Chapter II

Materials and Methods

Materials

1. Finest glutinous rice starch (Lot No. 0111124 , Erawan brand , Cho Heng Vermicelli Fac. Co. , Thailand)
2. Materials used in the modification of starch and characterization of modified starches
   - Isopropanol AR grade (Lot No. 9084-03 , J.T. Baker , USA.)
   - Monochloroacetic acid (Lot No. 34HD630 , Sigma Chemical Co. , USA.)
   - Sodium hydroxide (Lot No. 040991 , Eka Nobel Industries , Sweden)
   - Glacial acetic acid (Lot No. K18049863 , E. Merck , Germany)
   - Methanol anhydrous (Lot No. 3016KVDD , Mallinckrodt , USA.)
   - Sodium chloride (Lot No. K20420804-347 , E. Merck , Germany)
   - Hydrochloric acid (Lot No. K22703752-607 , BDH Laboratory , England)
   - Sulfuric acid (Lot No. 733696 , Fisher Scientific Co., USA.)
   - Sodium carbonate anhydrous (Lot No. 145 A614729 , BDH Laboratory , England)
   - Potassium biphthalate (Lot No. CH-9470 , Fluka Chemie AG , Switzerland)
3. Starting materials used in the production of pellets
   - Lactose hydrous (Lot No. 96291 , The Lactose Company Ltd., New Zealand)
   - Dicalcium phosphate dihydrate (Lot No. 360466/1 51897 , Fluka Chemie AG ,Switzerland)
   - Corn starch (Lot No. 9608852 , Supplied by Pharmaceutical Sciences , Bangkok , Thailand)
   - Avicel® PH101 (Lot No. 1722 , distributed by AMC Corporation)
   - Sucrose powder (Thailand)

Apparatus

1. High speed stirrer (Erweka , Germany)
2. Magnetic stirrer (Type MR 3001 , Heidolph , Germany)
3. Analytical balance (Model A200S , Sartorius , Germany)
4. Suction machine (Arthure H. Thomas Co., USA.)
5. Hot air oven (Memmert TYP UL80 , West Germany)
6. Moisture analyzer (Model MA30 , Sartorius , Germany)
7. Fourier Transform Infrared Spectrometer (Model 1760X , Perkin Elmer , USA.)
8. Brabender® Viskoamylograph (Model PT 100 , DISBURG , Germany)
9. Scanning electron microscope (Model JSM-5410LV , Jeol , Japan)
10. Planetary mixer (Kenwood Chef , Hampshire , UK.)
11. Screw extruder (Xtruder® , Model EXKS-1 , Fuji Paudal Co. , Japan)
12. Spheronizer (Model S320 , Niro Fielder , England)
13. Fluidized -bed dryer (Uni-Glatt Laboratory Units , Germany)
14. Mechanical sieve shaker (Josef Deckelmann , Aschaffenburg , West Germany)
15. Durometer (Shore A and D Type 3110 , 3116 , Zwick GmbH , Willich - Munchheide , Germany)
16. Roche Friabilator (Model D-63150 , Erweka , Germany)
17. Image analyzer (KS. 400 V.2 , Kontron Elektronik , Carl Zeiss Vision , Germany)
    - Stereomicroscope (No. 343863 , Olympus , Japan)
    - Color video camera (Model DXC-930P , 3CCD/IRIS , Sony Corp.,Japan)
18. Automated mercury porosimeters (PorSizer® 9320 , Micromeritics , USA.)
19. Particle size analyzer (Mastersizer S , Malvern , England)

Methods

In overall study , the following two steps were carried on. The first was a preparation of modified glutinous rice starches and evaluation of their properties. The second was the application of the modified starches as the aid in manufacture of the pellet which having different components.

A. Preparations and Evaluations of Sodium Carboxymethyl Starches

1. Preparation of Sodium Carboxymethyl Starches

Glutinous rice starch was the native starch used in this experiment. The modified starches were prepared by different carboxymethyl substitution using the procedures modified from those described by Filbert (1952). The following methods were used to obtained different three degrees of substitution (DS) of sodium carboxymethyl starch.

1.1 Method A (Degree of substitution about 0.3)

Eight hundred and seventy five parts of isopropanol and 59 parts of monochloroacetic acid were thoroughly mixed and heated to 45 °C. Two hundred and thirty one parts of finely divided glutinous rice starch (moisture content about 13 %) were added with continuous agitation , followed by 238 parts of a 50 % aqueous solution of sodium hydroxide. The temperature of reaction mixture was increased to 75 °C and maintained for 30 minutes with good agitation , the slurry was cooled to about 40 °C , and then neutralized with glacial acetic acid. After removal of the mother liquor , the product was washed several times with 80 % methanol until there was no sodium chloride in the filtrate (tested with $AgNO_3$) and washed finally with anhydrous methanol. The product was dried in a hot air

oven at 50 °C over night. The dried product was collected and sieved through No. 80 mesh screen. The final dried product about 220 gm/batch was obtained.

1.2 Method B (Degree of substitution about 0.2)

Two hundred and eighty six parts of ethanol (92.4 % by weight) , 29.2 parts of monochloroacetic acid , and 102 parts of glutinous rice starch were slurried , and 38.4 parts of flake 97 % sodium hydroxide dissolved in 69.0 parts of water were added. The temperature of slurry was maintained below 50 °C. Agitation at 50 °C was continued for 20 minutes. The slurry was cooled to about 40 °C , and neutralized with glacial acetic acid. The mother liquor was removed , and the product was washed several times with 80 % methanol until there was no sodium chloride in the filtrate (tested with $AgNO_3$) and washed finally with anhydrous methanol. After drying in a hot air oven at 50 °C over night , the product was sieved through No. 80 mesh screen.

