_0) so that the relative viscosity (η_r) is (Martin, 1993);

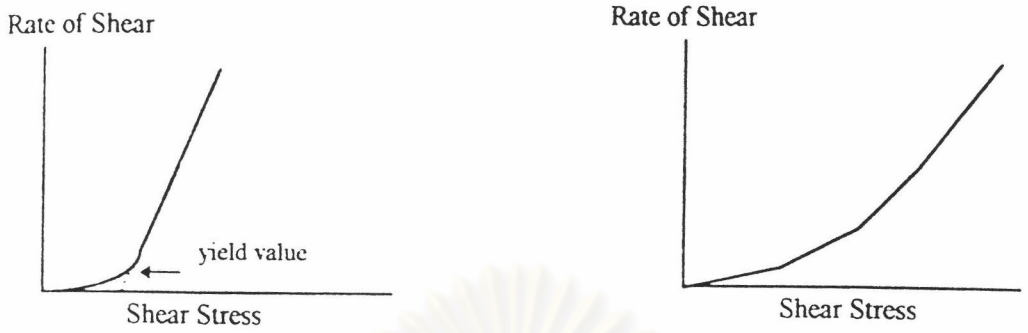
$$\eta_r = \frac{\eta}{\eta_0}$$

Non-Newtonian Flow

It is recognized that adherence to Newton's law is relatively uncommon. Flow behaviors that deviate from Newtonian are called "non-Newtonian". The essential characteristic of a non-Newtonian system is that the viscosity is not directly proportional to the shear rate. Several types of non-Newtonian rheological behavior were identified.

Plastic Flow

This is sometimes called Bingham flow and a material showing it is called a Bingham body. The most important characteristic of plastic flow is the existence of a yield value, an intercept of a straight line on the shear axis (Figure 6a), which implies solid like behavior under quiescent conditions (Zatz, 1985). This has the advantage of locking particles into a rigid network, which may effectively inhibit sedimentation altogether. Agitation temporarily disrupts the network, making it possible to do such things as shake, pour, or spread a preparation onto the skin.



(a) (b)

Figure 6 Typical non-Newtonian flow curves (Martin, 1993)

Pseudoplastic flow

The rheogram of a pseudoplastic system is usually a smooth curve (Figure 6b). Since the slope of the line represents the viscosity, it can be seen that the material becomes thinner the faster it is stirred. For this reason pseudoplastic flow is sometimes called shear rate thinning. In this type of flow the rate of shear is not directly proportional to the shearing stress. The curve passes through the origin, but is rarely linear. Pseudoplastic flow is characteristic of most natural and synthetic gums.

Thixotropy

Thixotropy is a rheological phenomenon of great industrial importance and is frequently observed in suspensions. It is described as a breakdown and reforming of the gel/sol/gel structure. A typical rheogram for a thixotropic material is shown in Figure 7, whereas the rate of shear is increased the ratio of stress to shear continually decreases. If at any point along the upcurve the rate of shear is decreased the ratio of stress to shear continually decreases. If at any point along the upcurve the rate of shear is decreased rapidly no further change in consistency is noted, and hysteresis loop is formed. The decrease in consistency differs from that seen with pseudoplastics in that there is a finite time required for reformation of sufficient yield value and viscosity, so that the shearing will produce a thinning which is persistent after shearing is discontinued. Thinning has been found to occur with increased rate

of shear and with time of shear at any given rate. Thixotropy is a desirable characteristic in liquid pharmaceutical systems that ideally should possess a relatively high consistency in the container, yet easily to pour or spread. A well-formulated thixotropic suspension will not settle out readily in the container, will resuspend upon shaking, and will remain long enough for an accurate dose to be withdrawn. Finally it will regain consistency rapidly enough so as to maintain the particles in a suspended state (Schramm, 1981). Thixotropic behavior can be observed in gelatin, latex, paint, and emulsion systems (Deem, 1988).

