CHARACTERIZATION OF TAPIOCA STARCH AND DERIVATIVES

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หัวข้อวิทยานิพนธ์	การวิเคราะห์สมบัติของแป้งมันสำปะหลังและอนุพันธ์
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บทคัดย่อ

ในระหว่างปี พ.ศ. 2517-2535 กรมวิชาการเกษตรได้แนะนำพันธุ์มันสำปะหลังให้แก่เกษตรกรจำนวน 7 พันธุ์ คือ ระยอง1 ระยอง3 ระยอง5 ระยอง60 ระยอง90 เกษตรศาสตร์50 และศรีราชา1 การศึกษาสมบัติทางเคมีและกายภาพ รวมถึงโครงสร้างของแป้งมันสำปะหลังในระดับโมเลกุล พบว่าแป้งมันสำปะหลังทั้ง 7 พันธุ์มีสมบัติแตกต่างกันบ้างเล็กน้อย เมื่อเปรียบเทียบสมบัติของแป้งมันสำปะหลังทั้งหมดนี้กับแป้งมันสำปะหลังซึ่งวางขายในตลาด พบว่าปริมาณของความชื้น ฟอสฟอรัส และสมบัติทางกายภาพบางอย่างแตกต่างกัน ทั้งนี้เป็นเพราะกรรมวิธีการผลิตแป้งที่ไม่เหมือนกัน ในส่วนของอนุพันธ์ของแป้งมันสำปะหลัง คือ แป้งกราฟท์โคพอลิเมอร์ ในการทดลองได้ศึกษาปัจจัยที่สำคัญต่อการกราฟท์โคพอลิเมอร์โดยใช้กระบวนการอิมัลชันและใช้โปแตสเซียมเปอร์ซัลเฟตเป็นตัวริเริ่มปฏิกิริยา ปัจจัยแรกที่ศึกษาคือ ชนิดของมอนอเมอร์ โดยใช้มอนอเมอร์ 4 ชนิดคือ MMA, EA, BA และ 2-EHA พบว่ามอนอเมอร์ทั้ง 4 ชนิดให้ผลผลิตของการกราฟท์ที่แตกต่างกันโดยเรียงลำดับดังนี้ คือ EA<MMA<BA<2-EHA ปัจจัยที่ 2 ที่ศึกษาคือ อัตราส่วนของมอนอเมอร์ โดยใช้ MMA เป็นมอนอเมอร์หลัก แล้วใช้มอนอเมอร์อะคริเลตอื่นๆ ได้แก่ EA, BA และ 2-EHA เป็นมอนอเมอร์ร่วมในอัตราส่วนที่กำหนด พบว่ามอนอเมอร์ผสม MMA/EA จะเพิ่มผลผลิตของการกราฟท์แป้งมันสำปะหลังให้สูงขึ้นกว่าการใช้มอนอเมอร์เพียงตัวเดียว แต่สำหรับมอนอเมอร์ผสมMMA/BA และ MMA/2-EHA จะเพิ่มผลผลิตของการกราฟท์แป้งมันสำปะหลังขึ้นเมื่อปริมาณของมอนอเมอร์ร่วมสูงขึ้น ปัจจัยที่ 3 ที่ศึกษาคือปริมาณของตัวริเริ่ม

ปฏิกิริยา พบว่าความเข้มข้นของโปแตสเซียมเปอร์ซัลเฟตที่ให้ผลของการกราฟท์สูงสุดคือ 1.50 x 10^{-2} โมล

