CHAPTER II

MATERIALS AND METHODS

1. Materials

1.1 Chemicals

The following substances were commercially available:
Hydroxypropyl methylcellulose 15 cps. (Methocel®E15, HPMC) was pharmaceutical grade obtained from Rama Production Co., Ltd., Bangkok, Thailand; crystalline fructose was food grade obtained from Rama production Co., Ltd., Bangkok, Thailand; isopropyl alcohol, ethyl alcohol USP, Tween 20®, Pluronic F-68® and triethanolamine were cosmetic grade purchased from Srichand United Dispensary Co., Ltd., Bangkok, Thailand; tocopheryl acetate (vitamin E acetate), menthol crystal, methyl paraben, propyl paraben, propylene glycol, glycerin were pharmaceutical grade from S. Tong Chemical Co., Ltd., Bangkok, Thailand; vitamin C palmitate was cosmetic grade obtained from Roche Co., Ltd. Thailand; peppermint oil, sorbic acid, sorbitol, Cremorphor RH 40®, carboxymethylcellulose (CMC) and carbomer 940® were pharmaceutical grade obtained from Namsiang Company limited., Bangkok, Thailand; sodium chloride, potassium chloride and calcium chloride were analytical grade obtained from Merck, Germany; benzoic acid was analytical grade obtained from Fluka Chemika.

1.2 Plant Material

Waste of fruit-hulls of durian was collected from local market and immediately washed, blended and dried in a hot air oven at 70 ± 5 °C. A dried-hull material was 20% fresh hull. Dried fruit-hull of durian was kept at 4 ± 5 °C in a cold room, until used.

1.3 Equipment

- Analytical Balance (Saturious Model A 200S, Germany)
- Micrometer (Thickness Gauge 0-10 mm., Code No.17389 Inspector 3)
- pH Meter (MP 230, Mettler Toledo, Switzerland)

- Viscometer (Brookfiled, USA)
- Magnetic stirrer (Model SP 46920-26, Barnstead/Hermolyne, USA)
- Instron Universal Tester (Instron®5565, USA)
- Hot air oven (Mammert, Germany)
- Sonicator (Bransonic 42, Branson cleansing equipment company, USA)
- Incubator Model 6 (Thelco)
- Refrigerator
- Rotary evaporator (BÜCHI Rotavapor R-114, Switzerland)
- Hot plate (E.G.O., Germany)
- Vortex mixer (VORTEX-2 GENIE, USA)
- Polytron Homogenizer (Heidolph DIAX 900, Germany)
- Rheological viscometer (Rheology International, USA)

2. Methods

2.1 Preparation of Polysaccharide Gel (PG) from Fruit-Hulls of Durian

The polysaccharide gel isolated from fruit-hulls of durian was partially purified according to the method modified by Pongsamart, S. (1998) and Gerddit, W. (2002). Chemical and physical properties of polysaccharide gel were characterized as previously described (Gerddit,W. 2002). Powder of polysaccharide gel (PG) was used for the determination of gel or film properties and preparation of gel and film.

2.2 Physical Properties of PG

2.2.1 Viscosity of PG

The viscosity of PG solutions in water at concentrations of 1 - 6% by weight was individually measured using a small sample viscosity (Brookfield® viscometer). A volume of 8 ml PG solution was poured into a cup, a cone was installed and the rate of shear was adjusted and the viscosity of PG solution was measured. The data were the average values of three determinations. Plots of viscosity against concentration of PG were drawned. The viscosity of 2.5 % CMC and 1.0 % Carbomer 940 were also measured and compared with that of PG.

2.2.2 pH

PG solutions were prepared by dissolving PG powder in distilled water to make concentration of 1-6 % by weight of PG. Their pHs were individually measured by a pH meter. The data reported were the means of three determinations. Plot of pH versus concentration of PG were presented.

2.2.3 Rheological Study

The viscosities of PG solutions at concentrations of 3 % and 4 %, respectively, were measured using a cone and plate viscometer at different values of rate of shear in the range of 0.5-100 rpm. Then, the viscosity values in the unit of cps, and the shearing stress values in the unit of dynes/cm^2 were calculated using the following equation:

$$\text{Viscosity (cps)} = (\text{Display reading x range}) / 100$$

$$\text{Shearing stress (dynes/cm}^2) = (\text{Shear rate x Viscosity}) / 100$$

The rheogram of shearing stress (x) versus rate of shear (y) was plotted and evaluated.

2.3 Compatibility Studies of PG

2.3.1 Acid and Base

The effect of acid and base on 3% and 4% PG solution in water is determined Sodium hydroxide (NaOH) (5M) was used to adjust the basic pH of PG solution, while 5M hydrochloric acid (HCl) was used to adjust the acid pH of PG solution. The viscosity values of PG solution was recorded at different pH. The effects on 2.5 % CMC and 1.0 % Carbomer 940 were also determined to compare with those of PG.

2.3.2 Electrolytes

The effects of electrolytes (NaCl, KCl, $CaCl_2$ and $MgCl_2$) on viscosity of the PG solutions were examined by dropping 10 M of the salt solutions into 50 ml of 3 % PG solution and the viscosity was recorded. The graph was plotted between the concentration of electrolyte and viscosity. The effects on 2.5 % CMC and 1.0 % Carbomer 940 were also determined to compare with those of PG.