1.3 Method C (Degree of substitution about 0.1)

Two hundred and fifty four parts of anhydrous methanol and 27.6 parts of monochloroacetic acid were thoroughly mixed and heated to 60 °C. One hundred and nine parts of glutinous rice starch were added with continuous mixing. After that , 110 parts of 50 % aqueous sodium hydroxide were added. The reaction mixture was held at 60 °C for 60 minutes with good agitation , and cooled to about 40 °C , and then neutralized with glacial acetic acid. After the removal of the mother liquor , the product was washed several times with 80 % methanol until there was no sodium chloride in the filtrate (tested with $AgNO_3$) and washed finally with anhydrous. The product was dried in a hot air oven at 50 °C over night. Finally , the dried product was passed through No. 80 mesh screen.

2. Evaluation of the Properties of Sodium Carboxymethyl Starches

2.1 Determination of a Degree of Substitution (DS.)

The procedure used to determine a degree of substitution of the above modified starches followed the method for Croscarmellose Sodium as described in USP XXIII. This procedure consists of two steps , the one is titration step and the other is residue on ignition step. The sample of each degree of substitution was determined in triplicate.

2.1.1 Titration Step

1. Weighed 1 gm of starches sample accurately and transfer to a glass-stoppered , 500-ml conical flask.
2. Added 300 ml of sodium chloride solution (1 in 10) and agitated until modified starch dissolved.
3. Twenty five ml of 0.1 N sodium hydroxide VS. were added.
4. Inserted the stopper , and allowed to stand for 5 minutes with intermittent shaking.
5. Five drops of m-cresol purple TS. were added.
6. Added about 15 ml of 0.1 N hydrochloric acid VS. from a buret , inserted the stopper and shaked.
7. If the solution is purple , 0.1 N hydrochloric acid VS. in 1-ml portions were added until the solution was yellow , shaking after each addition.
8. Titrated with 0.1 N sodium hydroxide VS. to a purple endpoint.
9. Calculated the net number of milliequivalent (M) of base required for the neutralization of 1 gm of sodium carboxymethyl starch , on dried basis.

2.1.2 Residue on Ignition Step

1. Weighed accurately 1 gm of sample in a suitable crucible which previously ignited , cooled and weighed.

2. Heated , gently at first , until the sample was thoroughly charred and cool.
3. Moistened the residue with 1 ml of sulfuric acid and then heated gently until white fumes were no longer evolved.
4. The sample was ignited at 800 $\pm$ 25 °C in a muffle furnace until the carbon was consumed.
5. Cooled in a desiccator , weighed , and the percentage of residue on ignition (C) was calculated.

   (Continued the ignition step until constant weight of sample was attained.)

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

$$DS = A + S$$

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 1 gm of modified starch

C = percentage of residue on ignition

2.2 Determination of Carboxymethyl Substitution in Modified Starches

Fourier-transform infrared spectrometer (FT-IR) was used to detect the carbonyl group in the prepared starches. The IR spectrums of modified starches was compared with that of glutinous rice starch. The presence of carbonyl group was used as evidence of substitution of carboxymethyl group in the modified starches.

2.3 Viscosity Measurement

The Brabender amylograph , a rotational instrument which permits continuous determination of viscosity or consistency while cooking and cooling

the starch paste with catridge 700 cmg. , was used to measure the viscosity of MGS compared with native glutinous rice starch by the procedure modified from that described by Smith (1967). The modified procedure was as follows.

A 15 gm of sample , on dry basis , was placed in a 500-ml beaker , distilled water was added to the sample to make a concentration of 3 % w/w , and the mixture was stirred to form a lump-free suspension. The sample slurry was then transferred to the measuring bowl rotated at 75 rpm and a measuring feeler with metal pins was projected down into the bowl to provide a good mixture and prevent sedimentation of the sample. The instrument was started , and the temperature of sample was increased at a rate of 1.5 °C per minute. Heating was continued until the sample temperature reached 95 °C. The sample was maintained at this temperature for 15 minutes and then cooled to 50 °C at the same rate. Heating and cooling at the specified rate and holding at the specified temperature were accomplished automatically by the instrument control. The obtained viscosity values were expressed in Brabender Units (BU).

2.4 Moisture Determination

The moisture content of carboxymethyl starches were determined by moisture analyzer. The sample (3 gm) was exposed to heat at 100 °C until the constant weight was obtained. The percentage of moisture content was based on the following equation.

$$\text{Moisture content (\%)} = \frac{(\text{Wet mass} - \text{Dry mass})}{\text{Wet mass}} \times 100$$

The experiment was repeated three times for each sample.

B. Application of Sodium Carboxymethyl Starches in the Preparation of Pellets.

This part can be subdivided into 4 steps.

1. Particle size analysis of starting materials used in the production of pellet.
2. Preparation of pellets using different DS of modified glutinous rice starch and various amounts of water. Extrusion/spheronization technique was used to prepare these pellets. Sucrose powder , anhydrous lactose , and dicalcium phosphate dihydrate were used as models for soluble and insoluble pellet bases.
3. Evaluation of the physical properties of pellets from step 2 which exhibited good characteristics , for example , round and less agglomeration. From these properties , one formulation from lactose model and one from dicalcium phosphate model which presented good physical properties were chosen for further studies.
4. Preparation and evaluation of pellets using different amounts of modified starch. The selected formulations from step 3 were used in pellet production .