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ABSTRACT

During 1975-1992, the Department of Agriculture had recommended 7 varieties of cassava, which were Rayong1, Rayong3, Rayong5, Rayong60, Rayong90, Kasetsart50, and Sriracha1, to farmers. For the study of their physico-chemical properties and molecular structure, found that all recommended-variety tapioca starches were slightly different in properties. The commercial brand tapioca starches, which were available in the market, found that amount of phosphorus and moisture and some physical properties were different from the recommended varieties. This due to the preparation process of starch. To produce tapioca starch derivatives, which were starch graft copolymers, the factors of graft copolymerization by emulsion process using potassium persulfate as initiator were studied. First factor was monomer types, four monomers (MMA; EA; BA; and 2-EHA) were used to study and were found that each monomer gave different values of graft yields by the following orders : EA <MMA<BA<2-EHA. The second factor was monomer ratios by using MMA monomer as the base monomer and other acrylate monomers (EA, BA, and 2-EHA) at set up ratios. It was found that tapioca starch graft copolymerizations with a mixture of MMA and EA monomers had higer graft yields than pure monomers. The graft yields of tapioca starch graft copolymerizations using a mixture of MMA and BA monomers and a mixture of MMA and 2-EHA monomers increase following to amount of BA or 2-EHA monomers increased in the

feed stock. The third factor was initiator concentration, it was found that the potassium persulfate concentration which gave highest graft yields was 1.50×10^{-2} mole.

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ABBREVIATIONS

AGU	:	anhydroglucose unit
BA	:	butyl acrylate
B.U.	:	brabender unit
BV	:	blue value
%C	:	percentage of conversion
DMSO	:	dimethyl sulfoxide
DP	:	degree of polymerization
dsb.	:	dry-starch basis
DSC	:	differential scanning calorimetry
EA	:	ethyl acrylate
2-EHA	:	2-ethyl hexyl acrylate
G	:	percentage of grafting
GE	:	percentage of grafting efficiency
GF	:	percentage of grafting frequency
GPC	:	gel permeation chromatography
IR	:	Infrared
KU50	:	Kasetsart 50
MMA	:	methyl methacrylate
R1	:	Rayong 1
R3	:	Rayong 3
R5	:	Rayong 5
R60	:	Rayong 60
R90	:	Rayong 90
RO	:	Rose Brand
SEM	:	scanning electron microscope
SR	:	Sriracha 1

ABBREVIATIONS (continued)

ST	:	Four Star Brand
T_c	:	complete temperature
T_g	:	glass-transition temperature
T_o	:	onset temperature
T_p	:	peak temperature
THF	:	tetrahydrofuran
TSC	:	total solid content

CHAPTER 1

INTRODUCTION

1.1 PROBLEM STATEMENT

Cassava is an economic plant in Thailand. The cultivation of cassava is in the north-eastern and eastern parts of the country. Most cassava roots are manufactured to industrial part. The products from cassava roots are cassava pellets and chips (about 70%) and cassava or tapioca starch (about 30%). The value of exported cassava products is more than twenty-three thousand million baht annually. However, Cassava products are almost exportation so the price of cassava is unstability depending on world market price. Because of non-balancing between demand and supply, the price decline of cassava products usually occur. Besides the situation of world market, the impact of the Common Agricultural Policy Reform of European Union is also cause of price decline [1, 32].

1.1.1 New Products from Tapioca Starch

Tapioca or cassava starch is manufactured from cassava roots. The number of export in 1993 shows that Thailand export tapioca and modified products in a total more than eight hundred thousand tons with the value more than seven thousand million baht [32].

Tapioca starch has been used in many industries in Thailand. The utilization of tapioca starch is used as raw materials and chemicals for improving properties of products. In general, native tapioca starch has unsuitable properties for application. The modification of starch is essential process to improve or modify native starch to desirable property.

The modification of starch are not only improve properties of starch, but also increase the value of starch. To investigate new products from tapioca starch is an interesting method to increase the value and amount of tapioca starch utilization in Thailand. These methods should be reduce the problem of cassava from exportation by increasing of amount of cassava utilization in the country.

