Chapter III

Materials and Methods

Materials

The following substances were obtained from commercial sources.

1. Starch materials
   - Glutinous rice starch (MFG. 15/02/02, New Grad, Thai Wan Food Products Public Co., Ltd, Thailand)
   - Rice starch (MFG. 10/02/02, New Grad, Thai Wan Food Products Public Co., Ltd, Thailand)
   - Tapioca starch (MFG. 02/04/02, New Grad, Thai Wan Food Products Public Co., Ltd, Thailand)
2. Commercial Modified starch
   - Ultrasperse®2000 (Batch No. BJ-7537, supplied by National Starch & Chemical (Asia) Pte Ltd.)
3. Model drug
   - Amoxicillin trihydrate (Batch No. M404375, DSM Anti-Infectives India Pvt. Ltd., Toansa, India,)
   - Cephalexin monohydrate (Batch No. 550510-0006-3, supplied by Siam Pharmaceutical, Thailand)
   - Calcium carbonate (Lot. No. 4967, Sichai Chemical, Thailand)
4. Material used for modification starch
   - Monochloroacetic acid (Lot No. 111H0240, Sigma, U.S.A.)
   - Absolute methanol (Batch No. 0398412, Fisher Scientific.)
   - Methanol (Batch No. 0398400, Fisher Scientific)
   - Absolute ethanol (Batch No. 0399375, Fisher Scientific)
   - Acetic acid (Lot No.124A14, Merck, Germany)
   - Glacial acetic acid (Lot No.148841, J.T. Beker Inc., USA)
   - Sodium hydroxide (J.T. Baker, U.S.A.)

5. Other dry syrup additives
   - Icing sugar (supplied by Thai Nakorn Patana, Thailand)
   - Acelsulfame potassium (supplied by Rama Production Co., Ltd, Batch No.0204011)
   - Anhydrous citric acid (Lot No. 217468, Samchai Chemical Co., Ltd)
   - Sodium citrate dihydrate (Lot No. 1253, Sichai Chemical, Thailand)
   - Methyl paraben (S.Tong Chemical Co., Ltd, Thailand)
   - Propyl paraben (S.Tong Chemical Co., Ltd, Thailand)
   - Colloidal silicon dioxide (Aerosil®200, Lot No. CO 6044, supplied by Rama Production Co., Ltd.)
   - Flavor: Raspberry powder (supply by, Adinop Co., Ltd.)
   - Flavor : Orange powder (Lot No. 12763, Bio-Industries, Indonesia)
   - Color: Sunset yellow (supply by, Adinop Co., Ltd.)
   - Color: Ponceua 4R (supply by, Adinop Co., Ltd.)
6. Others
   - Sulfuric acid (Lot No. 7333696, Fisher Scientific Co., U.S.A.)
   - Pyrazinamide (Lot No. pyz/P095/02, supply by Siam Pharmaceutical, Thailand)
   - Sodium dihydrogen phosphate (Lot No. 330630/1, BDH Chemical, England)
   - Monobasic potassium phosphate
   - Acetonitrile HPLC grade (Batch No. 0399975, Fisher Scientific)
   - Methanol HPLC grade (Batch No. 0398400, Fisher Scientific)
   - Water (Batch No. 0254216, Fisher Scientific)

Equipment

1. Analytical balance (Sartorius, Germany)
2. Hot Air Oven (Memmertt, type ULSO, Germany)
3. Fourier-Transformed Infrared Spectrophotometer (Perkin Elmer, 1760X, U.S.A.)
4. Magnetic stirrer (SP-18420, Nuova 7 Stir-Plate, Sabron Thermolyne, U.S.A.)
5. Karl Fischer apparatus (Model 716 DMS in connection with 703 DMS, Tritino, Metrohm, Switzerland)
6. Drum hoop mixer (Model AR400, Erweka, Germany)
7. Suction machine (Arthure H. Thomas Co., USA.)

8. Sonicater (Brasonic® Model B-1200EL, USA)
9. Viscometer (Brookfield DV-II+ Programmable Viscometer)
10. pH meter (SA 520 pH meter, Orion, USA.)
11. Micropipette (Socolex®)
12. High Performance Liquid Chromatography (HPLC)
    - High pressure pump (LC-10 AD VP, Shimadzu, Japan)
    - Degasser (DGU-14A VP, Shimadzu, Japan)
    - Variable UV-detector (SPD-10 AD VP, Shimadzu, Japan)
    - Chromatography data system computer integrator (SCL-10 AD VP , Shimadzu, Japan)
    - Auto Injector (SIL-10 AD VP, Shimadzu, Japan)
    - Column μ-Bondapack™ C18 ODS-1, 3.9x300 mm., particle size 5 μm. (Water, USA)

Methods

The experimental was divided into three parts. The first was a preparation and determination of modified starches. The second was an evaluation of modified starch as a suspending agent in dry syrup. The last was an application of selected modified starches in formulation.

