

CHAPTER 3

EXPERIMENTAL

3.1 Chemicals, Equipment, Glassware and Apparatus

3.1.1 Chemicals

Cassava starch was obtained, in one lot, from Thai Wah Co., Ltd. It was produced from tapioca cultivated in summer. It contained 13% moisture, 0.15% ash, pH value 4.5-7.0 and viscosity of 600.

Acrylamide, AM, and acrylic acid, AA, were provided by Siam Resin & Chemical Co., Ltd. They were used as received.

Acetone and methanol, commercial grade from BDH, were purified by fractional distillation at atmospheric pressure.

Other chemicals, as shown in Table 3.1, which were of analytical grade, were used without further purification.

3.1.2 Equipment and Glassware

Water bath circulator, reactor, hot plate and magnetic stirrer, heating mantle, mechanical stirrer, condenser, flat bottom flask, desiccator, centrifuge bottle, analytical balance, grinder, oven and other general laboratory glassware and equipment.

Table 3.1 Chemicals

Chemicals	Source
Hydrochloric acid (HCl)	Carlo Erba
Acetic acid (CH ₃ COOH)	Carlo Erba
Perchloric acid (HClO ₄)	Carlo Erba
Nitric acid (HNO ₃)	Carlo Erba
Maleic acid (C ₄ H ₄ O ₄)	Merck
Potassium hydroxide (KOH)	Carlo Erba
Sodium chloride (NaCl)	Carlo Erba
Magnesium chloride (MgCl ₂ .6H ₂ O)	Carlo Erba
Calcium chloride (CaCl ₂)	Carlo Erba
Boric acid (H ₃ BO ₃)	Merck
Citric acid (H ₃ C ₆ H ₅ O ₇ .H ₂ O)	Merck
Tri-sodium phosphate (Na ₃ PO ₄ .12H ₂ O)	Merck

3.1.3 Apparatus

Cobalt 60 Source, Gammabeam 650 of 42,750 Curies from Nordian International Inc., Canada

Fourier Transform Infrared Spectroscopy, Perkin Elmer 1760 X, U.S.A.

Elemental Analyzer, Perkin Elmer PE 2400 Series II, U.S.A.

Scanning Electron Microscope, JEOL, JSM-T 220 A, Japan

3.2 Procedure

3.2.1 Gelatinization of Cassava Starch

Into a 500-cm³ reactor, 10 g of cassava starch was mixed with 200 cm³ of distilled water. The system was stirred at 400 rpm and heated at approximately 85 ± 3 °C at the same time for one hour to form a paste-like slurry.

3.2.2 Graft Copolymerization of Acrylamide and Acrylic Acid onto Cassava Starch by Simultaneous Irradiation

The gelatinized starch was cooled at room temperature. Twenty grams of monomer mixture (10 g of AM and 10 g of AA) were added into the gelatinized starch. The mixture was stirred at 400 rpm at room temperature for 30 minutes. The gelatinized starch-AM/AA mixture was transferred into the 250-cm³ aluminium tube covered the inner wall with aluminium foil, and purged with nitrogen gas for 20 minutes. It was closed tightly with foil and paraffin film, and then irradiated under gamma rays. The reaction product was precipitated with acetone and dried in a vacuum oven at 56°C for 24 hours.

Several important parameters involved gamma-irradiation of cassava starch mixed with AM and AA were studied so as to produce a cassava starch graft copolymer with highest water absorption capacity. Those parameters were described in the following sections.

3.2.2.1 Effect of Monomer-to-Starch Ratio on Graft Copolymerization

Various amounts of monomer mixture as 5, 10, 15, 20, 25 and 30 g (monomer-to-starch ratio 0.5:1, 1:1, 1.5:1, 2:1, 2.5:1 and 3:1, respectively) were added to each of the gelatinized starches. The reaction mixture was irradiated at the

total dose of 6.0 kGy and the dose rate of 5.75 kGy hr⁻¹. The effect of monomer-to-starch ratio on grafting characteristics and water absorption were determined.

3.2.2.2 Effect of Total Dose (kGy) and Dose Rate (kGy hr⁻¹) on Graft Copolymerization

At this stage, pure AM and pure AA were used instead of a mixture of the two monomers. The optimum ratio of monomer to starch, which was derived from Section 3.2.2.1, was added into the gelatinized starch. The mixture was irradiated under gamma rays at various total doses and dose rates as shown in Table 3.2.

