CHAPTER III

EXPERIMENTAL

Chemicals, Equipment and Glassware

Chemicals

Cassava starch ($C_5H_9O_{10}$), was obtained from Thai Wah Co., Ltd., Thailand. It was produced from tapioca cultivated in summer. It contains 13% moisture, 0.15% ash, pH value of 4.50-7.00 and viscosity of 600.

Acrylic acid, was obtained from Siam Resin & Chemical Co., Ltd., Thailand. Acrylic acid is a clear, colorless liquid. The molecular weight is 72.06. The melting and boiling points are 13.5°C and 141°C, respectively. The heat of vaporization is 435 J/g and the heat of polymerization is 76.99 kJ/mol; the density at 25°C is 1.045 (kg/m^3) (42). The monomethyl ether hydroquinone (MEHQ) is frequently used as an inhibitor of acrylic acid (43). The inhibitor of acrylic acid monomer is particularly suitable for the manufacture of polymer without pretreatment. Acrylic acid polymerizes very easily. The polymerization is catalyzed by heat, light and peroxides to produce an insoluble polymer. The highly exothermic, spontaneous polymerization of acrylic acid is extremely violent. Poly(acrylic acid) is soluble at the extent of at least 1-2 pph (w/v) in water, dioxane, ethanol and methanol (44). The solubility of poly(acrylic acid) depends on concentration, temperature, molecular weight, and extent of neutralization.

Methanol (CH_3OH), commercial grade from BDH, was also purified by fractional distillation at atmospheric pressure.

Hydrogen peroxide (H_2O_2) 35%, analytical grade, was obtained from Merck. Hydrogen peroxide is a clear, colorless liquid which is miscible with water in all proportions. The molecular weight of hydrogen peroxide is 34.02. The melting and boiling temperatures are -33°C and 107.9°C. The heat of vaporization at 25°C is 1,519 $Jg^{-1}K^{-1}$. The heat of decomposition is 98.3 kJ/mol. The densities of the H_2O_2 solution at 0, 20, and 25°C are 1.1441, 1.1312, and 1.1282 kg/m^3, respectively. The decomposition of H_2O_2 is shown in eq 3.1.

$$H_2O_2(l) \rightarrow H_2O(l) + 1/2\ O_2(g) \qquad (3.1)$$

This reaction is extremely important in handing hydrogen peroxide during storage and in laboratoric work. This reaction is highly exothermic that takes place in the presence of small amounts of catalyst even in aqueous solution. In the absence of catalyst, it occurs only in the gas phase at high temperatures.

Decomposition can be catalyzed both homogeneously and heterogeneously (45). Several factors seem to influence the decomposition of hydrogen peroxide, the most importance of which are : (1) temperature; (2) trace catalysis of certain cations, the most active being those of elements with more than one valence, such as, iron, copper, vanadium, and manganese; (3) certain active surfaces, especially rough surfaces; (4) suspended matter, such as dust particles; (5) pH; and (6) radiation, especially of short wavelength.

Hydrogen peroxide can dissociate into free radicals by breaking either and H-O bond or O-O bond,

$$HOOH \rightarrow H^{\bullet} + {}^{\bullet}OOH \qquad (3.2)$$

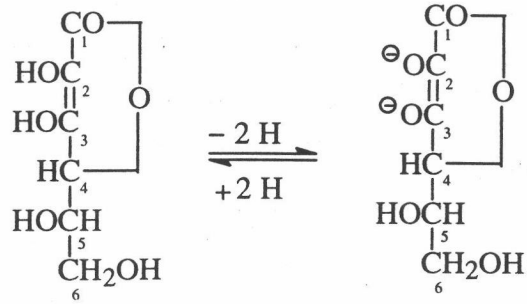
$$HOOH \rightarrow 2^{\bullet}OH \qquad (3.3)$$

As the heat for reaction 3.2 is 88 kcal/mole and for reaction 3.3 about 50 kcal/mole. The latter reaction predominates in uncatalyzed vapor-phase decomposition and photochemically initiated reactions. Hydrogen peroxide can oxidize a variety of organic and inorganic compounds (46).