One of interesting starch derivatives is starch graft copolymers. Several of these graft copolymers show promise as thickeners for aqueous systems, flocculants, clarification aids for wastewater, retention aids in paper making, and many other uses. In addition, some starch graft copolymers show thermoplastic properties. Research in this area is a result of current interest in conversing petrochemical-based thermoplastics by the substitution of annually renewable, plant-derived polymers, and also the desirability of introducing biodegradability into final products. Now, several starch graft copolymers are being marketed and used in many applications. For examples, "Super Slurper" is used as water absorbers which is being used commercially in incontinent pads, disposable diapers, bandages, etc. Biodegradable mulch is also one of applications of starch graft copolymers.

1.1.2 Structure of Starch and Derivatives

Molecular structure is one of important factors to determination of polymer properties. Polymers with different molecular structure or different molecular weight possess different properties. To study molecular structure of polymer, size exclusion chromatography (SEC) is the important apparatus. SEC technique is the modern technique and has been widely used for molecular weight determination.

Starch is a natural polymer so molecular structure gives effect to its properties. In Thailand, there are 7 varieties of cassava plants which have been recommended to farmers for starch manufacturing by the Department of Agriculture, Ministry of Agriculture and Cooperatives. The recommended varieties of cassava are Rayong1, Rayong3, Rayong5, Rayong60, Rayong90, Kasetsart 50, and Sriracha1. Each variety has different specification.

There was reported that starches from same plant source but different variety, season, etc., had different physico-chemical properties. This study was attended to prove that tapioca starches from different varieties had different molecular structure and different physico-chemical properties.

Starch derivatives by graft polymerization are polymers. SEC technique is also determined molecular weight and molecular structure of these starch derivatives. Molecular weight and molecular structure of starch graft copolymers show off their efficiency of graft polymerization and properties.

1.2 OBJECTIVE OF THE STUDY

The purpose of this study was, first, to modify tapioca starch by graft polymerization with synthetic monomers in order to investigate the starch derivatives and, second, to estimate molecular weight and molecular structure of tapioca starches and derivatives by modern size exclusion chromatography technique to gain better understanding of tapioca starches and derivatives properties and polymerization results.

1.3 SCOPE OF THE STUDY

In this study was divided in two parts: first, tapioca starches characterization and; second, synthesis of starch derivatives by graft polymerization and charac-terization.

The first parts, seven tapioca starch samples were made from recommended varieties of cassava plants: Rayong 1, Rayong 3, Rayong 5, Rayong 60, Rayong 90, Kasetsart 50, and Sriracha 1, and two tapioca starch samples were commercial brands (Four Star and Rose brands). All starch samples were characterized by size exclusion chromatography (SEC) technique and estimated physico-chemical properties by suitable methods.

The second parts, starch graft copolymers were investigated with emulsion polymerization by using starch from commercial Rose brand. Potassium persulfate was used as initiator and 4 monomers: MMA, EA, 2-EHA, and BA, were used as synthetic monomers to grafting onto tapioca starch. The parameters of graft polymerizations were determined. The starch derivatives were also characterized by SEC technique and thermal properties were characterized by differential scanning calorimeter (DSC).

1.4 PROCESS OF THE STUDY

The necessary procedure may be as followed:

1. Literature survey and in-depth study of this research work.
2. Preparing all starch samples, chemicals, and equipments for experimentation.
3. To characterize tapioca starch, molecular weight and molecular structure determinations by SEC technique and physico-chemical properties analysis by suitable methods and to compare all data.
4. To investigate starch graft copolymers by changing the following parameters :
   - initiator concentration
   - variety of monomers
   - monomer ratio in selected monomer combinations.
5. Studying effect of parameters on graft polymerization.
6. Starch derivatives were characterized by SEC technique and differential scanning calorimeter (DSC).
7. Summarizing the results.