Table 3.2 Various Quantities of Total Dose (kGy) and Dose Rate (kGy hr⁻¹) for Graft Copolymerization of Acrylamide and Acrylic Acid onto Cassava Starch

Dose Rate (kGy hr ⁻¹)	Total Dose (kGy)
2.24	2.9
	4.3
	6.0
	7.2
	8.6
5.75	2.9
	4.3
	6.0
	7.2
	8.6

Table 3.2 Various Quantities of Total Dose (kGy) and Dose Rate (kGy hr⁻¹) for Graft Copolymerization of Acrylamide and Acrylic Acid onto Cassava Starch (continued)

Dose Rate (kGy hr ⁻¹)	Total Dose (kGy)
14.75	2.9
	4.3
	6.0
	7.2
	8.6

Thereafter, the effects of total dose (kGy) and dose rate (kGy hr⁻¹) on grafting characteristics and water absorption were carried out.

3.2.2.3 Effect of Acid Additives on Graft Copolymerization

The amounts of acid to be mentioned were added in each batch of gelatinized starch-monomer mixture with the optimum monomer-to-starch ratio (Section 3.2.2.1): 10 cm³ of 0.01, 0.1 and 1 M HNO₃ and 2% maleic acid. Each batch of reaction mixture was irradiated at the optimum total dose (kGy) and dose rate (kGy hr⁻¹), which was derived from Section 3.2.2.2. The effect of the additives on grafting characteristics and water absorption was then evaluated.

3.2.2.4 Effect of Acrylamide-to-Acrylic Acid Ratio on Graft Copolymerization

Based on the experimental data obtained, of the optimum monomer-to-starch ratio (Section 3.2.2.1), and additives (Section 3.2.2.3), various AM-to-AA ratio

as 100:0, 70:30, 50:50, 30:70, and 0:100 were added to each batch of the gelatinized starch. The reaction mixture was irradiated at the optimum total dose (kGy) and dose rate (kGy hr^{-1}) of gamma rays, which was derived from Section 3.2.2.2. The effect of AM-to-AA ratio of grafting characteristics and water absorption was determined.

3.2.3 Removal of Homopolymer and Free Copolymer

The dried product (about 15 g) derived from the above sections that was ground previously into a powder form was stirred in $1,500\text{-cm}^3$ distilled water at room temperature for 24 hours. The mixture was centrifuged to separate the graft copolymer. The graft copolymer was washed with distilled water and centrifuged to allow another separation of the graft copolymer. Then it was precipitated with acetone, dried in the vacuum oven at 56°C for 24 hours and weighed to determine the amount of the homopolymer and free copolymer.

In the case of starch-g-poly(acrylic acid), the dried product (about 9 g) derived from the above sections, ground previously into a powder form, was extracted in $1,000\text{-cm}^3$ methanol by soxhlet at 65°C for 24 hours. The extracted product was dried in a vacuum oven at 65°C for 24 hours and weighed to determine the amount of the homopolymer.

3.2.4 Saponification of the Graft Copolymer

Into a 500-cm^3 reactor, 5 g of graft copolymer was mixed with 125 cm^3 of 5% potassium hydroxide solution. The mixture was stirred with a mechanical stirrer at 400 rpm and the reaction was carried out at room temperature. The reaction product was washed until pH 7 was reached and precipitated with methanol. It was dried at 65°C in a vacuum oven for 24 hours to remove any residual methanol. The dried product was ground into a powder form.

3.2.4.1 Determination of Optimum Time for Saponification of Acrylamide and Acrylic Acid Moieties

In order to determine the optimum time for saponification that only acrylic acid moiety was converted to acrylate salt and the acrylamide moiety should not be changed significantly, to be referred as “optimum saponification time”, the starch-g-polyacrylamide sample prepared by the dose rate of 2.24 kGy hr^{-1} to the total dose of 6.0 kGy was saponified with 5% potassium hydroxide solution for various periods of time: 30, 60 and 90 minutes. Saponified starch-g-polyacrylamide samples were subjected to the determination of the amount of nitrogen by elemental analysis. They were characterized by pyrolysis in high purity oxygen (a static-state oxidation), separated by frontal analysis in a gas chromatography and quantitatively detected by a thermal conductivity detector (CHNS/O analyser, Perkin Elmer PE 2400 Series II). The optimum saponification time found was then used for further saponification of the other products.