1. Particle Size Analysis of Starting Materials Used in the Pellet Production.

Particle size and size distribution of starting materials used in the preparation of pellet , anhydrous lactose , dicalcium phosphate dihydrate , corn starch and microcrystalline cellulose (Avicel PH 101) , were determined by using particle size analyzer. Each material was measured three times.

2. Extrusion and Spheronization Procedure

Batch size for the experiment was 350 gm of dry powder blend. Different mixtures consisting of various types and amounts of starting materials

were blended in a planetary mixer for 15 minutes and were granulated with appropriate amount of water for 5 minutes. The moist mass was extruded immediately after granulation using laboratory single-screw extruder (Figure 9). In this study , The set-ups of the extruder were kept constant , thus , throughout the study , the 1.00 mm screen and extrusion speed of 42 rpm were used. Three hundred grams of the extrudate was spheronized on a friction plate with radial geometry in a spheronizer (Figure 10) , and the plate was allowed to operate at a known speed for a fixed residence time. After that , the product (about 300 gm) was dried in a fluidized-bed dryer at 50 °C for 20 minutes.

3. Preparation of Pellets

3.1 Preparation of Sucrose Pellets

This section was a preliminary study for application of modified starch in pellet formulations. Sucrose pellets were prepared using various amounts of sucrose and modified glutinous rice starches with degree of substitution 0.32 ,i.e. 65 , 70 , 80 , 90 % w/w and 3 , 2 , 1 , 0.5 % w/w , respectively. Microcrystalline cellulose (Avicel PH101) was used in some formulations as another aid to produce spherical particles. Corn starch was used as a filler. The compositions and spheronization process conditions were shown in Tables 1 - 4. Alteration of the amounts of added water and process conditions were depended on the characteristics of outcome. General characteristics of process and product , particular of sphericity , were observed and recorded.

3.2 Preparation of Lactose Pellets

Lactose is one of the most widely investigated models in extrusion-spheronization process. The amounts of lactose used in this experiment were 65 and 80 % w/w which was replaced with 0.1-0.5 % w/w of modified glutinous rice starch as the aid in the pellet production. Corn starch and Avicel PH101 were used as a filler and extrusion aid , respectively. The modified starch at DS