CHAPTER 2

LITERATURE REVIEWS AND THEORY

2.1 LITERATURE REVIEWS

Jane and Chen (1992) studied effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. Amylose fractions from potato, normal, and high-amylose corn VII starches were used and molecular weight size (DP) were 1500, 667, and 530, respectively. Amylopectin from high-amylose corn V, waxy corn, and normal rice starches were used and length (DP) and distribution (%) of branch chain debranched with isoamylase were 43.8 (55.7%) and 18.6(44.3%); 29.6(23.9%) and 17.8(76.1%); and 51.7 (5.8%), 35.4(25%) and 14.3(69.2%), respectively. Both pure amylose and amylopectin viscosity were raised when solution concentration increased. To study synergistic effect from reconstituted starch found that normal corn amylose (an intermediate molecular size) had the greatest synergistic effect of the three amylose tested. To study synergistic effects among three amylopectins showed decreasing in order of high-amylose corn, rice and waxy maize. Gel strength of the reconstituted starches with amylopectin constitution from amylose corn formed gel, which indicated that long branch chain length amylopectin had a strong tendency to gel. The reconstituted starch from high-amylose corn V amylopectin and corn amylose had highest gel strength. However, the reconstituted starch form high-amylose cornV amylopectin (highest-branch chain length) and high-amylose cornVII amylose (smallest molecular size) had the greatest gel strength after storing at 4^{o}C for 72 hr. This could be ascribed to retrogradation of small amylose molecules. Light transmittance of reconstituted starches decreased with molecular size of amylose and branch chain length of amylopectin [20].

Wang, et al. (1993) studied physicochemical properties of starch from 17 mutant genotypes of the Oh43 inbred line. They found that amylose content is the important role in

properties of maize starch. Amylose content was positively correlated with blue value (BV), and iodine-absorption spectrum (λ_{max}) and negatively corrected with limiting viscosity number [η], swelling power, percent light transmittance (%T), and peak viscosity on the viscoamylogram. The high-amylose starch (amylose content > 30%) had significantly greater BV thận normal starch. An almost waxy starch (no amylose content) had similar BV and λ_{max}. The high-amylose starch had [η] < 200 ml/g, whereas waxy starch had [η] > 250 ml/g. The waxy starches were unrestricted swelling power, great solubility, high %T, low pasting temperature, high peak viscosity but low viscosity in set-back, and form only weak gel after several days storage. These were results from lack of amylose [35].

Asaoka and Rickard (1991) estimated physicochemical properties of starch from four cultivars of cassava, HMC-1, CM489-1, CM681-2, and CM1559-5, that four different occasions cultivating and harvesting at CIAT in Colombia. The relation between the physicochemical properties and organoleptic quality of cooked cassava roots were also studied. Both genetic and environmental factors that had an effect on organoleptic qualities of cooked roots. Cultivar or harvest time was no effect to the proximate composition of the minor components and no significant relation between these values and the organoleptic results. The size of starch granules was in ranged from 2.4-31.1 μm in diameter; the peak granular size was 12.3-15.6 μm in diameter. The X-ray diffraction patterns of all showed C_A pattern. The crystallinity of the starch granules varied between 15.3% and 17.8% which the results were collected over an angular range from 4° 20 to 54° 20, showing no significant relations with cultivar or harvesting time. The amylose content was in range from 16% to 20%. The elution patterns of isoamylase-debranched materials of all cultivars showed same trimodal distribution profile. The weight ratio of the short chains to long chains of amylopectin ranged from 2.2 to 2.4. The susceptibility of the starch granules to attack by glucoamylase was a similar degradation pattern to one another. The cultivar and growth condition were no influence on the susceptibility of cassava starch granules to glucoamylase. This result was attractive as a substrate for fermentation under industrial conditions [6].

Vera-Pacheco and others (1993) prepared and characterized hydrogels which obtained by grafting of acrylonitrile onto cassava starch (yucca starch or tapioca) by ceric ion initiation. The starch was pretreatment by heating at 65 $^{\circ}$C or 95 $^{\circ}$C for different lengths of time and were found that preheated temperature with relation on time effected to grafting parameters and water absorbency of alkaline hydrolysis of starch grafted polyacrylonitrile (H-SPAN). To increase monomer/starch (M/S) ratio was to increase compositional parameters of copolymer SPAN. At beginning, all grafting parameters increased with increasing initiator concentration, but when ceric (IV) initiator was higher, these parameters were very low increasing. The longer grafting time increased values of grafting parameters. These results were similar with other experiments which the starch were used from maize, potato, and wheat. However, % homopolymer decreased with increasing grafting time. To compare with other starches , i.e., wheat or maize starches, was found that similar results [33].