Part I: Preparation and Evaluations of Modified Starches

A. Preparation of Modified Starches

Three native starches were used in this study, glutinous rice starch, rice starch, and tapioca starch. Modification was done by carboxymethyl substitution and the modified methods based on those described by Filbert (1952). Ornanong Suwannapakul (1996) founded that modified glutinous rice starch (MGR), modified rice starch (MRS) and modified tapioca starch (MTS) at DS of 0.16. 0.26 and 0.38, respectively were suitable to be used as suspending agent in liquid preparation. To obtain three different degrees of substitution, methods of preparation were as follows;

Method 1: Modified Glutinous Rice Starch (MGS, DS = 0.16)

The absolute methanol of 254 g and monochloroacetic acid of 27.6 g were weighed and thoroughly mixed. Then, heat the mixture to 50 °C. Glutinous rice starch of 109 g was added together with continuous mixing. After that, 50 % aqueous solution sodium hydroxide of 110 g was added. The temperature of reaction mixture was maintained at 60 °C for 60 minutes, providing good agitation, then neutralized with acetic acid to pH 7.0. Removed mother liquor, then washed with 80 % methanol for several times and finally with 100% methanol until there was no sodium chloride in filtrate when tested with silver nitrate. The product was dried in a hot air oven at 50 °C overnight. The dried product was sieved through No.80 mesh screen and stored in desiccator.

Method 2: Modified Rice Starch (MRS, DS = 0.26)

The absolute ethanol of 286 g and monochloroacetic acid of 29.2 g were weighed and thoroughly mixed. Then, heat the mixture to 50 °C. Rice starch of 102 g was added together with continuous mixing. After that, the solution of 97 % sodium hydroxide flake 38.4 g in 69.0 g of water was added. The temperature of reaction mixture was maintained at 50 °C for 20 minutes, providing good agitation, then neutralized with acetic acid to pH 7.0. Removed mother liquor, then washed with 80 % methanol for several times and finally with 100 % methanol until there was no sodium chloride in filtrate when tested with silver nitrate. The product was dried in a hot air oven at 50 °C overnight. The dried product was sieved through No.80 mesh screen and stored in desiccator.

Method 3: Modified Tapioca Starch (MTS, DS = 0.38)

The absolute ethanol of 286 g and monochloroacetic acid of 29.2 g were weighed thoroughly mixed. Then, heat the mixture to 50 °C. Tapioca starch of 102 g was added together with continuous mixing. After that, the solution of 97 % sodium hydroxide flake 38.4 g in 69.0 g of water was added. The temperature of reaction mixture was maintained at 50 °C for 90 minutes, providing good agitation, then neutralized with acetic acid to pH 7.0. Removed mother liquor, then washed with

80 % methanol for several times and finally with 100 % methanol until there was no sodium chloride in filtrate when tested with silver nitrate. The product was dried in a hot air oven at 50 °C overnight. The dried product was sieved through No.80 mesh screen and stored in desiccator.

B. Determination of a Degree of Substitution (DS)

The procedure which was used to determine a degree of substitution of above modified starches was the same method as described for Croscarmellose Sodium monograph in USP 24. This method consists of two steps, the first is titration step and residue on ignition step is the second step. The sample of each degree of substitution was determined in triplicate.

Titration Step

The starch sample of 1.0 g was accurately weighed and transferred into a 500-ml glass-stopper conical flask. Sodium chloride solution (10 % w/v) of 300 ml was added together with agitated until modified starch was dissolved. After that, 0.1 N sodium hydroxide of 25 ml was added and the stopper was inserted. The mixture was allowed to stand for 5 minutes with intermittent shaking. Then, 5 drops of m-cresol purple TS were mixed. After that, 0.1 N hydrochloric acid of 15 ml was added and continuous shaking. When the solution was purple, 0.1 N hydrochloric acid in 1-ml portion was added until the solution was yellow, shaking after each addition. The solution was titrated to a purple endpoint with 0.1 N sodium hydroxide. Finally, the net number of milliequivalent (M) of base required for the neutralization of 1.0 g of sodium carboxymethyl starch was calculated on dried basis.

Residue on Ignition Step

The sample of 1.0 g was accurately weighed into suitable crucible which was previously ignited and cooled. The sample was gently heated until the charred sample was observed. The residue was moistened with 1 ml of sulfuric acid and then, gently heated until white fumes were no longer evolved. The sample was cooled in a desiccator. The ignition step was continued following equation in term as percentage of residue on ignition (C).

