CHAPTER II

EXPERIMENTALS

2.1 Materials

The following substances were obtained from commercial sources except for yeast extract powder

2.1.1 Substances for Tablet Making

2.1.1.1 spray dried yeast extract (supplied by Institute of Biotechnology and Genetic Engineering, IBGE, Chulalongkorn University)

2.1.1.2 magnesium carbonate light (Japan, supplied by Pharmaceutical Traders Co.,Ltd., Thailand)

2.1.1.3 calcium hydrogen phosphate (USA, supplied by Srichand United Dispensary Ltd. Partnership, Thailand)

2.1.1.4 corn starch (Pharmaceutical Science Ltd. Part., Thailand)

2.1.1.5 Cab-O-Sil® M-5 (Cabot, Belgium)

2.1.1.6 magnesium stearate (Peter Greven Fett-Chemie, Germany)

2.1.2 Substances for Microbiological Test

2.1.2.1 lactose broth (Difco, USA)

2.1.2.2 selenite broth (Difco, USA)

2.1.2.3 tetrathionate broth (Difco, USA)

2.1.2.4 Salmonella-Shigella agar (Difco, USA)

2.1.2.5 bismuth sulfite agar (Difco, USA)

2.1.2.6 brilliant green agar (Difco, USA)

2.1.2.7 triple sugar-iron sugar (Difco, USA)

2.1.3 Other Substances

2.1.3.1 lithium chloride (E.Merck, Germany)

2.1.3.2 potassium acetate (E.Merck, Germany)

2.1.3.3 magnesium chloride hexahydrate (E.Merck, Germany)

2.1.3.4 potassium carbonate (E.Merck, Germany)

2.1.3.5 magnesium nitrate hexahydrate (E.Merck, Germany)

2.1.3.6 sodium nitrite (E.Merck, Germany)

2.1.3.7 sodium acetate trihydrate (E.Merck, Germany)

2.1.3.8 ammonium sulfate (Fluka, Switzerland)

2.1.3.9 potassium nitrate (Riedel-De Häen Ag, Germany)

2.2 Methods

2.2.1 Size Screening

Spray dried yeast extract was received in various particle sizes. It was in the form of powder, granule, and lump. For further studies, the granule and lump part were milled using Moulinex grinder (Moulinex, Type 241.2.00, France) and passed through a 30-mesh screen. Then the powder part and screened part of yeast extract were mixed for 5 minutes in sigma-blade mixer (Viuhang Engineering, Thailand). The yeast extract powder was uniformly obtained.

2.2.2 Properties of Yeast Extract Powder

2.2.2.1 Morphology Determination

Shape and surface texture of yeast extract powder was characterized by using scanning electron microscope (JEOL®, JSM-T220A, USA) and photomicrographs were taken.

2.2.2.2 Particle Size Distribution and Specific Surface Area Determination

Size distribution, specific surface area and everage diameter of particle of yeast extract powder were examined by laser diffraction analysis (Malvern Particle Sizers, Series 2600 C, UK).

2.2.2.3 Thermal Analysis

Physico-chemical changes of yeast extract powder during heating from room temperature to 300 °C were determined by differential thermal analysis (Shimadzu, Japan).

2.2.2.4 Moisture Content Determination

The moisture content determination was carried out by applying from the method of examining moisture content in dried milk (Helrich, 1990). One gram of yeast extract powder was accurately weighed and evenly distributed over a 5.5 cm. diameter, round, flat-bottom, aluminium dish with tight-fitting slip-in cover (Figure 2-1). The dish was dried previously in hot air oven (Memmert, Germany) and weighed. The dish with the yeast extract powder and loosen cover was then place in vacuum oven (Precision Scientific, Inc., Model 01.9, USA) at 100 °C. The sample was dried to constant weight within about 16 hours under