L(+)-Ascorbic acid ($C_6H_8O_6$), analytical grade, was obtained from Carlo Erba. Ascorbic acid is a white, odorless, crystalline solid with a sharp acidic taste. The molecular weight is 176.13.

The most significant characteristic of ascorbic acid is its reversible oxidation to dehydroascorbic acid. Therefore, an estimation of the vitamin must provide means of determing both forms (eq 3.4).

Ascorbic acid has a lactone structure with an enediol configuration; thus its acidity is derived from the enolic character of the hydroxyl groups on C-2 and C-3. One gram of ascorbic acid dissolves in about 3 cm^3 of water, 50 cm^3 of absolute alcohol, or 100 cm^3 of glycerol; it is insoluble in benzene, chloroform, and fats. In aqueous solution, it is more sensitive to alkali than to acid; it is more stable at pH 5. Heat alone, in the absence of oxygen does not destroy the ascorbic acid; however, if air is not excluded, the ascorbic acid is readily oxidized at an increasing rate with a rise in temperature (47).



L-ascorbic acid

dehydroascorbic acid

(3.4)

Sodium hydroxide and calcium oxide, analytical grade from Merck, were used in grafting reacting.

Glacial acetic acid, analytical grade from J.T. Baker, and perchloric acid, analytical grade from Merck, were used in the hydrolysis reaction of starch-*g*-poly(acrylic acid).

Sodium dihydrogen phosephate and sodium azide, analytical grade from Merck, and disodium hydrogen phosphate and sodium sulphate analytical grade from Carlo Erba, were used in GFC technique.

Equipment and Glassware

4-necked round bottom flask, hot plate and magnetic stirrer, mechanical stirrer, heating mantle, RVT Brookfield viscometer, oven, water bath circulator, analytical balance, grinder, beakers, funnel, erlenmeyer flask, condenser, FTIR, FT-NMR and other general laboratory glassware and equipment.

Procedure

1. Gelatinization of Cassava Starch

60 g cassava starch was mixed with 600 cm^3 of distillated water in a 2,000 cm^3 4-necked round bottom flask. The system was stirred mechanically at 300 rpm under heating within the temperature range of 80±2°C for one hour under the nitrogen atmosphere to form a slurry and the viscosity was then measured.

2. Grafting of Acrylic Acid onto Cassava Starch

The gelatinized starch was then cooled to 35°C; 1.0 g calcium oxide, ascorbic acid 0.4 g, 4 cm^3 hydrogen peroxide 35%, and 80 cm^3 acrylic acid monomer were added in the reaction. The reaction mixture was stirred

mechanically at 300 rpm under heating within the controlled temperature of 35± 2°C under nitrogen atmosphere for 3 h and the viscosity was then measured.

3. Saponification of Starch-*g*-Poly(acrylic acid)

200 cm^3 of 25% sodium hydroxide solution was added in twenty equal portions to the starch-*g*-poly(acrylic acid) slurry at room temperature, while sodium hydroxide was added with one portion. The mixture was stirred mechanically. One could observe an exothermic heat developed during the reaction. It was allowed then to cool to room temperature and consequently added each of portions until sodium hydroxide was completed. It was precipitated with methanol completely filled in the burette which was added to the starch slurry at 0.0167 cm^3/s. The mixture was stirred mechanically overnight. The product was then filtered and washed thoroughly with methanol until pH 7 was reached. It was dried at 65°C in an oven for 24 h to remove any residual methanol. The dried product was ground into a powder form.

Influence of addition rate of methanol on gel properties were studied as a variable. Various levels of flow rate were 0.0167, 0.0333, and 0.05 cm^3/s and were used in the same condition. The addition rate of methanol that gave a free-flowing dispersion and micron-size gel fragments was chosen as another controlled parameter.

4. Homopolymer Extraction by Methanol

The product (about 20g) derived from Section 3, ground previous into a powder form, was extracted in 1,000 cm^3 methanol by soxhlet at 65°C for 24 hours. The extracted product was dried at 65°C for 24 hours and weighted to determine the amount of the homopolymer, poly(acrylic acid) and graft copolymer.