Patil and Fanta (1993) studied graft copolymerization of starch with methyl acrylate (MA) using ceric ammonium nitrate (CAN) as initiator by examination of reaction variables. Starch 20 g. and MA 30 g. were used in experiment which obtained starch-g-poly(methyl acrylate) (S-g-PMA) contained 55-60% PMA. Nitric acid concentration was found no significant in graft copolymerization. To increase CAN concentration showed increasing %add-on or grafting efficiency and %conversion until reached optimum level ($1.5x10^{-3}$ mol/l ,or 1 mol of ceric ion per 200 AGU). Percent conversion and add-on increased with reaction carried out with long time and low CAN concentration ($1.54 x10^{-3}$ mol/l). These values slightly decreased when reaction temperature was raised from 25 to 40 $^{\circ}$C. This could be responsible for temperature effect, i.e., starch may be hydrolyzed by acidic condition or oxidized by ceric ion at high temperature. Both percent add-on and conversion were slight difference in different method for gelatinization at reaction temperature was 25 $^{\circ}$C. To reduce amount of MA led to lower MW of grafted PMA, less frequent grafting, and fewer % add-on. Water content also resulted to reaction. To decrease amount of water from 400 to 50 ml. increased %conversion from 87 to 96 %. However, reaction carried out with 50 ml. of water occurred coagulation and difficult stirring in conventional glassware [26].

Lui, et al. (1993) used potato starch to prepare the starch graft copolymers with methyl acrylate (MA) by using ceric ammonium nitrate (CAN) as initiator. The grafting conditions were varied for studying the effect to graft polymerization. The first effect was reaction time; the optimum time was 2 hr. The second effect was reaction temperature; the optimum temperature was 50 $^{\circ}$C. The third effect was initiator concentration (C_{CAN}); the optimum concentration was 5.4×10^{-3} mol/l, the G and GE were highest while AGU/chain and molecular weight ($\overline{M}_v$) of grafted polymer were nearly lowest. The fourth effect was monomer concentration (C_{MA}); the optimum concentration was 1.082 mol/l. The last effect was nitric acid concentration (C_{HNO3}), The optimum concentration was 0.081 mol/L. This was due to ceric-ion in water formed as Ce^{4+}, $[Ce(OH)_3]^{3+}$, and $[Ce\text{-}O\text{-}Ce]^{6+}$ and Ce^{4+} formed at high C_{HNO3} which was in favor of graft copolymerization. Finally, Lui et al. reported new mechanism of the graft copolymerization of vinyl monomer onto polysaccharide by using ceric-ion initiator with reference the research of Ranby et al. as following:

$$R_p = K_1 [STOH] [Ce^{4+}] + K_2 [STOH] [M] \qquad (2.1)$$

where K_1 and K_2 = constant, and STOH, M, and Ce^{4+} represent starch, monomer, and ceric initiator, respectively [23].

Gao et al. (1994) used canna-starch to prepare starch graft copolymers with methyl methacrylate (MMA) by using manganic pyrophosphate ($[Mn(H_2P_2O_7)_3]^{3}$) as an initiator. The first effect to graft polymerization was initiator concentration; to increase concentration increased percentage of grafting (G) at the beginning and then decreased with further increasing over 3.0×10^{-3} mol/l. The second was monomer concentration (C_{MMA}); the highest percentage of grafting was C_{MMA} 3.0 mol/l. The third was canna-starch concentration [AGU]; G decreased with increasing [AGU] because ratio of monomer to canna-starch was lower, but the grafting efficiency (GE) increased with increasing [AGU]. The fourth was reaction temperature; both G and GE increased when temperature raised from 20 to 50 $^{\circ}$C. This may be raising temperature will (1) improve the swelling capability of canna starch, (2) increase the diffusion rate of the monomer and initiator onto and into starch granular, (3) raise the rate