The degree of substitution (DS) could be calculated by (USP 24):

$$DS = A + S$$

in which A was degree of acid carboxymethyl substitution

$$= 1150M / (7102 - 412M - 80C)$$

S was degree of sodium carboxymethyl substitution

$$= (162 + 58A)\, C / (7102 - 80C)$$

M = milliequivalent of base required for the neutralization of 1g of modified starch

C = percentage of residue on ignition.

C. Detection of Carboxymethyl Substitution in Modified Starches

Fourier-transform infrared spectrometer (FT-IR) was used to detect the carbonyl group in the prepared starches. The samples were prepared as KBr pellets and scanned with the speed of three seconds per scan. The comparison was made between IR spectrum of native starch and IR spectrum of modified starch. The presence of carbonyl group was used as evidence of substitution of carboxymethyl group in modified starches.

D. Determination of Reconstitution Time

The each of modified starches (MGS, MRS and MTS) of 0.1, 0.2 and 0.3 g and Ultrasperse®2000 (UT) of 0.1, 0.2, 0.3 and 0.4 g were weighed and transferred into 15-ml test tube. Then, ten ml of distilled water was added. The time used for complete dispersion 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 completely dispersion was determined as reconstitution time. If the sample was not completely dispersed after shaking vigorously for 30 seconds, the mixture was described as "lump". The reconstitution time determination was done in triplicate.

E. Viscosity Measurement of Pure Dispersions

The each of modified starch (MGS, MRS and MTS) of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g and Ultrasperse®2000 of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 g were dispersed in water. In order to completely hydrate the modified starches was kept at room temperature overnight. The measurement of viscosity was performed with viscometer (Brookfield viscometer Model LDV-II^{+}). The viscosities were determined in triplicate with shear rate of 10 rpm at room temperature.

F. Rheological Studies of Pure Dispersions

1. Rheological Determination of Each of Modified Starches and Ultrasperse®2000 Dispersions

The rheological behaviors of the dispersion of Ultrasperse®2000 and three modified starches were studied by Brookfield viscometer at room temperature. The complete cycle of rheological behaviors study was eight minutes. The shear rate was increased by step from 0 to 10 rpm within two minutes and maintained at the shear rate 10 rpm for 4 minutes. After that, the shear rate was decreased by step until shear rate of 0 rpm was reached within two minutes. The viscosities were transformed to shear stress and used for plotting a rheogram. The flow pattern of a particular dispersion suspension can be determined by examining a plot of shear stress versus rate of shear (Falkiewicz, 1996).

2. Measurement of Thixotropic

The rheogram was constructed of shear stress (dye/cm^2) versus shear rate (sec^{-1}) shows thixotropic loops for materials sheared for two different times intervals (Deem, 1996). The formation of a thixotropic loop is the excerpted criteria of thixotropy. The area enclosed within up-curve and down-curve is know as hysteresis loop (Deem, 1996; Falkiewicz, 1996) The area of the hysteresis loop had been mentioned as one way of measuring thixotropic value (Deem, 1996). The thixotropic value could be calculated from the area of hysteresis loop by integrating area between up-curve and down-curve with computer program (AutoCad®).

Part II: Evaluation of Modified Starch as Suspending Agent in Calcium Carbonate Suspension

Suitable concentration of each modified starch (MGS, MRS and MTS) and Ultrasperse®2000 as good suspensions formulation were investigated. Application of modified starches and Ultrasperse®2000 as suspending agent in calcium carbonate suspensions was assessed.

A. Preparation of Calcium Carbonate Suspension

The 10% w/v calcium carbonate suspension was prepared with varying concentrations of suspending agents, each of modified starches (1.0 %, 2.0 % and 3.0 % w/v) and Ultrasperse®2000 (1.0 %, 2.0 %, 3.0 % and 4.0 % w/v). The formulation was given below.

Calcium carbonate	10 % w/v
Methyl Paraben (MP)	0.18 % w/v
Propyl Paraben (PP)	0.02 % w/v
Suspending Agent	1.0 % or 2.0 % or 3.0 % or 4.0 % w/v
Purified Water qs. ad	100 ml

Methyl paraben and propyl paraben were dissolved in water and added mixture of calcium carbonate and modified starch together. Shaking until all ingredients were dispersed. Then, the volume was adjusted with water and mixed thoroughly.

B. Determination of Reconstitution Time

The reconstitution time determination of reconstituted calcium carbonate suspension was performed using the same procedures as mentioned in Part ID.