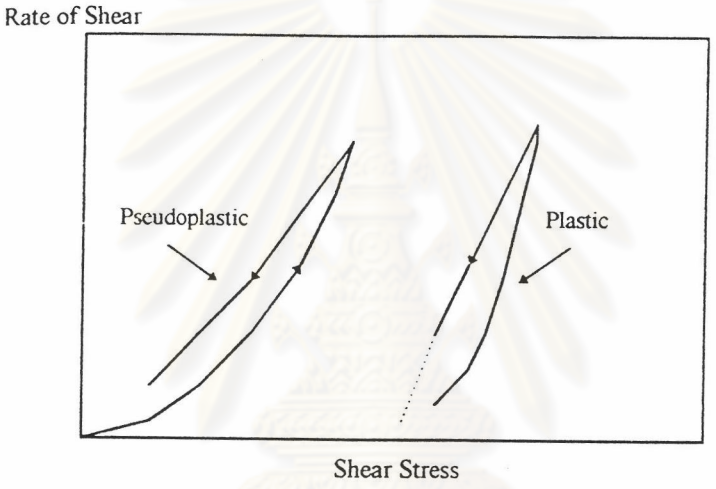


Figure 7 Rheogram of thixotropic material (Martin, 1993)

Model Dry Syrup Formulation

Oral liquid penicillin formulations are widely prescribed as a means of increasing patient acceptability. Penicillins are generally too labile for an adequate shelf-life to be assigned to liquid preparation by manufacturer. Such preparations are formulated as granules for reconstitution, incorporated a sugar based (usually sucrose) and require the addition of fixed volume of water. The reconstitution syrup may be in the form of a solution, e.g. phenoxymethylpenicillin (penicilinV), or a suspension, e.g. ampicillin. A 7- or 14-day expiry date is normal on such preparations (Hempenstall et al., 1985).

1. Amoxicillin Trihydrate

1.1 Description

Amoxicillin trihydrate is a broad spectrum penicillin antibiotic that was first marked by Beecham Pharmaceutical in 1972 (Bird, 1994). Basic structure of the group of penicillins includes a double ring thaiazolidine β-lactam moiety, which has been named 6-aminopenicillaic acid (6-APA). The differences in physical, chemical and biological properties of the various penicillins are due to their side chain (R) (Figure 8).

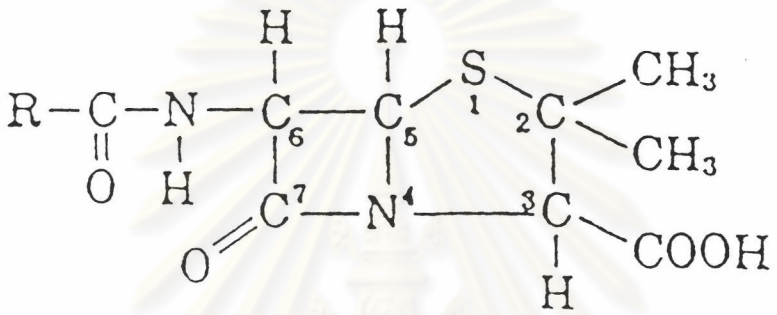
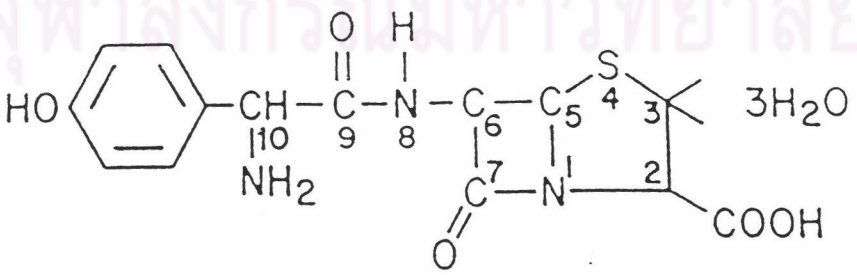


Figure 8 Basic structure of penicillin group (Bird, 1994)

The β-lactam ring is quite labile in all the penicillins known at present. Differences in stability of the several penicillins are determined by the side chains, the structural characteristics of which confer varying degree of protection against degradation (Bhattacharyya and Cort, 1978).

Amoxicillin trihydrate is generally referred to 6-aminopenicillianic acid trihydrate and in early studies is called hydroxyampicillin. Unlike ampicillin, the anhydrous form of amoxicillin have been not studies extensively (Schwartz and Buckwalter, 1962). The formula and molecular weight of amoxicillin trihydrate are shown in Figure 9.



$C_{16}H_{19}N_3O_5S.H_2O$ Molecular weight. 419.46

Figure 9 Structural formula of amoxicillin trihydrate (Bird, 1994)

1.2 Physical Properties

Appearance

Amoxicillin trihydrate is a white or almost white crystalline powder. Amoxicillin trihydrate may have the slightly sulfurous odor that is typical of many penicillins (Bird, 1994; Lund, 1994; Schwartz and Buckwalter, 1962).