of grafting. In conclusion, Gao et al. established rate equation of graft copolymerization from the experiment as following:

$$Rg = K\,[MMA]\,[Mn(III)]^{1/2}[AGU]^{1/2} \qquad (2.2)$$

where K is constant, and MMA, Mn(III), and AGU represent monomer MMA, initiator [$Mn(H_2P_2O_7)^{3}]^{3}$ and starch, respectively. X-ray diffraction patterns indicated that graft copolymerization involved both in the amorphous and crystal phase. The scanning electron microscope photos showed deformed structure of starch granular after graft copolymerization [14].

Goni et al. (1993) used ^{13}C n.m.r. spectroscopic methods to characterize the graft copolymers of methyl methacrylate (MMA) with ethyl acrylate (EA) onto amylose cornstarch by using ceric ammonium nitrate initiator. The monomer composition of EA/MMA was 80/20, 60/40, 50/50, 40/60, 20/80, and 10/90. To compare grafting efficiency (GE) between pure monomer and mixture monomer used in graft copolymerization found that GE of graft copolymerization with mixture of monomer had higher value than with pure monomer. However, grafting of reaction with a mixture of monomers lower than pure monomer. These results were same as percentage of grafted acrylic copolymer. Therefore, the presence of another monomers leads to a decrease in the polymerization propagation rate (under these reaction condition). The grafting values obtained by gravimetric methods and by ^{13}C n.m.r. spectroscopic methods agree to within an error of < 2%. To characterize grafted chains by ^{13}C n.m.r. spectroscopic methods showed that percentage of grafted PMMA composition had higher values than feed composition while percentage of grafted EA composition had lower values than feed composition. This could be explained by reactivity ratio which MMA (r_{MMA}=2.03) is higher than EA (r_{EA}=0.24) [17].

2.2 STARCH

Starch is a carbohydrate, which is generated in granular form and is deposited in the organs of plants. Although starch occurs in all plants, there are only a limited number of plants which available for commercial sources. The sources of commercial starch are seeds of cereals grain (corn, wheat, sorghum, and rice), tubers (potato), roots (tapioca, sweet potato, and arrowroot), and the pith of sago palm. The major sources for commercial starch are difference in different regions depend on the abundance of plants and climate. While maize is the major source for starch products in the U.S., potatoes and wheat are the major sources in Europe. In tropical countries, tapioca starch and sago starch are produced as the major starch products. The starch manufacturing and properties depend on the plant sources [9].

2.2.1 Chemical Structure of Starch

Starch is a mixture of α-glucan. The major component, amylopectin, is an α-1,4-linked, α-1,6-branched (4-6% branching) polymer with an average molecular weight close 10^8. The minor, amylose, is a smaller mostly linear polymer, and also composed of α-1,4-linked glucose units with α-1,6 branches less than 1% of the glucose unit connections. The molecular weight of amylose is approximately 10^5 [9]. Starches of different origins have different amylose-amylopectin ratios, as shown in Table 2.1. The Table 2.1 shows also the average degree of polymerization (DP) of both fractions in various starches.

2.2.1.1 Amylose : Amylose is a linear glucose polymer, connected by α-1,4-linkages (see Fig. 2.1). Enzyme studies show a few amount of branching in the amylose molecules. The branched amylose molecules may contain 3-20 chains, with an average chain length of about 500 glucose units. Starches in different species have different ratio of amylose to amylopectin by fairly constant of ratio. Cereal starches have higher amylose content than tuber and root starches. The waxy starches contain no amylose fraction, but amylomaize starches may contain up to 80% amylose.