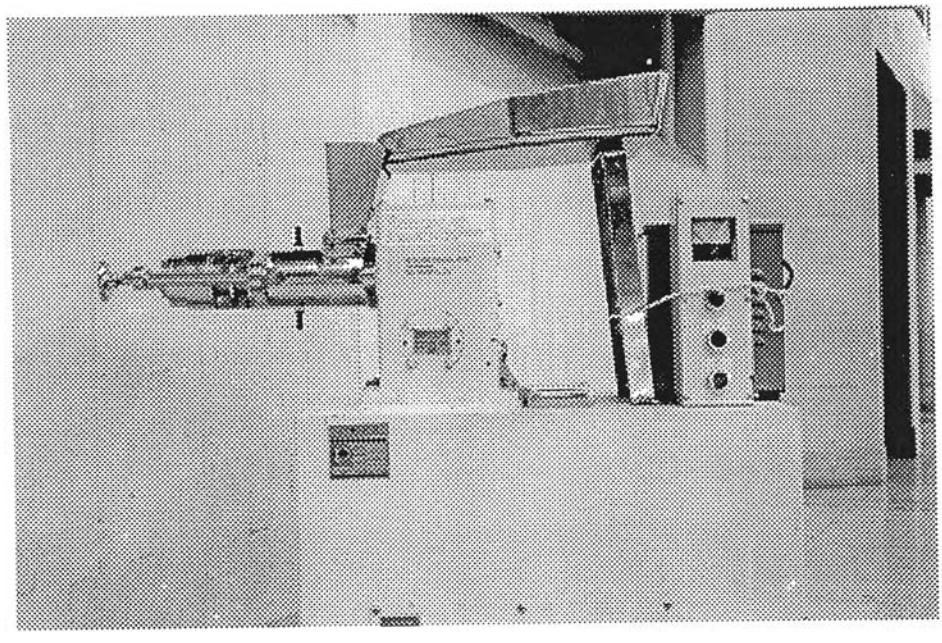

Figure 9 Single - screw extruder

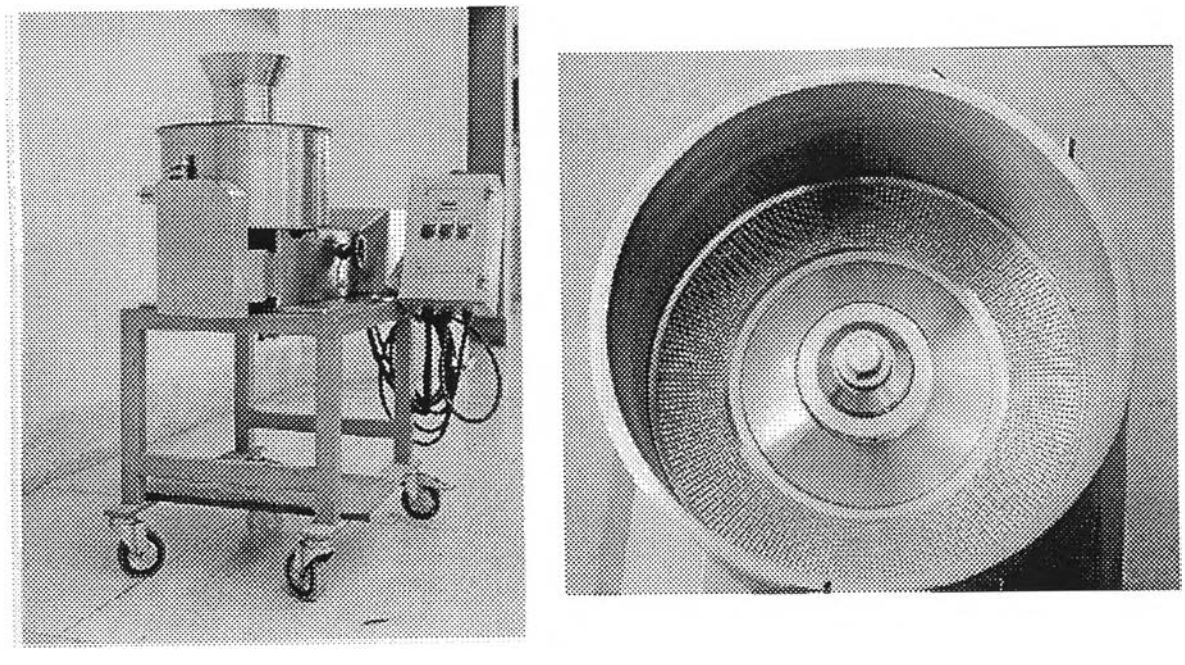

Figure 10 Spheronizer (Left) with radial geometry of friction plate (Right)

Table 1 Compositions and spheronization process conditions in 65 % sucrose pellet formulations.

Title	Formulations						
	$6S_{31}$	$6S_{32}$	$6S_{33}$	$6S_{34}$	$6S_{35}$	$6S_{36}$	$6S_{37}$
Ingredients (%w/w)							
Sucrose powder	65.0	65.0	65.0	65.0	65.0	65.0	65.0
Corn starch	32.0	32.0	34.0	34.0	17.0	17.0	14.5
Avicel PH 101	-	-	-	-	17.0	17.0	20.0
MGS (DS 0.32)	3.0	3.0	1.0	1.0	1.0	1.0	0.5
H_2O	10.3	8.0	8.0	8.0	9.5	8.0	9.5
Conditions							
Spheronizer speed (rpm)	500	500	500	500	800	500	500
Residence time (min)	5	5	5	5	5	5	10
Spheronizer load (gm)	300	300	300	300	300	300	300

$6S_{3(\ )}$: 65 % sucrose pellet formulations prepared with MGS at DS 0.32.

Table 2 Compositions and spheronization process conditions in 70 % sucrose pellet formulations.

Title	Formulations				
	$7S_{31}$	$7S_{32}$	$7S_{33}$	$7S_{34}$	$7S_{35}$
Ingredients (%w/w)					
Sucrose powder	70.0	70.0	70.0	70.0	70.0
Corn starch	29.0	29.0	29.0	29.0	28.0
Avicel PH 101	-	-	-	-	-
MGS (DS 0.32)	1.0	1.0	1.0	1.0	2.0
H_2O	12.0	10.5	9.0	9.0	9.0
Conditions					
Spheronizer speed (rpm)	500	500	500	500	500
Residence time (min)	5	5	10	5	8
Spheronizer load (gm)	300	300	300	400	300

$7S_{3(\ )}$: 70 % sucrose pellet formulations prepared with MGS at DS 0.32.

Table 3 Compositions and spheronization process conditions in 80 % sucrose pellet formulations.

Title	Formulations					
	$8S_{31}$	$8S_{32}$	$8S_{33}$	$8S_{34}$	$8S_{35}$	$8S_{36}$
Ingredients (%w/w)						
Sucrose powder	80.0	80.0	80.0	80.0	80.0	80.0
Corn starch	18.0	18.0	19.0	19.5	19.5	19.5
Avicel PH 101	-	-	-	-	-	-
MGS (DS 0.32)	2.0	2.0	1.0	0.5	0.5	0.5
H_2O	9.0	7.0	9.0	6.5	8.0	9.0
Conditions						
Spheronizer speed (rpm)	500	500	500	500	500	500
Residence time (min)	5	5	5	5	5	5
Spheronizer load (gm)	300	300	300	300	300	300

$8S_{3(\,)}$: 80 % sucrose pellet formulations prepared with MGS at DS 0.32.

Table 4 Compositions and spheronization process conditions in 90 % sucrose pellet formulations.

Title	Formulations						
	$9S_{31}$	$9S_{32}$	$9S_{33}$	$9S_{34}$	$9S_{35}$	$9S_{36}$	$9S_{37}$
Ingredients (%w/w)							
Sucrose powder	90.0	90.0	90.0	90.0	90.0	90.0	90.0
Corn starch	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Avicel PH 101	-	-	-	-	-	-	-
MGS (DS 0.32)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	3.5	6.0	8.0	7.0	7.5	7.0	7.0
Conditions							
Spheronizer speed (rpm)	500	500	500	500	500	400	300
Residence time (min)	2	5	5	5	5	10	15
Spheronizer load (gm)	300	300	300	300	300	300	300

Table 4 (Cont.)

Title	Formulations					
	$9S_{38}$	$9S_{39}$	$9S_{310}$	$9S_{311}$	$9S_{312}$	$9S_{313}$
Ingredients (%w/w)						
Sucrose powder	90.0	90.0	90.0	90.0	90.0	90.0
Corn starch	9.5	9.5	7.0	5.0	5.0	-
Avicel PH 101	-	-	2.5	4.5	4.5	9.5
MGS (DS 0.32)	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	7.5	7.5	7.5	7.5	7.5	7.5
Conditions						
Spheronizer speed (rpm)	400	500	500	500	500	400
Residence time (min)	10	10	5	5	5	10
Spheronizer load (gm)	300	300	300	300	300	300

$9S_{3(\ )}$: 90 % sucrose pellet formulations prepared with MGS at DS 0.32.