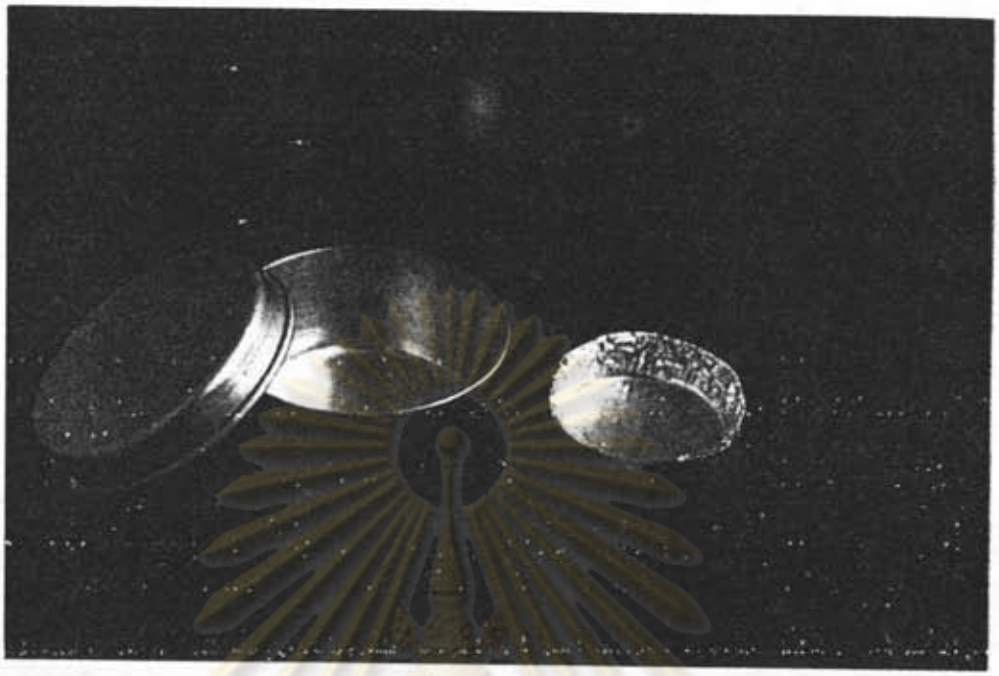

Figure 2-1 Moisture Dish and Hand-made Dish

Figure 2-2 Apparatus Used for Moisture Adsorption Isotherm Determination

absolute pressure not over 100 mm. (4 in.) of Hg. While the sample was drying, slow current of dried air which was passed through sulphuric acid was admitted into the oven about 2 bubbles/second. Before removing the dish from the oven, its cover was pressed tightly. Then it was cooled in a desiccator filled with silica gel. The percent moisture content (dry basis) was calculated from 3 determinations.

2.2.2.5 Moisture Adsorption Isotherm Determination

Yeast extract powder was accurately weighed 300 mg. and evenly spread over a dish which had approximately 3.5 cm. in diameter and 1.0 cm. in height, made by compressing 7 layers of Diamond® aluminium foil (Figure 2-1). Nine dishes of yeast extract powder were put into 9 glass bottles. Then they were kept in desiccators (Figure 2-2) containing saturated salt solutions : lithium chloride (LiCl), potassium acetate ($KC_2H_3O_2$), magnesium chloride hexahydrate ($MgCl_2.6H_2O$), potassium carbonate (K_2CO_3), magnesium nitrate hexahydrate ($MgNO_3.6H_2O$), sodium nitrite ($NaNO_2$), sodium acetate trihydrate ($NaC_2H_3O_2.3H_2O$), ammonium sulfate ($(NH_4)_2SO_4$), potassium nitrate (KNO_3) at room temperature (approximately 30°C) which could give relative humidity at 11.2, 22.0, 32.8, 43.6, 52.0, 63.3, 71.3, 80.8, and 90.7 percent, respectively (Wink, 1963). After 24 hours, each dish of yeast extract powder was checked for equilibrium condition using humidity recorder (Yamato Scientific Co., Ltd., Model YH-33R, Japan). The bottle was taken out of the desiccator and covered immediately with its bottle cover which was connected with a probe of the hygrometer (Figure 2-3). While measuring, the surrouding temperature was controlled (Figure 2-4). Then the sample was determined