C. Determination of Sedimentation Volume

The sedimentation volume of calcium carbonate suspension was determined at room temperature over a period of 14 days using the cylindrical graduate method (Nasipuri and Ogumlana, 1978). The procedure is as follows. : suspensions of 50 ml were stored for 14 days in 60 ml calibrated glass cylinders. Each sample was shaken

to ensure uniformity prior to the study. Sedimentation height was measured and recorded everyday without disturbing the suspension. The sedimentation volume (SV) was calculated from the ratio of the ultimate height (Hu) to the initial height (Ho) of the total suspension (Martin, 1961). The sedimentation volume determination was done in triplicate.

$$\text{Sedimentation Volume (SV)} = \frac{Hu}{Ho}$$

D. Determination of Redispersibility

The ease of redispersion was determined from the number of the times which each test tube had to be inverted by hand under reasonable-controlled conditions until the suspension was re-suspended (Farley and Lund, 1976). Ten ml of suspensions was poured into the calibration test tubes. Then, they were allowed to settle for 7 and 14 days. The redispersibility of the formulation were evaluated. Based on the number of inversion and the effort required to obtain homogeneous suspension. If the complete redispersion was not achieved after 12 inversions, it was vigorously shaken for 15 seconds. After shaking vigorously, the sediment was still presented; the suspension was described as "caked".

E. Viscosity Measurement of Calcium Carbonate Suspension

The viscosities of calcium carbonate suspension using each of modified starches at concentration of 1.0 %, 2.0 %, and 3.0 % w/v and Ultrasperse®2000 at concentration of 1.0 %, 2.0 %, 3.0 % and 4.0 % w/v were determined by Brookfield viscometer. The sample were hydrated overnight to allow complete hydration. The experiments were repeated in triplicate with a shear rate of 10 rpm at room temperature.

Part III: Application of Selected Modified Starches in Dry Syrup Formulation

A. Development of Model Dry Syrup Formulation

After the preliminary study, there were different types of modified starch that exhibited the best properties as suspending agent at different percentages in formula. Since pH of the formulation affects the drug stability, it is necessary to control the pH of the model drug formulation. Therefore, buffer system was used to control pH of the formulation. However, buffer is a salt which might be incompatible with modified starches. The addition of salt into the formulation might cause the decrease in viscosity. Before model dry syrup formulation was formulated, it was necessary to study effect of the buffer concentration on model drugs stability and viscosity of the formulation.

Buffers are used to maintain the optimum pH for all ingredients and sodium citrate is a common buffer used in suspensions for reconstitution (Ofner et al., 1996). In liquid formulation, citrate buffer is widely used in pharmaceutical formulation to adjust the pH of solution at concentration of 0.05 - 0.5 molar (Gordon, 1993; Wade and Weller, 1994; Kibbe, 2000).

In this study model dry syrup formulation were amoxicillin trihydrate dry syrup at concentration 125 mg/5ml and cephalexin monohydrate dry syrup at the concentration of 125 mg/5ml. Suitable pH for amoxicillin trihydrate dry syrup and cephalexin monohydrate dry syrup followed the pharmaceutical requirement and pH-rate profile of active ingredients.

Amoxicillin trihydrate and cephalexin monohydrate were stable at pH 5.0-7.0 (Tsuji et al., 1978) and 2.0-5.0 (Yamana and Tsuji, 1976), respectively. According to USP 24, the required pH value for Amoxicillin for Oral Suspension was between 5.0 and 7.5; Cephalexin for Oral Suspension was between 3.0 and 6.0. Consequently, the controlled pH values for amoxicillin trihydrate and cephalexin monohydrate dry syrup were at 6.0 and 4.5 respectively.

1. Effect of Buffer Concentration on Content of Model Drugs

The dispersions of model drugs in various concentrations (0, 0.05, 0.10 and 0.20 molar) of buffer solution were prepared. The citrate buffer was used to control pH of the model drugs dispersions. The pH of amoxicillin trihydrate and cephalexin monohydrate dispersions were adjusted to obtain the values of 6.0 (Tsuji et al., 1978) and 4.5(Yamana and Tsuji, 1976), respectively. The dispersions of model drugs were protected from light and kept at room temperature and refrigerator (8.0 ± 1 °C). The content of model drugs in dispersions was quantitatively determined at 1, 3, 5, 7, 10 and 14 day by HPLC method (PartIIIC). The drug content determination was done in triplicate.

Buffer concentration which might affect the stability of model drugs was studies by varying buffer concentration (0, 0.05, 0.10, and 0.20 molar). Therefore, lowest buffer concentration with stabilized drugs dispersion in buffer solution when stored at room and refrigerator (8 ± 1 °C) was selected.