3.2.5 Characterization of Copolymer

3.2.5.1 Existence of Functionalities by IR Spectroscopy

The infrared spectra of cassava starch, graft copolymer, and saponified graft copolymer were measured by IR spectroscopy on an FT-IR spectrophotometer using a KBr pellet.

3.2.5.2 Determination of Percentage Conversion

The experimental procedures described in the above section were carried out. The weight of the graft copolymer along with homopolymer was regarded

as the total amount of polymer obtained from the weight of monomer charged. Percentage conversion was subsequently calculated.

3.2.5.3 Determination of Percentage Homopolymer

The weights obtained from Section 3.2.3 were the amount of free polyacrylamide, poly(acrylic acid) and poly(acrylamide-co-acrylic acid) produced as by-product, which were then calculated.

3.2.5.4 Determination of Percentage Add-on

In a 500-cm³ flat bottom flask equipped with a condenser, 0.8 g of the graft copolymer was stirred in 250 cm³ of 1.5 M HCl and the mixture was refluxed for 2 hours. The polymer was filtered and it was then washed until pH 7 of the washed solution was reached and then it was dried.

In the case of starch-g-poly(acrylic acid), determination of percentage add-on was based on Dennenberg and Abbott's method [31]. The graft copolymer (0.8 g) was added to 100-cm³ glacial acetic acid that was then heated to 90-100°C. The mixture was stirred for 1 hour to swell the grafted side chains. Perchloric acid (60%) of 2 cm³ was added dropwise, and the reaction was completed within 1-2 minutes. The reaction was cooled to room temperature and centrifuged to separate the poly(acrylic acid) side chains.

To observe the completion of reaction, the residue starch (substrate) after acid hydrolysis were checked with iodine solution.

The weight percentages of polyacrylamide, poly(acrylic acid) and poly(acrylamide-co-acrylic acid) in the graft copolymer or the so called "percentage add-on" were computed from the weight difference between the graft copolymer and soluble starch, which was removed by the acid hydrolysis.

3.2.5.5 Determination of Percentage Grafting Ratio

The experimental procedure of Section 3.2.5.4 also gave the weights of the polymer in grafts and the substrate (starch) was regarded as the percentage grafting ratio.

3.2.5.6 Determination of Percentage Grafting Efficiency

The experimental procedures of Sections 3.2.1-3.2.3 and 3.2.5.4 were carried out with the grafted product. The percentage of the total synthetic polymer formed that had been grafted to starch or the so called “percentage grafting efficiency” was computed.

3.2.5.7 Determination of Surface Morphology of the Copolymers

The surface morphology of the copolymers were investigated using a scanning electron microscope (SEM), model JSM-T 220 A JEOL without cross-section. The thickness of gold coated on the copolymer was 25 nm and SEM was operated with a 15 or 20 kV accelerating voltage.

3.2.6 Water Absorption/Retention Capacities of the Copolymer

3.2.6.1 In Distilled Water

One hundred gram of distilled water was added to 50 mg of dried and saponified graft copolymer in a 150-cm³ glass beaker and allowed to stand for 30 minutes for swelling. The fully swollen polymer was filtered through a 100-mesh aluminium screen for 3 hours and the drained water was weighted. The amount of water retained by the saponified graft copolymer was calculated as in gram per gram of the dry modified starch.

3.2.6.2 In Sodium Chloride, Magnesium Chloride, and Calcium Chloride Solutions

The same experimental procedure as described in Section 3.2.6.1 was carried out, except that a series of sodium chloride, magnesium chloride, and calcium chloride solutions of 0.9% w v⁻¹ was used instead of distilled water.

3.2.6.3 In Buffer Solutions

The same experimental procedure as described in Section 3.2.6.1 was carried out, except that a series of buffer solutions ranging from pH 3 to 11 was used instead of distilled water. The buffer solutions of pH 3 to 11 were prepared from mixing of 0.20 M boric acid, 0.05 M citric acid, and 0.10 M tri-sodium phosphate [32].