0.32 was initially used. The amount of added water and spheronization process conditions were altered to obtain spheres with good appearances. When the product became sphere and showed a good characteristic , MGS with DS 0.26 and 0.16 were respectively applied using the same amount of materials and processing conditions as formulations of DS 0.32.

3.2.1 Formulations of 65 % Lactose Pellets

Table 5 showed the ingredients and spheronization process conditions of 65 % Lactose pellet formulations using modified glutinous rice starches at degree of substitution of 0.32. At first ,0.1-0.5 % of MGS of DS 0.32 was used with different quantities of starting materials - 20-30 % of corn starch and 5-15 % of Avicel PH101, and added water and various process conditions were employed until pellets of good characteristics were produced (formulation $6L_{316}$). After that , the pellets were prepared by increasing and decreasing the amounts of water to determine the effect of added water on physical properties of

pellets , using appropriate processing conditions of formulation $6L_{316}$. Then , the pellets which used the modified starches produced from method B and C (DS 0.26 and 0.16) were respectively performed and the formulations were shown in Tables 6 . For MGS at DS 0.26 , the formulations were $6L_{21}$, $6L_{22}$ and $6L_{23}$ which employed the same compositions and spheronization conditions as of $6L_{316}$, $6L_{317}$ and $6L_{318}$ in Table 5 , respectively . In the same manner , the formulations using MGS of DS 0.16 were coded as $6L_{11}$, $6L_{12}$ and $6L_{13}$.

3.2.2 Formulations of 80 % Lactose Pellets

The pellet formulations using 80 % w/w of lactose were prepared by the procedure similar to that of 65 % lactose pellet formulations. Modified glutinous starch at degree of substitution 0.32 was initially used. Corn starch at 5 % w/w and Avicel PH101 at 15 % w/w were used in these formulations. The compositions and processing conditions were presented in Table 7. Consequently , MGS at DS 0.26 and 0.16 were employed and the formulations were coded as $8L_{21}$, $8L_{22}$, $8L_{23}$ for DS 0.26 , and $8L_{11}$, $8L_{12}$, $8L_{13}$ for DS 0.16. The ingredients and processing conditions of these formulations (in Tables 8) were the same as those of $8L_{32}$, $8L_{33}$ and $8L_{34}$.

3.3 Preparation of Dicalcium Phosphate Pellets

Dicalcium phosphate was used as a model for insoluble pellet base. The quantities of dicalcium phosphate used in this section were 65 and 80% w/w which were partly substituted by 0.5 % w/w of modified glutinous rice starch as an extrusion aid. The fundamental steps of preparation were similar as previously described in preparation of lactose pellets.

Table 5 Compositions and spheronization process conditions in 65 % lactose pellet formulations using MGS at DS 0.32

Title	Formulations																	
	$6L_{31}$	$6L_{32}$	$6L_{33}$	$6L_{34}$	$6L_{35}$	$6L_{36}$	$6L_{37}$	$6L_{38}$	$6L_{39}$	$6L_{310}$	$6L_{311}$	$6L_{312}$	$6L_{313}$	$6L_{314}$	$6L_{315}$	$6L_{316}$*	$6L_{317}$*	$6L_{318}$*
Ingredients (%w/w)																		
Lactose	64.5	64.5	64.5	64.5	64.5	64.5	64.7	64.7	64.8	64.9	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5
Corn starch	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	25.0	25.0	25.0	25.0	20.0	20.0	20.0	20.0
Avicel PH 101	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	10.0	10.0	10.0	15.0	15.0	15.0	15.0
MGS (DS 0.32)	0.5	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.2	0.1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	35.5	32.0	34.0	34.0	32.0	30.0	32.0	33.0	33.0	33.5	36.0	38.0	37.0	37.0	38.0	38.0	37.0	36.0
Conditions																		
Spheronizer speed	500	500	500	800	500	500	500	600	500	500	500	500	800	900	500	500	500	500
Residence time (min)	10	10	5	3	2	2	2	2	2	2	2	2	5	5	5	8	8	8
Spheronizer load (gm)	300	250	200	300	300	150	150	300	150	150	150	150	150	150	300	300	300	300

$6L_{3(\ )}$: 65 % lactose pellet formulations prepared with MGS at DS 0.32.

* : Different amounts of water were used to determine the effect of added water on the physical properties of pellet.

The extrudates of $6L_{314}$ to $6L_{318}$ were left for 10 minutes before processing in spheronizer.

Table 6 Compositions and spheronization process conditions in 65 % lactose pellet formulations using MGS at DS 0.26 and 0.16.

Title	Formulations (DS 0.26)			Formulations (DS 0.16)		
	$6L_{21}$	$6L_{22}$	$6L_{23}$	$6L_{11}$	$6L_{12}$	$6L_{13}$
Ingredients (%w/w)						
Lactose	64.5	64.5	64.5	64.5	64.5	64.5
Corn starch	20.0	20.0	20.0	20.0	20.0	20.0
Avicel PH 101	15.0	15.0	15.0	15.0	15.0	15.0
MGS	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	38.0	37.0	36.0	38.0	37.0	36.0
Conditions						
Spheronizer speed (rpm)	500	500	500	500	500	500
Residence time (min)	8	8	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300	300

The extrudates of all formulations were left for 10 minutes before processing in spheronizer.