2. Effect of Suspending Agent and Buffer Concentrations on Viscosity of Each Modified Starches (MGS, MRS and MTS) and Ultrasperse®2000 Dispersion

The suitable viscosity of modified starches and Ultrasperse®2000 dispersions had based on that of calcium carbonate suspension in preliminary study. In order to investigate the appropriate concentration of modified starches and Ultrasperse®2000 that gave the suitable viscosity, for modified starches and Ultrasperse®2000 dispersions with various concentrations in buffer solution were prepared.

The concentration of each of modified starch (MGS, MRS and MTS) in buffer solution were 0.5 %, 1.0 %, 1.5 %, 2.0 %, 2.5 % and 3.0 % w/v and those of Ultrasperse®2000 were 0.5 %, 1.0 %, 1.5 %, 2.0 %, 2.5 %, 3.0 % and 4.0 % w/v.

As the result of the effect of buffer concentration which could stabilizing model drug in buffer solution at room temperature and refrigerator (8.0 ± 1 °C) for 14 days was selected and used for preparing the each of modified starch and Ultrasperse®2000 dispersions. Samples were prepared by hydrating the suspending agents (MGS, MRS, MTS and UT) in buffers solution overnight. The viscosities of each of modified starch Ultrasperse®2000 dispersions were determined by Brookfield

viscometer. The measurement was done in triplicate with a shear rate of 10 rpm at room temperature.

Moreover, in order to study the effect of buffer concentration on viscosity of each modified starch (MTS, MRS and MTS) and Ultrasperse®2000 dispersions, the dispersions of each modified starch (MTS, MRS and MTS) and Ultrasperse®2000 various concentrations (0, 0.05, 0.10 and 0.2 molar) of buffer solution were prepared by hydrated dispersions overnight. The concentration of each of modified starch (MGS, MRS and MTS) in two pH of citrate buffer solution (pH 4.5 and 6.0) were 0.5 %, 1.0 %, 1.5 %, 2.0 %, 2.5 % and 3.0 % w/v and those of Ultrasperse®2000 were 0.5 %, 1.0 %, 1.5 %, 2.0 %, 2.5 %, 3.0 % and 4.0 % w/v. The viscosities of the dispersions were determined by Brookfield viscometer. The measurement was done in triplicate with a shear rate of 10 rpm at room temperature.

3. Formulation of Model Dry Syrups

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 (Ofner et al, 1996).

In this study, the excipients used for drugs formulation design were anticaking agent, sweetener, preservative, buffer, suspending agent, flavor and color.

Therefore, concentration of citrate buffer in dry syrup formulation was selected on basis result obtained from the study in A1 and percentage for suspending agent in formulation of model dry syrup was chosen on the basis of the result obtained from the study in Part IIIA2.

Colloidal silicon dioxide (Aerosil®200) was used as anticaking agent in all model dry syrup formulations because it was recommended for using in reconstituted oral suspension (Ofner et al, 1996). Colloidal silicon dioxide was used as anticaking agent at the concentration of 0.5 - 2.0 % w/v (Wade and Weller, 1994). According to study, colloidal silicon dioxide was used as anticaking agent in amoxicillin trihydrate and cephalexin monohydrate dry syrups at the concentration of 0.5 % and 1.0 % w/v, respectively.

For amoxicillin trihydrate dry syrup The used of aspartame, saccharin sodium, acelsulfame potassium and sodium cyclamate as sweeteners at concentration of

0.02 - 2.0 %, 0.02 - 0.05 %, 0.05 - 0.7 % and 0.01 - 0.2 % w/v, respectively was compared. The taste and appearance of selected formulations were observed. It was found that acelsulfame potassium at concentration of 0.5 % w/v and aspartame at concentration of 2.0% w/v might be used. However, aspartame was not stable, thus acelsulfame potassium was selected. For cephalexin monohydrate dry syrup, the used of saccharin sodium, acelsulfame potassium and sodium cyclamate at concentration of 0.02 - 4.0 %, 0.05 - 1.0% and 0.01 - 0.5 % w/v, respective was compared. Acelsulfame potassium at concentration of 0.8 % w/v was selected because it gave the acceptable.

Icing sugar was bulking agent with taste sweet and low cost (Wade and Weller, 1994), therefore it was selected used to as bulking agent. This weight was gave uniformity of drug and give good appearance of powder formulation in container. The dry powder at the weigh of 30 g was suitable in dry syrup formulation. Consequently, the weight of dry powder was adjusted to weight of 30 g.

Methyl paraben and propyl paraben at concentration of 0.18 % and 0.02 % w/v, respectively were used as preservative in model dry syrup formulation. They are widely used as an antimicrobial preservative in pharmaceutical formulation. The parabens are effective over a wide pH range and have a broad spectrum of antimicrobial activity although they are most effective against yeasts and molds (Wade and Weller, 1994).