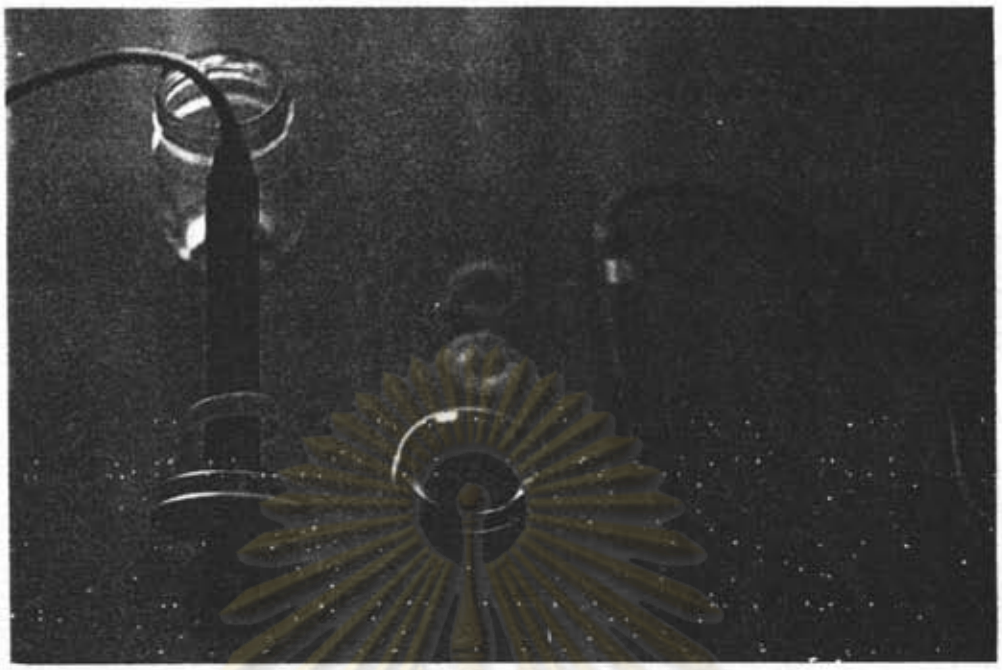

Figure 2-3 Probe of Hygrometer Connecting to the Bottle Cover

Figure 2-4 Relative Humidity Measuring Condition

for its moisture content as in section 2.2.4. The percent moisture contents (dry basis) were plotted against the percent equilibrium relative humidity. Each point of the percent moisture content on the graph was calculated from 3 determinations.

2.2.2.6 Flowability Determination

Yeast extract powder was weighed 50.00 g. and filled into a Nalgene® 60 powder funnel with 100-mm. maximum diameter cone, *ca.* 70 mm. long and 18-mm. diameter outlet tube, *ca.* 34 mm. long (Figure 2-5). Time was recorded as soon as the yeast extract powder was permitted to flow. The flow rate was calculated in g./sec.. The mean flow rate was the average of 3 determinations.

2.2.2.7 Solubility Determination

Yeast extract powder, approximately one gram, was excessively dissolved in 10 ml. of distilled water which was filled into a 16x125 mm. screw-cap test tube. The closed test tube was clamped in agitation equipment (Figure 2-6) and turned round about 40 rpm. by a motor (Induction motor, Department of Manufacturing Pharmacy, Chulalongkorn University). The temperature of the agitation equipment was maintained at 37±2 °C by using thermoregulator (Techne (Cambridge) Ltd., Model FAP6, England). After 4 hours, the saturated solution of yeast extract was filtered through No.1 Whatmann filter paper into a beaker. The filtration was set in incubator (Memmert, Germany) to maintain the temperature at 37 °C. Five milliliters of filtrate was pipeted into an evaporating dish and continuously kept in the incubator till most of the water was evaporated. Then the semi-