Table 7 Compositions and spheronization process conditions in 80 % lactose pellet formulations using MGS at DS 0.32.

Title	Formulations			
	$8L_{31}$	$8L_{32}$	$8L_{33}$	$8L_{34}$
Ingredients (%w/w)				
Lactose	79.5	79.5	79.5	79.5
Corn starch	5.0	5.0	5.0	5.0
Avicel PH 101	15.0	15.0	15.0	15.0
MGS (DS 0.32)	0.5	0.5	0.5	0.5
H_2O	35.0	31.0	30.0	29.0
Conditions				
Spheronizer speed (rpm)	500	500	500	500
Residence time (min)	5	8	8	8
Spheronizer load (gm)	300	300	300	300

$8L_{3()}$: 80 % lactose pellet formulations prepared with MGS at DS 0.32.

* : - Different amounts of water were used to determine the effect of added water on the physical properties of pellet.

The extrudates were left for 10 minutes before processing in spheronizer.

Table 8 Compositions and spheronization process conditions in 80 % lactose pellet formulations using MGS at DS 0.26 and 0.16.

Title	Formulations (DS 0.26)			Formulations (DS 0.16)		
	$8L_{21}$	$8L_{22}$	$8L_{23}$	$8L_{11}$	$8L_{12}$	$8L_{13}$
Ingredients (%w/w)						
Lactose	79.5	79.5	79.5	79.5	79.5	79.5
Corn starch	5.0	5.0	5.0	5.0	5.0	5.0
Avicel PH 101	15.0	15.0	15.0	15.0	15.0	15.0
MGS	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	31.0	30.0	29.0	31.0	30.0	29.0
Conditions						
Spheronizer speed (rpm)	500	500	500	500	500	500
Residence time (min)	8	8	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300	300

The extrudates of all formulations were left for 10 minutes before processing in spheronizer.

3.3.1 Formulations of 65 % Dicalcium Phosphate Pellets

Similarly to the experiments of lactose pellets , MGS at DS 0.32 was initially used as shown in formulations $6D_{31}$-$6D_{315}$ (Table 9). Alteration of added water and spheronization process parameters were made to produce a good appearance of spheres. The pellets employing different amounts of water (52 , 50 and 48 %) were obtained to study the influence of adding water on the physical properties of pellets. Corn starch at 20-35 % and Avicel PH101 at 5-15 % were used as filler and another extrusion aid , respectively. After that , MGS at DS 0.26 and 0.16 were used and code of formulations were $6D_{21}$, $6D_{22}$, $6D_{23}$ and $6D_{11}$, $6D_{12}$, $6D_{13}$ (Table 10). The ingredients and processing conditions of these formulations were similar to those of $6D_{314}$, $6D_{312}$ and $6D_{315}$ but with MGS of DS 0.26 and 0.16.

3.3.2 Formulations of 80 % Dicalcium Phosphate Pellets

MGS at DS 0.32 was initially used according to formulations and processing conditions as shown in Table 11. Corn starch (5 %) and Avicel PH101

Table 9 Compositions and spheronization process conditions in 65 % dicalcium phosphate pellet formulations using MGS at DS 0.32.

Title	Formulations														
	$6D_{31}$	$6D_{32}$	$6D_{33}$	$6D_{34}$	$6D_{35}$	$6D_{36}$	$6D_{37}$	$6D_{38}$	$6D_{39}$	$6D_{310}$	$6D_{311}$	$6D_{312}$*	$6D_{313}$	$6D_{314}$*	$6D_{315}$*
Ingredients (%w/w)															
Dicalcium phosphate	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5
Corn starch	35.0	35.0	35.0	30.0	30.0	30.0	25.0	25.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Avicel PH 101	-	-	-	5.0	5.0	5.0	10.0	10.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
MGS (DS 0.32)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	41.3	39.0	36.0	40.5	42.0	42.0	44.0	47.0	48.0	50.0	50.0	50.0	51.0	52.0	48.0
Conditions															
Spheronizer speed (rpm)	500	500	500	500	500	800	500	500	500	500	800	800	800	800	800
Residence time (min)	8	2	2	3	3	3	3	5	5	5	5	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300

$6D_{3(\ )}$: 65 % dicalcium phosphate pellet formulations prepared with MGS at DS 0.32.

* : Different amounts of water were used to determine the effect of added water on physical properties of pellet.

The extrudates of $6L_{312}$ to $6D_{315}$ were left for 15 minutes before processing in spheronizer.

Table 10 Compositions and spheronization process conditions in 65 % dicalcium phosphate pellet formulations using MGS at DS 0.26 and 0.16.

Title	Formulations (DS 0.26)			Formulations (DS 0.16)		
	$6D_{21}$	$6D_{22}$	$6D_{23}$	$6D_{11}$	$6D_{12}$	$6D_{13}$
Ingredients (%w/w)						
Dicalcium phosphate	64.5	64.5	64.5	64.5	64.5	64.5
Corn starch	20.0	20.0	20.0	20.0	20.0	20.0
Avicel PH 101	15.0	15.0	15.0	15.0	15.0	15.0
MGS	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	52.0	50.0	48.0	52.0	50.0	48.0
Conditions						
Spheronizer speed (rpm)	800	800	800	800	800	800
Residence time (min)	8	8	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300	300

The extrudates of all formulations were left for 15 minutes before processing in spheronizer.

Table 11 Compositions and spheronization process conditions in 80 % dicalcium phosphate pellet formulations using MGS at DS 0.32.