5. Influential Parameters on Molecular Weight Averages and Water Absorption

To obtain a good copolymer with an appropriate molecular weight distribution and a relatively low water absorption value, important effects on graft copolymerization were carried out as follows:

5.1 Effect of Acrylic Acid Concentration on Graft Copolymerization

All other variables were fixed except acrylic acid monomer. Various amounts of acrylic acid of 80, 100, 120, and 140 cm^3 (1.70, 2.06, 2.40, 2.73M) were added to each of the gelatinized starch.

The acrylic acid concentration at which the water absorption capacity was minimum would be used for the subsequent experiments.

5.2 Effect of Hydrogen Peroxide Concentration on Graft Copolymerization

All other variables were fixed except hydrogen peroxide initiator. Various amounts of hydrogen peroxide of 2, 4, 6, and 8 cm^3 (0.097, 0.194, 0.290, and 0.386M) were added to each of the gelatinized starch.

The hydrogen peroxide concentration at which the water absorption capacity was minimum would be used for the subsequent experiments.

5.3 Effect of Amount of Starch on Graft Copolymerization

All other variables were fixed except amounts of cassava starch. Various amounts of starch of 50, 60, 70, and 80 g were added to each of the gelatinized starch.

The amount of starch at which the water absorption capacity was minimum would be used for the subsequent experiments.

5.4 Effect of Ascorbic Acid on Graft Copolymerization

All other variables were fixed except ascorbic acid. Various amount of ascorbic acid of 0.2005, 0.4005, 0.6004, and 0.8002 g (0.16, 0.33, 0.50, and 0.67% weight based on added to each of the optimum amount of acrylic acid) were added to each of the gelatinized starch.

The percentage of ascorbic acid at which the water absorption capacity was minimum would be used for the subsequent experiments.

5.5 Effect of Reaction Temperature on Graft Copolymerization

In this experiment, the temperature of the grafting reaction was taken as a variable. Various levels of temperature for the graft copolymerization were 25, 30, 35, and 40°C.

The reaction temperature which gave the water absorption capacity with a minimum value would be used for the subsequent experiments.

5.6 Effect of Reaction Time on Graft Copolymerization

In this experiment, the time of the grafting reaction was also taken as a variable. Various levels of time were 2, 3, 4, and 5 h.

The reaction time, which gave the water absorption capacity with a minimum value would be used for the subsequent experiments.

5.7 Effect of Addition Rate of Acrylic Acid-Hydrogen Peroxide mixture on Graft Copolymerization

In this experiment, the addition rate of acrylic acid-hydrogen peroxide of the grafting reaction was studied as a variable. Various levels of flow rate were 0.0167, 0.0250, 0.0333, and 0.0417 cm^3/s.

The addition rate at which the water absorption capacity was minimum would be used for the subsequent experiments.

All variable parameters were repeated as mentioned in Sections 1, 2, 3 and 4. The reaction products from Sections 5.1 to 5.7 were then characterized as described in Sections 6.1-6.3, and the investigation of their water absorption capacity was carried out. The minimum water absorption capacition of the hydrolyzed starch-*g*-poly(acrylic acid) indicates the appropriate of condition.

6. Copolymer Characterization

6.1 Infrared Spectroscopy

The infrared spectra of cassava starch, and the hydrolyzed starch-*g*-poly(acrylic acid) copolymers were measured with an FT-IR Spectrophotometer (Perkin Elmer Model 1760X) using KBr pellet.

6.2 Nuclear Magnetic Resonance Spectroscopy

The NMR spectra of acrylic acid, cassava starch and the hydrolyzed starch-*g*-poly(acrylic acid) copolymers were measured with an NMR Spectrophotometer (AC-F200, 200 MHz, Bruker Spectospin) using D_2O as a solvent.

6.3 Determination of Average Molecular Weights of The Grafted Polymer

6.3.1 Standard Calibration Curve

Gel filtration chromatography was used with liquid chromatography (C-R7A, Shimadzu Corporation) equipped with a TSK-GEL G2000SW, G3000SW and G4000SW columns (30 cm x 7.5 mm I.D.). A differential refractometer detector (RID-6A) and an ultraviolet detector (SPD-10AV) were used to detect the separated peaks at 25°C.