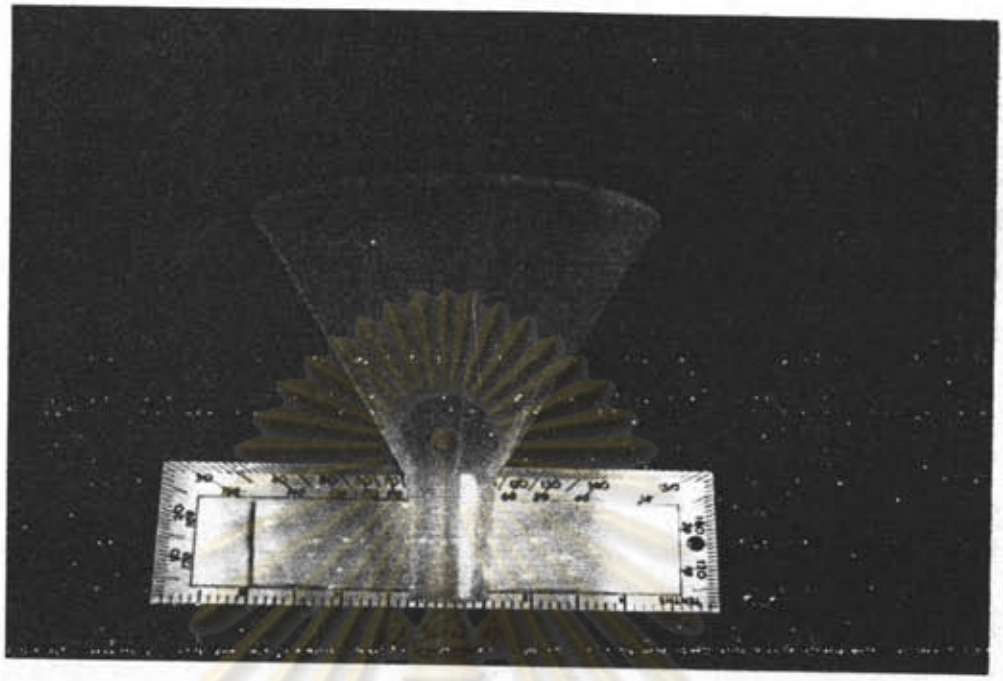

Figure 2-5 Powder Funnel

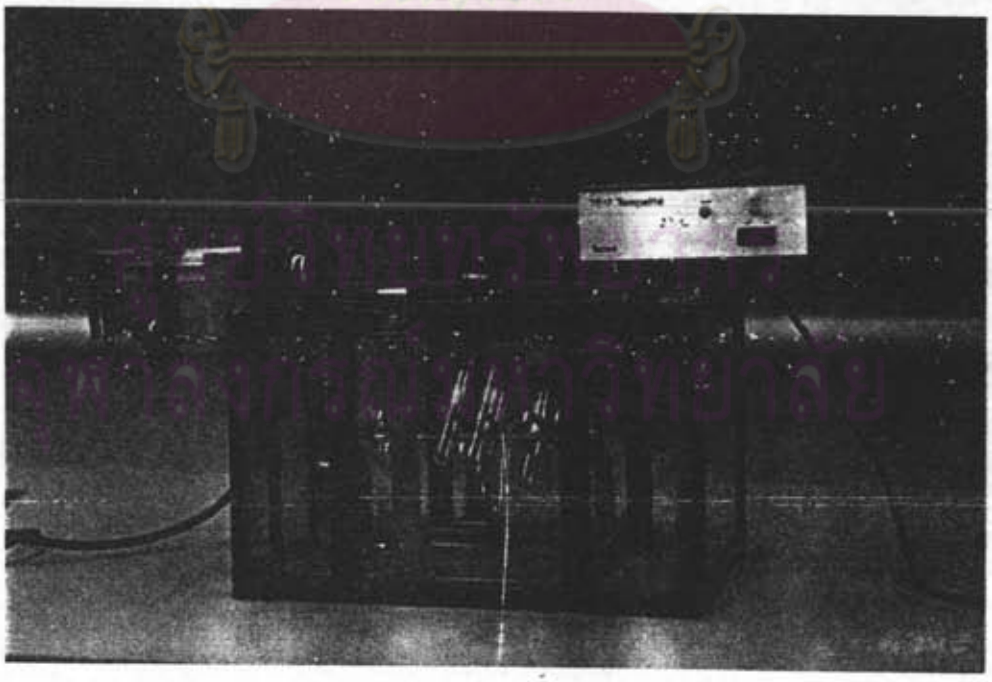

Figure 2-6 Agitation Equipment

solid filtrate was dried in vacuum oven (Precision Scientific Inc., Model 01.9, USA) as in section 2.2.4. The residue was calculated from 3 determinations and reported in g./5 ml. of filtrate.