Furthermore, the compatibility of flavoring and coloring agent was considered. Orange flavor powder and sunset yellow was used in amoxicillin trihydrate dry syrup formulation at concentration of 2.0 % and 0.005 % w/v, respectively. Raspberry flavor powder and ponceua 4R was used in cephalexin monohydrate dry syrup formulation at concentration of 0.50 % and 0.004 % w/v, respectively. Then, the good appearance of formulation was observed.

3.1 Amoxicillin Trihydrate Dry Syrup

Amoxicillin trihydrate dry syrup (125 mg/5ml) was prepared according to the following formulation.

Amoxicillin trihydrate	2.5	g
Citrate buffer pH 6.0	*a	% w/v
Suspending agent	**b	% w/v
Aerosil®200	0.50	% w/v
Acelsulfame potassium	0.50	% w/v
Orange flavor powder	2.0	% w/v
Sunset yellow	0.005	% w/v
Methyl paraben (MP)	0.18	% w/v
Propyl paraben (PP)	0.02	% w/v
Icing sugar qs to	30	g
Purified Water qs ad	100	ml

*a = buffer concentration dependent on PartIIIA1

**b = Percentage of each suspending agent (MGS, MRS, MTS and UT) dependent on PartIIIA2

3.2 Cephalexin Monohydrate Dry Syrup

Cephalexin monohydrate dry syrup (125 mg/5ml) was prepared according to the following formulation.

Cephalexin monohydrate	2.5	g
Citrate buffer pH 6.0	*a	% w/v
Suspending agent	**b	% w/v
Aerosil®200	1.0	% w/v
Acelsulfame potassium	0.08	% w/v
Raspberry flavor powder	0.50	% w/v
Ponceua 4R	0.004	% w/v
Methyl paraben(MP)	0.18	% w/v
Propyl paraben(PP)	0.02	% w/v
Icing sugar qs to	30	g
Purified Water qs ad	100	ml

*a = buffer concentration dependent on PartIIIA1

**b = Percentage of each suspending agent (MGS, MRS, MTS and UT) dependent on PartIIIA2

The preparation procedure as following:

A. Passed model drugs through a sieve #60 (0.25 mm).

B. Passed suspending agents and flavor powder through a sieve #60.

C. Dried citrate buffer (anhydrous citric acid and sodium citrate dihydrate), Aerosil®, acelsulfame potassium and color powder for 2 hours at 80 ^{0}C by hot air oven. Levigated and passed through a sieve #60.

D. Dissolved methyl paraben and propyl paraben in water. Spray the preservative solution on icing sugar and mixed and heated. Dried for 6 hours at 80 ^{0}C by hot air oven. Passed through a sieve #60.

E. Mixed thoroughly of A, B, C and D together by geometric dilution method.

4. Evaluation of the Formulation

4.1 Dry Powder

4.1.1 Appearance of Model Dry Syrup

Appearances of dry powder formulations were color, powder smoothness and cohesiveness of powder, were visually observed.

4.1.2 Content Uniformity of Drugs in Model Dry Syrup

An amount of the sample powder equivalent to 125 mg/5 ml of drugs was accurately weighed and drug content was determined by HPLC method (Part IIIC). The determination was done in triplicate.

4.1.3 Determination of Water Content Model Dry Syrup

The water content of sample was determined by Karl Fisher titration using a Metrohm model 716 DMS Titrino, Switzerland. The Karl Fisher instrument was standardized using 10 µl water. Hydranol®-Composite 5 was used as the Karl Fisher reagent and dry methanol was used as the solvent. The sample powder of approximately 100 mg was directly dispensed into the titration vessel. Then, the end point was determinated amperometrically. The water content was reported in w/w percentage.

4.2 Reconstitution Model Dry Syrup

4.2.1 Physical Property Determinations of Model Reconstituted Suspension

The amoxicillin trihydrate and cephalexin monohydrate suspensions were freshly prepared and storaged in refrigerator (8.0 ±1 °C) for 14 days. The appearances of suspensions such as color and pH were observed.

4.2.2 Determination of Reconstitution Time

The reconstitution time of model dry syrup formulations were carried out by adding distilled water into 100-ml calibrated glass bottle with 30 g of model dry syrup formulation; adjusted final volume to 100 ml. The method was followed the same procedure as mentioned in PartID.

4.2.3 Viscosity Measurement of Model Reconstituted Suspension

The viscosities of reconstituted suspensions formulations were determined when the dry powder was dispersed homogeneously in water at 7 and 14 days by Brookfield viscometer. Air bubble was removed from the suspension by sonicating. The measurements were repeated in triplicate with a shear rate of 10 rpm at room temperature.

4.2.4 Redispersibility of Model Reconstituted Suspension

The redispersibility of model reconstituted suspension was measured using the same procedure as mentioned in Part IID.