In order to ease understanding the entire process, the preparation of graft copolymer and the characterization is summarized in Figure 3.1.

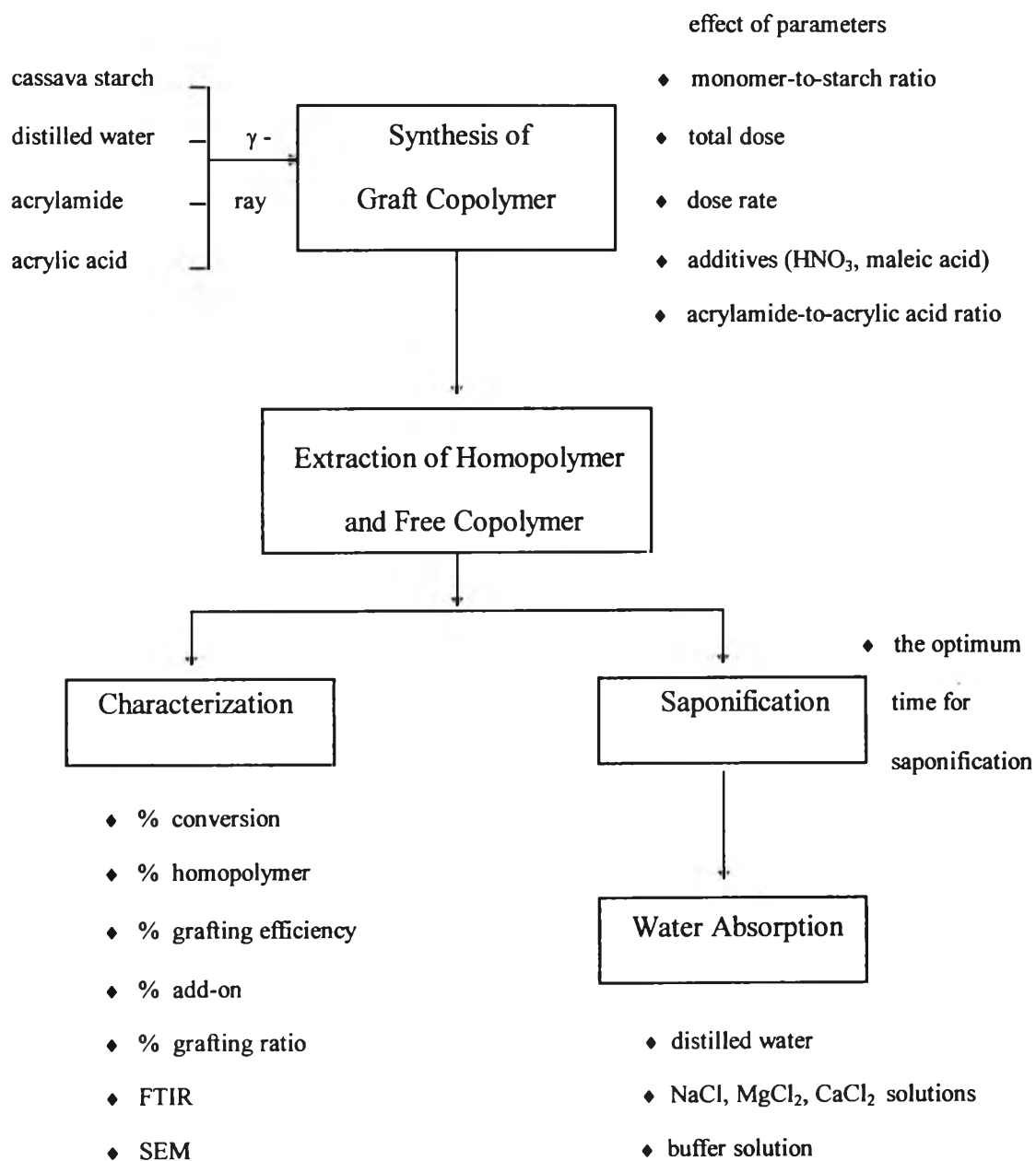


Figure 3.1 Overall Schematic Experimental Process