2.2.2.8 Density Determination

2.2.2.8.1 Bulk Density (Carstensen, 1977)

Bulk density was determined by pouring 50.00 gram of yeast extract powder into 100 ml. graduated cylinder which was tilted to an angle of 60 degree. The volume occupied by the yeast extract powder was read to the nearest 0.5 ml.. The density was calculated from 3 determinations and reported in g./ml..

2.2.2.8.2 Tapped Density (Carstensen, 1977)

Following the determination of the bulk density, tapped density was measured by dropping the cylinder onto the hard wood surface from the height of 5 cm. until the constant volume of yeast extract powder was obtained (about 100 tramps). The volume was read to the nearest 0.5 ml.. The density was calculated from 3 determinations and presented in g./ml..

Percent compressibility of yeast extract powder was calculated as follow :

$$\% \text{ compressibility} = \frac{(\text{tapped density} - \text{bulk density}) \times 100}{\text{tapped density}}$$

2.2.2.8.3 True Density

One gram of yeast extract powder was accurately weighed and measured for its true volume using Multivolume Pycnometer (Micromeritics Instrument Corp., Model 1305, USA). The true density was calculated from 3 determinations following the equation mentioned in the Multivolume Pycnometer manual and reported in g./ml..

2.2.3 Method of Granulation

2.2.3.1 Preparation of Corn Starch Paste

At first, the water was divided into 4 parts. Weighed corn starch was suspended in one part of cold water. The rest of the water was heated to boiling then added to the corn starch suspension. This mixture was heated again with constant stirring to 70 °C till translucent paste occurred. Some cold water was added into the paste when its weight was lost by water vaporization.

2.2.3.2 Preparation of Granules

Granules were prepared according to the formulations presented in Table 2-1. The yeast extarct powder was active ingredient. Magnesium carbonate, corn starch, and calcium hydrogen phosphate (or dibasic calcium phosphate) were diluents. Corn starch paste was binding agent. The certain amount of the yeast extract powder and the diluents used in the formulations were weighed and thoroughly mixed by mortar and pestle for 9 minutes. Firstly, the yeast extract powder and magnesium carbonate were mixed together then added with corn starch and dibasic calcium phosphate, respectively. The interval time for each mixing period was 3 minutes. The mixture was kneaded into damp

Table 2-1 Formulations of Yeast Extract Tablets

Ingredients (mg./tab.)	Formula No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Yeast extract powder	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Magnesium carbonate	22.0	16.0	22.0	16.0	22.0	16.0	22.0	16.0	22.0	16.0	22.0	16.0	22.0	16.0	22.0	16.0
Corn starch	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0
Calcium phosphate dibasic	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0
Binder (Dry weight)	6.8*	6.8*	3.4**	3.4**	6.8*	6.8*	3.4**	3.4**	6.8*	6.8*	3.4**	3.4**	6.8*	6.8*	3.4**	3.4**
Dried corn starch	24.0	24.0	24.0	24.0	14.5	14.5	14.5	14.5	24.0	24.0	24.0	24.0	14.5	14.5	14.5	14.5
Cab - O - Sil	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Magnesium stearate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Total weight	345.3	339.3	341.9	335.9	335.8	329.8	332.4	326.4	343.9	337.9	340.5	334.5	334.4	328.4	331.0	325.0

* Prepared to 10% wt./wt. corn starch paste

** Prepared to 5% wt./wt. corn starch paste

Both corn starch paste concentration were used in equal amount.

mass with the binding agent till uniforming damp mass was occurred. The damp mass was passed then through a 12-mesh hand screen onto an aluminium tray. The tray was placed in tray dryer (Lytzen, Denmark) at 55±5°C. Two hours later, the dried mass was granulated by using oscillating granulator (Viuhang Engineering, Thailand) through a 16-mesh screen. Then it was again dried in the tray dryer for about 4 hours. Approximately one gram of granules was sampled to determine the moisture content by using moisture determination balance (Ohaus Scale Corp., Model No.6100-H, USA). The lamp was set at the height of 1.5 inches from the sample pan and the dial was set at No.4 for 15 minutes (about 85°C). Percent moisture content was calculated from the following equation :

$$\% \text{ moisture} = \frac{(\text{wet mass} - \text{dry mass}) \times 100}{\text{wet mass}}$$

If percent moisture (wet basis) was less than 2 percent, the drying process was stopped.