Title	Formulations				
	$8D_{31}$	$8D_{32}$	$8D_{33}$	$8D_{34}$	$8D_{35}$
Ingredients (%w/w)					
Dicalcium phosphate	79.5	79.5	79.5	79.5	79.5
Corn starch	5.0	5.0	5.0	5.0	5.0
Avicel PH 101	15.0	15.0	15.0	15.0	15.0
MGS (DS 0.32)	0.5	0.5	0.5	0.5	0.5
H_2O	45.72	48.0	47.0	46.0	44.0
Conditions					
Spheronizer speed (rpm)	800	800	800	800	800
Residence time (min)	8	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300

$8D_{3()}$: 80% dicalcium phosphate pellet formulations prepared with MGS at DS 0.32.

* : Different amounts of water were used to determine the effect of added water on physical properties of pellet.

The extrudates were left for 15 minutes before processing in spheronizer.

(15%) were used in these formulations. Then MGS of DS 0.26 and 0.16 were tested as shown in formulations $8D_{21}$ - $8D_{23}$ and $8D_{11}$ - $8D_{13}$, respectively (Table 12). These formulations consisted of the same amount of materials and spheronization process parameters as $8D_{32}$, $8D_{34}$ and $8D_{35}$ as presented in Table 11.

3.4 Preparation of Blank Pellets

In this study , the physical properties of pellets prepared as described in 3.2.1 - 3.3.2 were compared with blank pellets which consisted of the same compositions and processing conditions as those formulations but no modified glutinous rice starch was added. Table 13 and 14 display the formulations of lactose and dicalcium phosphate blank pellets , respectively.

4. Evaluation of the Physical Properties of the Pellets

4.1 Particle Size Analysis

One hundred grams of each pellet formulation was sieved through a nest of sieves (Endecotts test sieves , UK.) to separate them into various size fractions. Sieves series of aperture sizes of 1400 , 1000 , 710 , 500 and 250 μm. (No. 14 , 18 , 25 , 35 and 60 mesh) were used and placed on a mechanical sieve shaker for 10 minutes. The amount of spheres remaining on each sieve was calculated as percentage of total weight. After that , geometric mean diameter and geometric standard deviation were calculated from cumulative percent undersize plot.

4.2 Bulk and Tapped Densities

Bulk density of pellets was determined by pouring 50 gm of sample into a 100 ml graduated cylinder. Then , the initial volume was recorded , and bulk density could be calculated from division of weight by initial volume.

Table 12 Compositions and spheronization process conditions in 80 % dicalcium phosphate pellet formulations using MGS at DS 0.26 and 0.16.

Title	Formulations (DS 0.26)			Formulations (DS 0.16)		
	$8D_{21}$	$8D_{22}$	$8D_{23}$	$8D_{11}$	$8D_{12}$	$8D_{13}$
Ingredients (%w/w)						
Dicalcium phosphate	79.5	79.5	79.5	79.5	79.5	79.5
Corn starch	5.0	5.0	5.0	5.0	5.0	5.0
Avicel PH 101	15.0	15.0	15.0	15.0	15.0	15.0
MGS	0.5	0.5	0.5	0.5	0.5	0.5
H_2O	48.0	46.0	44.0	48.0	46.0	44.0
Conditions						
Spheronizer speed (rpm)	800	800	800	800	800	800
Residence time (min)	8	8	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300	300

The extrudates of all formulations were left for 15 minutes before processing in spheronizer.

Table 13 Compositions and spheronization process conditions in 65 % and 80 % lactose blank pellet formulations.

Title	Formulations					
	B.1	B.2	B.3	B.4	B.5	B.6
Ingredients (%w/w)						
Lactose	65.0	65.0	65.0	80.0	80.0	80.0
Corn starch	20.0	20.0	20.0	5.0	5.0	5.0
Avicel PH 101	15.0	15.0	15.0	15.0	15.0	15.0
H_2O	38.0	37.0	36.0	31.0	30.0	29.0
Conditions						
Spheronizer speed (rpm)	500	500	500	500	500	500
Residence time (min)	8	8	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300	300

Table 14 Compositions and spheronization process conditions in 65 % and 80 % dicalcium phosphate blank pellet formulations.

Title	Formulations					
	B.7	B.8	B.9	B.10	B.11	B.12
Ingredients (%w/w)						
Dicalcium phosphate	65.0	65.0	65.0	80.0	80.0	80.0
Corn starch	20.0	20.0	20.0	5.0	5.0	5.0
Avicel PH 101	15.0	15.0	15.0	15.0	15.0	15.0
H_2O	52.0	50.0	48.0	48.0	46.0	44.0
Conditions						
Spheronizer speed (rpm)	800	800	800	800	800	800
Residence time (min)	8	8	8	8	8	8
Spheronizer load (gm)	300	300	300	300	300	300

Tapped density was measured by dropping the graduated cylinder on a hard surface from a height of 5 cm until the volume did not further decreased. This final volume divided by weight of pellets was tapped density. Both densities were averaged from three measurements.