2.3.3 Organic solvents

The effects of organic solvents (ethyl alcohol, isopropyl alcohol and butyl alcohol) on viscosity of the PG solutions were examined by dropping each solvent into 50 ml of 3 % PG solution, the viscosity and volume of solvent were recorded. The graph was plotted between the solvent concentration and viscosity of PG. The effects on 2.5 % CMC and 1.0 % Carbomer 940 were also determined to compare with those of PG.

2.3.4 Humectants

The effects of humectants (glycerin and propylene glycol) on viscosity of the PG solutions were examined by dropping each reagents into 50 ml of 3% PG solution, the viscosity and volume of humectant were recorded. The graph was plotted between the humectant concentration and viscosity of PG. The effects on 2.5 % CMC and 1.0 % Carbomer 940 were also determined to compare with those of PG.

2.3.5 Preservatives

The effects of preservatives (benzoic acid, sorbic acid and paraben concentrate) on viscosity of the PG solutions were examined by dropping each preservative solution into 50 ml of 3 % PG solution, the viscosity and volume of preservative were recorded. The graph was plotted between the preservative concentration and viscosity of PG. The effects on 2.5 % CMC and 1.0 % Carbomer 940 were also determined to compare with those of PG.

2.3.6 Heat

Effect of heat on viscosity of 3 % and 4 % PG solution was determined. The viscosity of PG solutions was repeatedly measured before heating, after heating for minutes at 50, 70 and 100 °C, respectively and after coling down to ambient temperature. The effects on 2.5 % CMC and 1.0 % Carbomer 940 were also determined by the same procedure to compare with those of PG.

2.4 Preparation Vitamin E gel

2.4.1 Solubility Study of Vitamin E Acetate

The solubility of 1% vitamin E acetate with the other solubilizers such as Tween 20, Pluronic F-68® and Cremophor RH 40® in propylene glycol was tested by mixing the ingredients by using vortex.

2.4.2 Preparations of 1.0% w/w Vitamin E Acetate Gel.(อุษณีย์ คำประกอบ, 2543)

Formulation of vitamin E gel using PG as a gelling agent.

Ingredients	Function	Amount (g)
PG	Gelling agent	2.5-3.5
Tocopheryl acetate (Vitamin E acetate)	Antiaging, Antioxidant	0.5
Cremorphor RH-40®	Solubilizing agent	10
Propylene glycol	Humectant	15
Amerchol L-101®	Emollient	0.5
$CaCl_2$ solution(0.05 M)	Electrolyte	0-0.2
Triethanolamine (TEA)	pH adjustment to 4.5-5.5	qs
Paraben concentrate	Preservative	1
Deionized water	Vehicle to	100

Formulation of vitamin C gel using PG as a gelling agent.

Ingredients	Function	Amount (g)
PG	Gelling agent	2.5-3.5
Ascorbyl palmitate	Antiaging, Antioxidant	0.1
Cremorphor RH-40®	Solubilizing agent	10
Propylene glycol	Humectant	15
Amerchol L-101®	Emollient	0.5
$CaCl_2$ solution (0.05 M)	Electrolyte	0-0.2
Triethanolamine (TEA)	pH adjustment to 4.5	qs
Paraben concentrate	Preservative	1
Deionized water	Vehicle to	100

Formulation of vitamin E&C gel using PG as a gelling agent.

Ingredients	Function	Amount (g)
PG	Gelling agent	2.5-3.5
Tocopheryl acetate (Vitamin E acetate)	Antiaging, Antioxidant	0.5
Ascorbyl palmitate	Antiaging, Antioxidant	0.1
Cremorphor RH-40®	Solubilizing agent	10
Propylene glycol	Humectant	15
Amerchol L-101®	Emollient	0.5
$CaCl_2$ solution(0.05M)	Electrolyte	0-0.2
Triethanolamine (TEA)	pH adjustment to 4.5-5.5	qs
Paraben concentrate	Preservative	1
Deionized water	Vehicle to	100

Preparation Procedure

PG powder was dispersed in an appropriate amount of water with continuous stirring until uniform. An accurate amount of humectant, solubilizer, vitamin E acetate was mixed with continuous stirring until a clear solution was obtained. The clear vitamin E solution was slowly added into the PG solution and the mixture was stirred continuously. Paraben concentrate and $CaCl_2$ solution were added and stirred until homogeneous. The gel pH was adjusted to a desired pH with TEA. Distilled water was added to make a total weight of 100 gram with continuous stirring. Gel viscosity was measured and PG gel preparations were evaluated. Physical appearances flow (pourability), air bubbles, colour, pH and viscosity after freshly prepared, after 6 temperature cyclings tested and after 30 days stored at ambient temperature were determined.

2.5 Preparation of PG Films

2.5.1 Effects of Plasticizer Types and Concentrations on PG Film

Three types of plasticizer (propylene glycol, glycerin and sorbitol) were used at 0-15 % based on dry weight of PG. Tensile properties and thickness were determined.