2^2 factorial design experiment with 4 replicates was used in this granulation studies (Table 2-2). The variables and their corresponding levels for this experiment are:

1. Magnesium carbonate light content of 22.0 and 16.0 mg. per tablet
2. Corn starch paste, binding agent, using at the level of 6.8 and 3.4 mg. per tablet

Table 2-2 Experimental Arrangement for 2^2 Factorial Experiment

Treatment Combination	Factor A	Factor B	Formula Number
1	-	-	4, 8, 12, 16
a	+	-	3, 7, 11, 15
b	-	+	2, 6, 10, 14
ab	+	+	1, 5, 9, 13

A = magnesium carbonate light

B = corn starch paste

2.2.4 Physical Properties Determinations of Granules

2.2.4.1 Morphology Determination

Shape and surface texture of granules were characterized by using the scanning electron microscope (JEOL®, Model JSM-T220A, USA) and photomicrographs were taken.

2.2.4.2 Particle Size Distribution Determination

Fifty grams of granule was put on the top of the sieve series (Endecotts Ltd., England) stacking vertically in the order of decreasing aperture size : 840, 420, 250, 177, 149 μm.. The nest of sieve was placed on sieve shaker (Josef Deckelman, Germany). The amount remaining on each screen after 5-minute shaking is then weighed. The results were calculated from 3 determinations and reported as histogram plot in which percent distirbution (by weight) is plotted as a function of the size range.

2.2.4.3 Moisture Content Determination

Granules were determined by using the same procedure as in section 2.2.2.4.

2.2.4.4 Flowability Determination

Granules were determined as in section 2.2.2.6.

2.2.4.5 Density Determination

Approximately 25.00 g. of granules were determined as in section 2.2.2.8.

2.2.5 Preparation of Tablets

2.2.5.1 Tabletting Machine

A single punch tablet machine (Viuhang Engineering, Thailand), driven at constant speed of 900 rpm. electric motor was used for tablet compression. The compression force could be measured by strain gauges (Kyowa, Type YFLA-5, Japan) which were connected to strain meter (Tokyo Sokki Kenkyojo Co.,Ltd., Model DA-12A, Japan) with recorder (Instrument Corp., Model Linear 252, USA). Two strain gauges were mounted to the upper plunger with an adhesive, then coated with moisture proofing wax (Figure 2-7). They were calibrated by using Universal Testing Machine (Shimadzu, Japan). When stress was applied, gauge resistance was changed and responses were shown by the recorder.

2.2.5.2 Compression of Tablets

All disintegrants and lubricants were passed through 80-mesh screen. Dried corn starch was prepared by drying in hot air oven (Memmert, Germany) at 70 °C for 30 minutes. Following the formulations in Table 2-1, each batch of granules was thoroughly mixed with the disintegrant by using manual bottle tumbling method. After mixing by rotating the 1000 ml. cylindrical plastic bottle for 100 rounds, Cab-O-Sil® was added, then mixing procedure was continued for another 100 rounds. Finally, magnesium stearate was mixed in the same procedure. The mixture was compressed into tablets using the single-punch tablet machine equipped with 3/8 in., round flat-face punch at the compression force of 600 pounds. The tablet weight of each formulations was shown in Table 2-1.