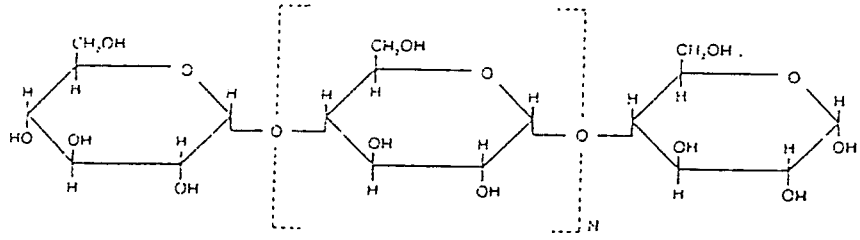


Fig.2.1 Linear-Chain Structure of Amylose Molecule [9].

The degree of polymerization of amylose molecules depend on the source of the starch. Potato and tapioca amylose molecules have higher molecular weight than corn and wheat amylose molecules.

Amylose forms inclusion complexes with iodine and various organic compounds, for example butanol, fatty acids, etc. The complexing agents are bound within the α-helical structures of amylose. the complex of amylose with iodine give a characteristic blue color which is widely used for determining the percentage of amylose [9,18].

2.2.1.2 Amylopectin : Amylopectin is also α-1,4-linked glucose polymer, but it has highly branched structure by connecting through α-1,6-linkage (see Fig. 2.2). The model of amylopectin which the most widely accepted models that proposed by Robin et al. is shown in Fig. 2.3.

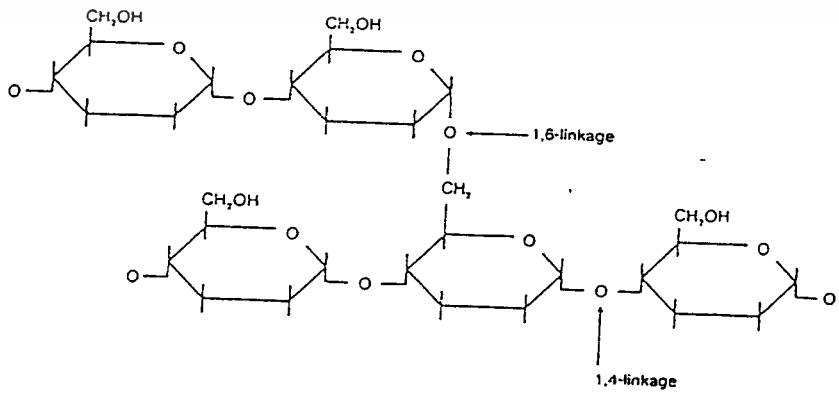


Fig. 2.2 Structure of Amylopectin Branching Points [9].

Table 2.1 Amylose and Amylopectin Contents and Degree of Polymerization of Various Starches [16]

Starch	Amylose		Amylopectin
	Content [(a)]	DP	DP[(b)]
Cereals			
Oats	27	1,300	20
Wheat	26-31	2,100	19 -20
Maize	28	940	25 - 22
Amylomaize	52 - 80	1,300	23
Waxy maize	0 - 1	-	20 - 22
Barley	22 -29	6,850	26
Roots and tubers			
Manihot	17	-	-
Potato	23	3,200	24

(a) Amylose content (as % of amylose and amylopectin) ; for cereal starches, some literature values for amylose content are low if account has not been taken of amylose in lipid-complexed form.

(b) Average degree of polymerization determined by pullanase or isoamylase debranching.

The proportion of amylopectin in starch varies depending on starch source as amylose. The Table 2.1 shows the degree of polymerization (DP) of amylopectin after debranching with pullulanase or isoamylase. Amylopectin has molecular weights in the range 10^7-10^9, depending upon the source. The structure contains approximately 5% of α-1,6-branch points that occurs in a biomodal distribution of A-chains (DP ≈ 15) and B-chains (DP ≈ 45) after debranching [16].