2.5.2 Thickness Measurement (Bozdemir, 2003)

A micrometer was used to measure film thickness (an accuracy of 0.01 mm). Five measurements were made at different locations on the film and an average value was calculated.

2.6 Formulation of Confectionery PG Film. (Peh, K.K. and Wong, C.F., 1999 Remunan-lopez,1996; Vemuri, 2000; Bozdemir, 2003)

PG films of 20 x 25 x 0.04 mm containing different concentration of HPMC based on PG were prepared by the following formula.

Ingredients	%w/w of PG	mg/strip (2x2.5 cm^2)
Water phase:		
PG	1.5	1.24
Fructose	1.5	1.24
Sorbitol solution	0.2	13.33 based on PG
HPMC	0-0.5	0-33.3 based on PG
Oil phase:		
Menthol	1.0	0.82
Peppermint oil	1.0	0.82
Food color		
Purified water qs to	100	

A mouth refreshing film was prepared by a casting/solvent evaporation method. All ingredients in water phase were mixed and stirred for 1 hour at 50° C, then the oil phase and water phase were mixed until homogeneous by polytron homogenizer at 8000 rpm for 5 mins and air bubbles were removed by a sonicator. The mixture was casted using petri dish, and dried at 50 °C for 5 hours. The film was carefully removed from the glass plates and cut to the size designed for the analysis.

2.6.1 Evaluation of PG films.

2.6.1.1 Mechanical Properties (Nunthanid, *et al.*, 2001)

The mechanical properties of PG films were evaluated using a universal tensile testing instrument (Instron5565®, USA) equipped with a 100N tension load cell. A PG film strip having a dimension of 2 x 20 mm and was free from air bubbles or physical imperfections, was held between two clamps positioned at a distance of 5 mm. During measurement, the strip was pulled by the top clamp at a rate of 10 mm/min and returned to the starting points. The force and elongation were measured when the film broke. The mechanical properties of PG films were evaluated experimentally for % elongation, stress at break and Young's modulus (megapascals). Measurements were oparated in five replicates for each film.

2.6.1.2 Moisture Sorption Study (Nunthanid, *et al.*, 2001)

The determination of moisture sorption of the PG strips was performed by the following procedure. A film which was carefully cut into pieces of rectangular size of 2 x 2cm^2 were placed inside the desiccator filled with silica gels for 1 week at room temperature. The initial dry weight (W_0) of the strips was determined and then the films were stored in a securely closed desiccator containing the saturated sodium chloride solution in the well to make about 75% relative humidity (RH), at ambient temperature. The weight of the films was recorded after exposure to moisture (W_1) for 7, 10 and 15 days, respectively, and the percentage of the moisture sorption was calculated using the following equation. The measurement was made in triplicate.

$$\% \text{ Moisture sorption} = \frac{W_1 - W_0}{W_0} \times 100$$

where W_0 = the initial weight of the strip film(mg)

W_1 = the weight of the strip film after exposed to the moisture(mg)

2.6.1.3 Swelling Study (Peh, K.K. and Wong, C.F., 1999)

The swelling studies of the films were conducted using two media, namely, distilled water and simulated saliva solution that consisted of phosphate buffer saline solution. Each film sample with surface area about 1x1cm^2 was cut. The film was then submerged into 15 ml medium contained in a container. The increased area of the film was determined at preset time intervals until a constant area was observed. Each measurement was repeated three times. The percentage of swelling was calculated using parameters,

$$\% \text{ of swelling} = \frac{(A_1 - A_o)}{A_o} \times 100$$

where A_1 is the constant area of film after soak in the medium

A_o is the initial area of film.

2.7 Sensory Analysis

The instrument used in this study was volunteers.

2.7.1 PG Film Preparation

Twenty-seven healthy adult volunteers (10 male and 17 female), aged between 20 and 35 years old were asked to evaluated the mouth refreshing film before and after trying the product. The following parameters were obtained from the questionnaire answered by volunteers.

Physical appearance: color, thickness, clearness, smoothness and sample size.

Sensory after trying the product: solubility, flexibility, sweetness, coolness, hotness, freshness, overall satisfactory.

The opinions were analyzed by interpreting the opinion frequency according to the following criteria: 1 = least satisfy, 2 = less satisfy, 3 = satisfy, 4 = more satisfy, 5 = most satisfy. The questionnaires is presented in Appendix D.

2.7.2 PG gel preparation

Twenty-seven healthy adult volunteers (27 female), aged between 18 and 25 years old were asked to evaluated PG gel before and after applying the product.

Physical appearance before applying the product: color, clearness, viscosity, smoothness, firmness and overall satisfactory.

Sensory after applying the product: coolness, spreadability, tackiness while applying, time of disappearance, tackiness after disappearance, amount of residue, moisture after disappearance and overall satisfaction after applying.

The opinion were analyzed by interpreting the opinion frequency according to the following criteria: 1 = least satisfy, 2 = less satisfy , 3 = satisfy, 4 = more satisfy, 5 = most satisfy. The questionnaires is presented in Appendix D.