4.2.5 Stability of Drugs in Model Reconstituted Suspension

Dry powder was reconstituted by distilled water to get the concentration of 25 mg/ml. The suspension was shaken until the homogeneous dispersion was observed. The suspensions were stored at room temperature and refrigerator (8±1 °C). The 0.1 ml of suspensions which were allowed to settle every 0, 1, 3, 5, 7, and 10 and 14 days were withdrawn and tested for content of model drugs by HPLC method (Part IIIC). The assay was done in triplicate.

B. Stability Studied of Selected Dry Syrup Formulation

Stability studied was determined at two storage conditions for four months after selection of suitable model dry syrup formulations from the study in PartA4. One set of the sample was evaluated under normal condition at room temperature (27.0 ± 2.0 °C). The other set was evaluated under stress condition at 45 °C and 75% relative humidity. (Nyqvist,1983)

All formulations were kept in finish closed bottle and were tested at the time interval of one month for four months.

1. Preparation of Select Model Drugs Dry Syrup Formulation.

Selected model drugs formulations were scaled up. The preparations of dry syrup formulation were performed at controlled temperature below 27.0 °C and relative humidity not more than 27.0 %. The dry syrup formulation of 4.5 g was weighed in glass bottle size 20 ml and closed with rubber and aluminum cap.

1.1 Amoxicillin Trihydrate Dry Syrup

Amoxicillin trihydrate dry syrup (125 mg/5ml) was prepared by the following formulation.

Amoxicillin trihydrate	2.5	g
Citrate buffer pH 6.0	*a	% w/v
Suspending agent	**b	% w/v
Aerosil®200	0.50	% w/v
Acelsulfame potassium	0.50	% w/v
Orange flavor powder	2.0	% w/v
Sunset yellow	0.005	% w/v
Methyl paraben (MP)	0.18	% w/v
Propyl paraben (PP)	0.02	% w/v
Icing sugar qs to	30	g
Purified Water qs ad	100	ml

*a = buffer concentration dependent on PartIIIA1

**b = Percentage of each selected suspending agent dependent on PartIIIA3 and PartIIIA4

1.2 Cephalexin Monohydrate Dry Syrup

Cephalexin monohydrate dry syrup (125 mg/5ml) was prepared by the following formulation,

Cephalexin monohydrate	2.5	g
Citrate buffer pH 6.0	*a	% w/v
Suspending agent	**b	% w/v
Aerosil®200	1.0	% w/v
Acelsulfame potassium	0.08	% w/v
Raspberry flavor powder	0.50	% w/v
Ponceua 4R	0.004	% w/v
Methyl paraben(MP)	0.18	% w/v
Propyl paraben(PP)	0.02	% w/v
Icing sugar qs to	30	g
Purified Water qs ad	100	ml

*a = buffer concentration dependent on PartIIIA1

**b = Percentage of each selected suspending agent dependent on PartIIIA3 and PartIIIA4

The preparation procedure was followed the same procedures as mentioned in PartIIIA3.

2. Evaluation of Selected Model Dry Syrup

2.1 Dry Powder

The appearances, content of model drug and moisture of powder formulation were evaluated. The evaluation procedure was followed the same as mentioned in PartIIIA4.

2.2 Reconstituted Model Dry Syrup

The physical property, reconstitution time, viscosity and ease of redispersion were measured using the same procedures as previously described in PartIIIA4.

For stability study, the dry powder which was stored at both room temperature and 45 °C, 75 % RH for 4 months were used for evaluating drug content every month. Model dry syrup was reconstituted by water and determining drug content of model reconstructed suspensions was followed the some as mentioned in Part IIIA4.2.5.

C. Assay of Model Drugs.

The drug content was determined by high performance liquid chromatography (HPLC) method.

1. Assay of Amoxicillin Trihydrate (USP 24)

1.1 Chromatographic Condition and Instrumental Settings

Column : μ-Bondapack™ C18 ODS-1, 3.9x300 mm., particle size 5 μm (Water, USA.)

Mobile phase : Mixture of acetonitrile and 0.01 M monobasic potassium phosphate buffer, pH 5, (4:96 by volume).

Flow rate : 1.8 ml/min.

Detector : UV at 230 nm.

Injection volume : 10 μl.

Temperature : ambient

1.2 Mobile Phase Preparation

Monobasic potassium phosphate of 13.6 g was dissolved in 2,000 ml of water. The solution was adjusted to pH of 5.0 by 45 % potassium hydroxide solutions. The buffer was filtered through a cellulose acetate filter paper (pore size 0.45 μm), mixed with acetonitrile to obtain the solvent ratio of 94:6 v/v. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm) and sonicated for 30 minutes.