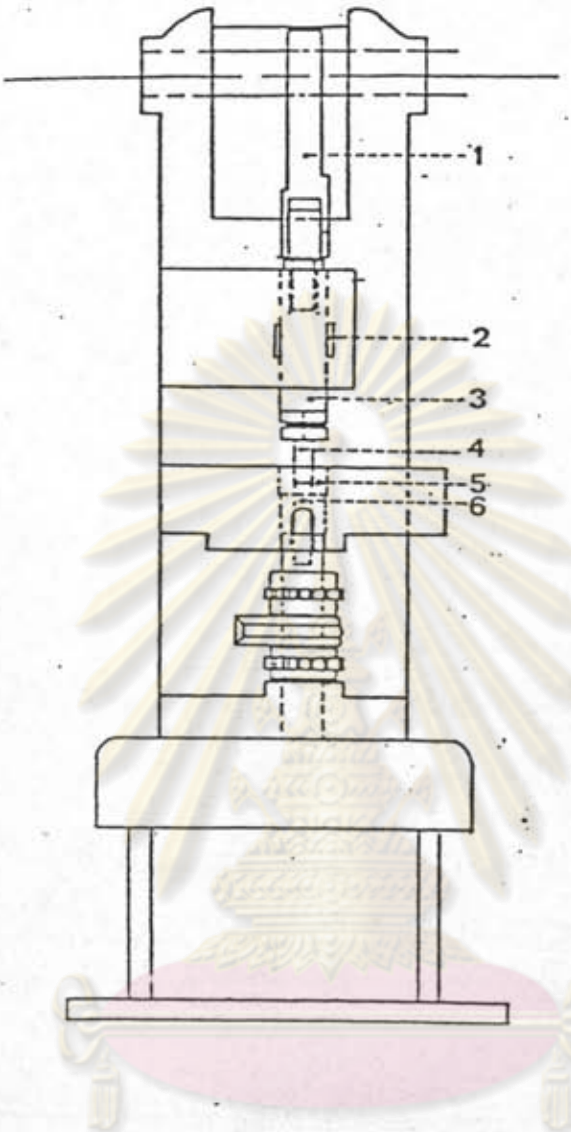

Figure 2-7 Schematic Drawing of the Tablet Machine

(1) eccentric sheaf
(2) strain gauge
(3) upper plunger
(4) upper punch
(5) die
(6) lower punch

2^4 factorial experiment was designed for the tablet making studies (Table 2-3) and in addition to the Yate's method (Box, Hunter, and Hunter, 1978; Montgomery, 1991), normal probability plot (Box, Hunter, and Hunter, 1978; Montgomery, 1991) was used for statistical analysis.

2.2.6 Tablet Evaluation

After compression, the following physical properties of yeast extract tablets were immediately assessed.

2.2.6.1 Weight Variation

Twenty tablets from each batch were individually weighed using analytical balance (Sartorius, Model A-200S, Germany). Average weight, standard deviation and percent coefficient of variation were calculated. The weight variations were checked according to the United States Pharmacopeia 22th edition (USP XXII).

2.2.6.2 Hardness

Ten tablets were individuallly determined using Strokes-Monsanto hardness tester (Manesty Machines Ltd., England) which expressed in kilogram unit. Means and standard deviations were calculated.

2.2.6.3 Friability

Twenty tablets was accurately weighed and placed in friabilator (Erweka, Type TAP, Germany) rotated at 25 rpm. for 4

Table 2-3 Experimental Arrangement for 2^4 Factorial Experiment

Treatment Combination	Factor A	B	C	D	Formula Number
1	-	-	-	-	16
a	+	-	-	-	15
b	-	+	-	-	14
ab	+	+	-	-	13
c	-	-	+	-	12
ac	+	-	+	-	11
bc	-	+	+	-	10
abc	+	+	+	-	9
d	-	-	-	+	8
ad	+	-	-	+	7
bd	-	+	-	+	6
abd	+	+	-	+	5
cd	-	-	+	+	4
acd	+	-	+	+	3
bcd	-	+	+	+	2
abcd	+	+	+	+	1

A = magnesium carbonate light

B = corn starch paste

C = dried corn starch

D = magnesium stearate

minutes. Then the tablets were dedusted with a soft brush to remove adhering particles and reweighed. Percent friability was calculated as follow :

$$\% \text{ friability} = \frac{\text{weight loss} \times 100}{\text{initial weight of 20 tablets}}$$

2.2.6.4 Thickness

Ten tablets were individually measured to the nearest 0.01 mm. using thickness tester (Teclock Corp., Germany). Means, standard deviations and percent coefficient of variation were reported.