4.3 Hardness

The determination of hardness was performed by measuring the force required to break a pellet using Shore A and D Durometer , fitted with 10 and 100 N load cell , respectively. Ten pellets of each formulation were tested. The obtained data were in shore unit. Force expressed in Newton (N) could be calculated from the following equations.

$$\text{Force (N)} = 0.550 + (0.075 + \text{Shore Units}) \quad \text{.....Shore A}$$

$$\text{Force (N)} = \text{Shore units} \times 0.4445 \quad \text{.....Shore D}$$

4.4 Friability

Pellet friability was determined by placing 1 gm of pellets together with 20 metallic beads (average diameter 0.5 mm) , to increase the mechanical stress on the pellet , in a 30 ml-plastic bottle. Then , the pellets were rotated in Roche friabilator at 25 rpm for 4 minutes. After that , the pellets were put on a 425 μm (No. 40 mesh) sieve to remove dust and smaller particles , and weighed again. Percent friability was calculated from the following equation.

$$\text{Friability (\%)} = \frac{\text{Weight loss}}{\text{Initial weight}} \times 100$$

4.5 Sphericity

Sphericity of 20 pellets with a specific size was analyzed using an image analyzer. The image analyzer consisted of a computer system linked to a video camera and a stereomicroscope. Each individual pellet was inspected and data were processed automatically. The feature parameters , longest diameter or $feret_{max}$ (R_1) , smallest diameter or $feret_{min}$ (R_2) , area , and perimeter were determined. Aspect ratio and form factor which gave a measure of the degree of pellet sphericity were derived from those four basic parameters and could be calculated by the following equations.

$$\text{Aspect ratio} = \frac{\text{Longest diameter}}{\text{Smallest diameter}} = R_1 / R_2$$

$$\text{Form factor} = \frac{4\pi[\text{Area}]}{[\text{Perimeter}]^2}$$

These two values of unity describe a perfect circle.

In the determination of hardness , friability and sphericity , three different sieve size fractions of 1000-1400 , 710-1000 and 500-710 μm. were tested.

4.6 Morphology by Scanning Electron Microscopy (SEM)

The pellets were placed on the stub using adhesive tape and coated with gold before being photographed using a Jeol JSM - 5410LV SEM. Size fractions of 1000-1400 , 710-1000 and 500-710 μm. of each pellet formulation were photographed individually. In addition , SEM pictures were taken of surface and cross-sectioned pellets. SEM pictures of unsieved pellets were also taken.

4.7 Porosity

Porosity determination was performed utilizing mercury intrusion porosimeter (PorSizer® 9320) by forcing mercury into pellet void space under pressure. A tested sample of about 2 gm was put in 5 ml- penetrometer. Then , the sample was measured using low pressure operation and automated high pressure operation. The obtained result was expressed as percent porosity.

Porosity of one formulation from lactose model and one from dicalcium phosphate model were measured and compared with that of blank pellets to determine the influence of incorporated modified glutinous rice starch on porosity of pellets.

5. Preparation and Evaluation of Pellet Formulations Using Different Amounts of Modified Starches

In this part , the different amounts of modified glutinous rice starch were used while the other parameters were kept constant to evaluate this effect on the physical properties of pellet. After consideration of the physical properties

of all previous described formulations , one formulation from lactose model and one from dicalcium phosphate model which exhibited good physical properties were chosen for varying the quantity of modified starch.

5.1 Formulations of Lactose Pellets Using Different Amounts of Modified Starch

The formulation composed of 65 % lactose , 0.5 % MGS at DS 0.26 and 37 % of added water , formulation $6L_{22}$, was selected to study in this part. Preparation of pellets were performed by decreasing and increasing the quantity of MGS to 0.3 and 0.8 % w/w. These pellet formulations were presented in Table 15 which also included blank pellet formulation as shown in formulation B.2.

5.2. Formulations of Dicalcium Phosphate Pellets Using Different Amounts of Modified Starch

In this part , formulation $8D_{35}$ composed of 80 % dicalcium phosphate , 0.5 % MGS at DS 0.32 and 44 % of added water was selected for varying the quantity of modified starch. Table 16 shows the formulations using different amounts of MGS together with blank pellet formulation (B.12) and formulation $8D_{35}$.

The physical properties of formulation L.1 , L.2 , D.1 and D.2 were evaluated by the same procedures as previously described (in step 4) except porosity determination and compared with those of basic ($6L_{22}$ and $8D_{35}$) and blank (B.2 and B.12) pellets. Consequently , one formulation of lactose model and one of dicalcium phosphate model were used for porosity determination (see 4.7 in step 4). The porosity of these final formulations were compared with their blank pellets.

Table 15 Compositions and spheronization process conditions in lactose pellet formulations using different amounts of modified starch.

Title	Formulations			
	L.1	$6L_{22}$	L.2	B.2
Ingredients (%w/w)				
Lactose	64.7	64.5	64.2	65
Corn starch	20.0	20.0	20.0	20.0
Avicel PH 101	15.0	15.0	15.0	15.0
MGS (DS 0.26)	0.3	0.5	0.8	-
H_2O	37.0	37.0	37.0	37.0
Conditions				
Spheronizer speed (rpm)	500	500	500	500
Residence time (min)	8	8	8	8
Spheronizer load (gm)	300	300	300	300

The extrudates of all formulations were left for 10 minutes before processing in spheronizer.

Table 16 Compositions and spheronization process conditions in dicalcium phosphate pellet formulations using different amounts of modified starch.

Title	Formulations			
	D.1	$8D_{35}$	D.2	B.12
Ingredients (%w/w)				
Dicalcium phosphate	79.7	79.5	79.2	80.0
Corn starch	5.0	5.0	5.0	5.0
Avicel PH 101	15.0	15.0	15.0	15.0
MGS (DS 0.32)	0.3	0.5	0.8	-
H_2O	44.0	44.0	44.0	44.0
Conditions				
Spheronizer speed (rpm)	800	800	800	800
Residence time (min)	8	8	8	8
Spheronizer load (gm)	300	300	300	300

The extrudates of all formulations were left for 15 minutes before processing in spheronizer.