Solubility

Solubility of amoxicillin trihydrate is about 2.5-4.0 mg/ ml in water, 1.0-3.4 mg/ ml in ethanol and 5.0-7.5 mg /ml in methanol, practically insoluble in chloroform and in ether. The solubility of amoxicillin trihydrate in water depends on pH. Amoxicillin trihydrate dissolves in dilute solutions of acids and alkali hydroxides (Bird, 1994; Lund, 1994). The pH of a 0.2 % w/v aqueous solution of amoxicillin trihydrate is in the range of 3.5 to 5.5 (BP 1993) or 3.5 to 6.0 (USP 24). The pH solubility profile of amoxicillin trihydrate was determined at 37 °C and ionic strength 0.5, to be a U-shaped curve with minimum solubility at pH close to isoelectric point. Van't Hoff plots indicated a linear relationship between the equilibrium solubility of amoxicillin and temperature in the range of 20 to 50 °C (Bird, 1994; Lund, 1994).

Dissociation Constant

The dissociation constants (pK_a) for the three ionisable groups in water are 2.4 (caroxyl), 7.4 (aromatic hydroxyl) and 9.6 (α- ammonium) (Bird, 1994; Lund, 1994).

Partition Coefficient

A calculated log P value of 0.87 (equivalent to a P value of 7.4) for a partition in to n-octonol has been reported (Bird, 1994; Lund, 1994).

1.3 Compatibility of Amoxicillin Trihydrate with Some Excipients.

During development of pharmaceutical dosage formulation, excipient compatibility studies are routinely performed to look for possible interactions of the formulation component with the active ingredient both physical and chemical interaction. In particularly, the solid dosage forms such as tablet and capsule, usually contain diluents, binders, disintegrates and lubricants. The physicochemical study on the interaction between active ingredient and selected excipients must be considered.

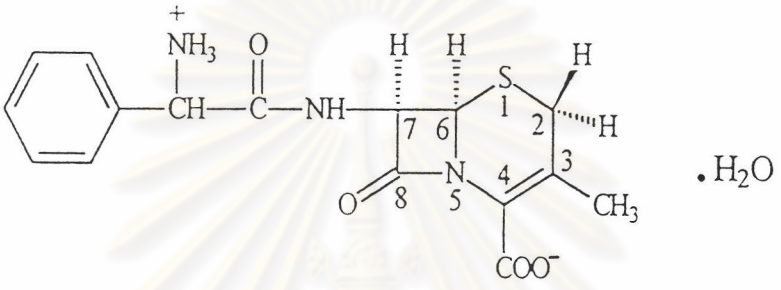
Hassan et al. (1995) studied the interaction between amoxicillin trihydrate and commonly used tablet and capsule excipients. Dissolution tests, differential scanning calorimetry (DSC) and IR spectroscopy of powder mixture were used to investigate the interaction of the formulation component with amoxicillin trihydrate. The results obtained from the dissolution tests indicated that most of the insoluble excipients such as Avicel® PH 101, Avicel® PH 102, Emcocel®, dicalcium phosphate, starch, titanium dioxide and magnesium stearate, decrease the dissolution rate of amoxicillin trihydrate, but Aerosil® 200 did not affect on the dissolution rate. On the other hand, most of the soluble excipients (sodium starch glycolate, sorbitol and manitol) had no significant effect on the dissolution rate of the drug. In the case of sorbitol, the ability of sorbitol (instant and crystalline) to adsorb amoxicillin trihydrate was examined. The adsorption capacity and binding strength of instant sorbitol was greater than that of crystalline form in dry powder blends (Lund,1994). PEG 6000 and sodium carboxymethylcellulose decreased the dissolution rate of the drug to different levels. These decreases may be due to the possible complex formulation between PEG systems and the drug as well as the formulation of a gel between the drug and sodium carboxymethylcellulose.

In study of the uptake of amoxicillin by some adsorbants (Khalil, Mortada, and Khawas, 1984), the results obtained from this study indicated that amoxicillin trihydrate in solution was adsorbed by attapulgite and aluminium magnesium silicate (Veegum®) but not by kaolin or magnesium trisilicate in the pH range of 2.1 to 3.2. For the effect of the presence of either kaolin (4 g) or veegum (1 g) on the in vitro availability of amoxicillin trihydrate from hard gelatin capsule by dissolution experiment, it was found that the presence of both adsorbants in the dissolution medium (0.01N HCl) had no apparent effect on the level of drug in solution.