The mobile phase was 0.05% NaN_3 and 0.1M Na_2SO_4 in 0.1M phosphate buffer, at a flow rate of $8.33 \times 10^{-3} cm^3/s$. All standard samples concentration were prepared to 0.1% w/v and were filtered through a 0.45-micron millipore sartoron cellulose nitrate filter prior to injection, and the injection volume was 10^{-1} cm^3(100 µl). The data were processed with the C-R7A software GPC program.

The molecular weight calibration curve was determined and constructed with Shodex Standard P-82 (P-1600, P-400, P-200, P-100, P-50, P-5 with molecular weights of 1,660,000; 380,000; 186,000; 100,000; 48,000; 5,800; respectively) of a narrow molecular weight distribution in distilled water. A 10^{-1} cm^3 (100 µl) of 0.1% solution of each standard was injected.

6.3.2 Acid Hydrolysis of Starch-*g*-PAA Thickeners

The following technique is based on Dennenberg and Abbott's method on hydrolysis of amylopectin and side chain recovery (38).

Hydrolyzed starch graft copolymer (2 g) was weighted accurately and added to 100 cm^3 glacial acetic acid which was then heated to 90-100°C. The sample was stirred for 1 h to swell the grafted side chains.

Perchloric acid (60%) 2 cm^3 was added dropwise, and within 1-2 minute the reaction was completed. The reaction mixture was immediately poured into ice water to precipitate the acrylic polymer side chains from the polysaccharide backbone.

The ratio of starch (g): glacial acetic acid (cm^3): perchloric acid (cm^3) as a variable were:

- 2:100:2, 2:100:4, 2:100:6, 2:100:8, 2:100:10
- 2:150:2, 2:150:4, 2:150:6, 2:150:8, 2:150:10
- 4:100:2, 4:100:4, 4:100:6, 4:100:8, 4:100:10
- 4:150:2, 4:150:4, 4:150:6, 4:150:8, 4:150:10

6.3.3 Blank Test Preparation

In this work, there is a problem of separating the acrylate grafted chains from the polysaccharide backbone because the solubility of acrylic acid in water is enormous. A blank test by the acid hydrolysis starch for comparing with those of the graft copolymers of the GFC results was carried in the same manner.

The gelatinized starch was repeated as mentioned in Section 6.3.2. The acid hydrolysis starch solution 1 cm^3 was pipetted and water was added until 10 cm^3. The solution was then filtered with 0.45-micron millipore sartoron cellulose nitrate fiber prior to injection and the injection volume was 10^{-1} cm^3(100 μl).

6.3.4 Sample Preparation for GFC Injection

The HSPAA samples were treated with glacial acetic acid and perchloric acid as mentioned in Section 6.3.2. The sample concentration was prepared to 0.01% w/v (double distilled water used as

solvent). A 10^{-1} cm^3(100 µl) of each sample was used the same condition as standard was injected (see Section 6.3.1).

7. Water Absorption Capacity of Saponified Starch-*g*-Poly(acrylic acid)

75 g of deionized distilled water was added to 0.1 g of dried saponified cassava starch-*g*-poly(acrylic acid) in a 250 cm^3 beaker and it was allowed to stand for 30 min. The fully swollen polymer was filtered through a 100 mesh aluminium screen for 2 h and the drained water was weighed. The amount of water retained by the starch-*g*-poly(acrylic acid) was calculated as in gram per gram of dried modified starch.

8. Measurement of the Viscosity of Thickener

8.1 Various amounts of the dried HSPAA: 6%, 8%, 10%, and 12% w/w (0.3, 0.4, 0.5, and 0.6 g) were added to 5 g deionized distilled water. Thickener solutions so prepared were measured for the viscosity profile.

8.2 The viscosity of the above solution of saponified cassava starch-*g*-poly(acrylic acid) was measured by a Brookfield viscometer (model RVT, with a small sample adapter and spindle number 14) at 25°C under various shear rates: 1, 2, 4, 8, and 20 (sec^{-1}) were claculated by multiplying 0.4 with 2.5, 5, 10, 20, and 50 rpm, respectively.