1.3 Standard Preparation of Amoxicillin Trihydrate

Amoxicillin trihydrate of 100.0 mg was dissolved in distilled water in a 100 ml volumetric flask to obtain a concentration of stock solution of 1.0 mg/ml. Each concentration of amoxicillin trihydrate standard solution was prepared by pipetting 0.1, 0.3, 0.5, 0.7 and 1.0 ml of stock solution into 10-ml volumetric flasks and

adjusted to volume with distilled water, so that the final concentration of standard solution were 0.01, 0.03, 0.05, 0.07 and 0.10 mg/ml respectively.

1.4 Calibration Curve of Amoxicillin Trihydrate Aqueous Solution

The calibration curve was constructed by plotting the peak area and amoxicillin trihydrate concentration.

1.5 Preparation of Sample Solution

Amoxicillin trihydrate dry syrup was reconstituted by distilled water at concentration of 125 mg/5 ml. Shaking to have homogeneous dispersed suspension and accurately sampling 0.1 ml of suspension into a 100-ml volumetric flask. Distilled water was added for nearly full volume and sonicated for 30 minutes. The sample was held until cool and adjusted to volume with distilled water. Sample solution was filtered by cellulose acetate (pore size 0.45 μm) before injection and 10 μl was injected in column. The peak area ratio was converted into the percentage of active ingredient by means of calibration curve.

Amount of amoxicillin trihydrate in dry syrup was determined by taking sample powder equivalent to 2.5 mg. of amoxicillin trihydrate. The sample was weighed accurately in a 100-ml volumetric flask and the assay was tested in triplicate.

2 Assay of Cephalexin Monohydrate

2.1 Chromatographic Condition and Instrumental Settings

Column : μ-Bondapack™ C18 ODS-1, 3.9x300 mm., particle size 5 μm (Water, USA)

Mobile phase : A mixture of methanol and 0.01 M sodium dihydrogen phosphate buffer, pH 5, (1:3 by volume).

Flow rate : 1.8 ml/min.

Detector : UV at 262 nm.

Injection volume : 10 μl

Temperature : ambient

2.2 Mobile Phase Preparation

Sodium dihydrogen phosphate of 1.2 g was dissolved in 1,000 ml of water. The solution was adjusted to pH of 5.0 by phosphoric acid. The buffer was filtered through a cellulose acetate filter paper (pore size 0.45 μm), mixed with methanol to obtain the solvent ratio of 3:1 v/v. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm) and sonicated for 30 minutes.

2.3 Internal Standard Solution

Pyrazinamide of 20 mg. was accurately weighed and dissolved in distilled water in a 100-ml volumetric flask to obtain a concentration of 0.2 mg/ml. It used as internal standard stock solution.

2.4 Standard Preparation of Cephalexin Monohydrate

Cephalexin monohydrate of 100 mg. was accurately weighed and dissolved in distilled water in a 100-ml volumetric flask to obtain a concentration of stock solution of 1.0 mg/ml. Each concentration of cephalexin standard solution was prepared by pipetting 0.1, 0.3, 0.5, 0.7 and 1.0 ml of stock solution to 10-ml volumetric flasks. Internal standard stock solution of 0.1 ml was added to each volumetric flask and adjusted to volume with distilled water, so that the final concentration of standard solution were 0.01, 0.03, 0.05, 0.07 and 0.10 mg/ml, respectively. A 0.01 ml of internal standard stock solution was added throughout the whole concentration range of cephalexin monohydrate before diluted with distilled water to volume.

2.5 Calibration Curve of Cephalexin Monohydrate Aqueous Solution.

The calibration curve was plotted between the ratio of the peak area of cephalexin monohydrate to the peak of pyrazinamide and cephalexin monohydrate concentration.

2.6 Preparation of Sample Solution

Cephalexin monohydrate dry syrup was reconstituted by distilled water as concentration of 125 mg/5ml. Shaking to have homogeneous suspension and accurately sampling 0.1 ml of suspension in a 100-ml volumetric flask. Internal

standard stock solution of 0.1 ml was added to each volumetric flask before adjusting to volume by distilled water. The sample was added with distilled water nearly full volume and sonicated for 30 minutes. After this, the sample was held until cool and adjusted to volume with distilled water. Sample solution was filtered by cellulose acetate (pore size 0.45 μm) before injection and 10 μl was injected in column. The peak area ratio was converted into the percentage of active ingredient by means of calibration curve.

Amount of drug in cephalexin monohydrate syrup was determined by taking sample powder equivalent to 2.5 mg. of amoxicillin trihydrate. The sample was weighed accurately in a 100-ml volumetric flask and the assay was tested in triplicate.