2. Cephalexin Monohydrate

2.1 Description

Cephalexin, 7 α- (D-Amino-α-phenylacetamide) –3-methylcephemcarbozylic acid, is classified as a first generation cephalosporin C is replaced by phenylglycine and the ester-linked acetic acid is condensed to a simple methyl group. Its chemical structure is such that it probably represents the derivative of 7- aminocephalosporanic acid most closely resembling the broad-spectrum penicillin ampicillin (Figure 10).



$C_{16}N_{17}O_3S_{4+}H_2O$ Molecular weight: 365.41

Figure 10 Structure formula of cephalexin monohydrate (Marrelli, 1975)

Cephalexin was found to occur in several solvated crystal forms, and often in widely varying mixtures of these forms, some of solvated crystal forms prepared were the dihydrate, monohydrate, diacetonitrilate, formamidate, methanolate, and acetonitrile hydrate (Marrelli, 1975; Pfeiffer, Yang and Tucker, 1970). Hendriksen, Preston and Yorkz (1995) found that crystalline cephalexin shows discrete formation of mono and dihydrate forms, and their formation is easily reversible, indicating that water plays little part in the stabilization of the lattice. Spray and freeze-drying both lead to a form of cephalexin which continuously sorbs water without formation of a stoichiometric hydrate, and does not reversibly dehydrate; this indicates the sample is highly disordered and that the water can access a wide range of lattice sites which play a significant part in the overall solid state stability. Otsuka, Otsuka and Kaneniwa (1994) studied the polymorphic transformation pathway during grinding and the physicochemical properties of cephalexin. It was indicated the cephalexin is converted into noncrystalline solid at room temperature. The noncrystalline solid of cephalexin is very stable at 35 °C under lower than 66% of relative humidity.

2.2 Physical Properties

Appearance

Cephalexin is a white to cream-colored crystalline powder, having a characteristic order (BP 1998).

Solubility

The solubility of cephalexin monohydrate in the following solvent is shown in Table 6.

Table 6 Solubility of cephalexin monohydrate at 25 °C

Solvent	Cephalexin monohydrate (mg)/Solvent(ml)
Water	13.50
Methanol	3.40
N-octanol	0.03
Chloroform	< 0.01
Ether	< 0.01

Dissociation Constant

The following dissociation constants (pKa) were reported

pK_a = 2.56, 6.88 (Yamana and Tsuji, 1976)

pK_a = 2.50, 5.20, 7.30 (Moffat, 1986)

pK_a = 5.2, 7.3 (Budavari,1996)

2.3 Compatibility of Cephalexin with Some Excipients

Hassan et al. (1995) studied the compatibility between cephalexin monohydrate and some excipients. DSC revealed cephalexin to be compatible with Avicel® PH 101 and PH 102, Emcocel®, dicalcium phosphate, titanium dioxide, magnesium stearate, starch, Explotab® and sodium carboxymethyl cellulose. The interaction of cephalexin was observed with polyethylene glycol 6000, sorbitol and mannitol.

2.4 Stability

The stability of cephalexin in solution is dependent on pH, degrading rapidly in basic media and remaining stable under mild acidic conditions. No loss in cephalexin activity occurred in 72 hours at 25 °C in the pH range from 3 to 5. The rate of degradation found at pH 6 and pH 7 (25 °C) was approximately 3% and 18%

per day, respectively. With refrigeration, no appreciable loss occurs between pH 3 and pH 7 after 72 hours, in hydrochloric acid buffer pH 1.2, cephalexin lost 5% activity in 24 hours at 37 °C as compared to a 45% loss in phosphate buffer at pH 6.5. The antibiotic retains activity well in serum and urine as no loss in activity was noted after storage at 20 °C for 14 days. Cephalexin in serum was found to loss 10%, 50% and 75% activity, respectively, after storage at 5 °C, 25 °C and 37 °C for 48 hours (Yamana and Tsuji, 1976; Marrellie, 1975). Some organisms have been found to produce a β-lactamase (cephalosporinase) which can rapidly degrade cephalexin. Degradation of cephalexin also results from heat, strong alkali, strong acids and ultraviolet light (260 nm).