2.2.6.5 Disintegration Time

Disintegration time of yeast extract tablets was determined in purified water at 37 $\pm$ 2 °C by using disintegration test apparatus (Hanson Research Corp., Model QC-21, USA) procedure according to the USP XXII. The disintegration time of 6 tablets was individually observed.

2.2.7 Salmonella Test

Salmonella test for yeast extract powder and selected yeast extract tablets was examined according to the procedure mentioned in the National Formulary 13th edition (NF XIII) which was the test for *Salmonella* in dried yeast tablets. The results were observed duplicatedly.

2.2.8 Aging Studies of Yeast Extract Tablets

2.2.8.1 Aging in Closed Containers

Yeast extract tablets from the same production batch were kept in closed amber glass bottles (Figure 2-8) and stored in desiccators which were given relative humidity condition of 32.8, 52.0, and 71.3 percent at room temperature (about 30°C). These conditions were prepared by using saturated salt solutions of magnesium chloride hexahydrate, magnesium nitrate hexahydrate, and sodium acetate trihydrate, respectively. The sample of storage tablets were taken at 30, 60, and 90 days period for the evaluations mentioned in section 2.2.6.

2.2.8.2 Aging in Opened Containers

Yeast extract tablets from the same production batch were placed in opened containers (Figure 2-9) and exposed to three relative humidity conditions used in section 2.2.8.1. Representative tablets were removed and evaluated at 7, 15, and 30 day period as in section 2.2.6 except for percent friability. The appearance by visual inspection and photography was additionally observed.

Single-factor experiment with three aging periods as variables was designed for aging studies of the yeast extract tablets.

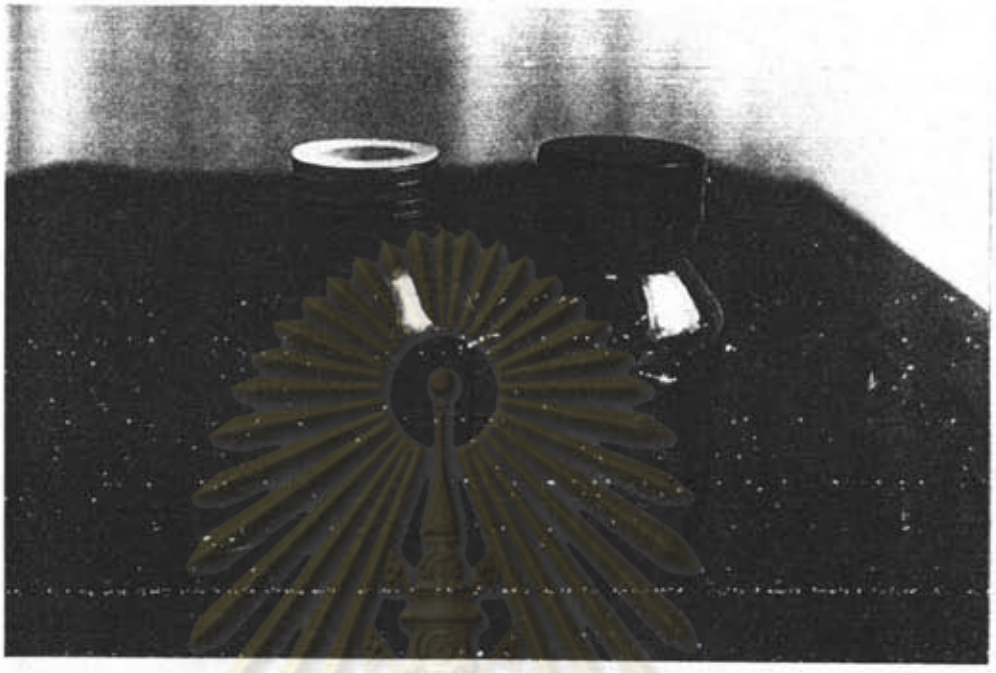

Figure 2-8 Closed Container Used for Aging Studies

Figure 2-9 Opened Container Used for Aging Studies