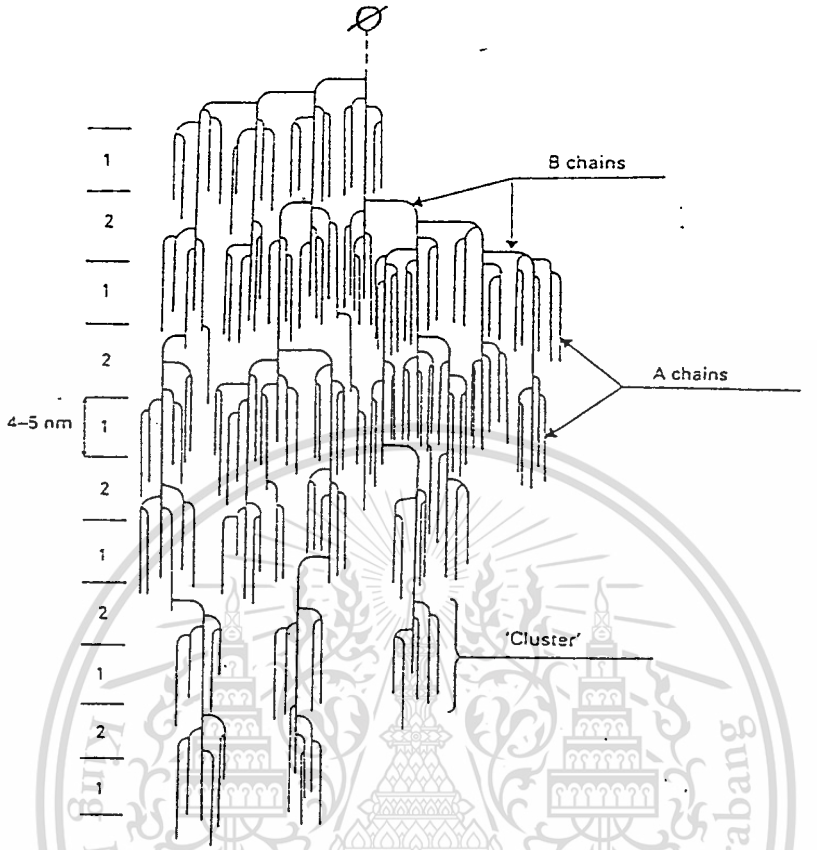


Fig. 2.3 Racemose Model of Amylopectin According to Robin et al. [16].

2.2.2 The Minor Composition

In general, starch granules contain 10-20% (w/w) moisture and small amount of minor components (Table 2.2). The higher valve of minor components may be found in crude commercial starches and the lower value of minor components are found in well-washed starch samples as prepared in the laboratory.

The amount of minor components varies according to such factors as age, soil, variety, climate, and manufacture processes of starches. These minor components have a profound effect on the physical properties of starches. For example, lipid which exit as an amylose-lipid inclusion complex in starch granules tend to repress the swelling and solubilization of the cereal starch granules. The fatty substance can create problems in

cornstarch products utilization because of the tendency to become rancid on storage. The residual protein in cereal starches may have a mealy flavor and odor and also a tendency to foam [9,16].

Table 2.2 Average Minor Chemical Composition of Starch Granules [9]

Starch	Moisture at 65% RH[(a)] and 20°C	Lipid, % on DS[(b)]	Proteins, % on DS[(b)], N[(c)] x6.25	Ash, % on DS[(b)]	Phosphorus, % on DS[(b)]
Corn	13	0.6	0.35	0.1	0.015
Potato	19	0.05	0.06	0.4	0.08
Wheat	14	0.8	0.4	0.15	0.06
Tapioca	13	0.1	0.1	0.2	0.01
Waxy maize	13	0.2	0.25	0.07	0.007
Sorghum	13	0.7	0.3	0.08	-
Rice	-	0.8	0.45	0.5	0.1
Sago	-	0.1	0.1	0.2	0.02
Amylomaize	13	0.4	-	0.2	0.07
Sweet potato	13	-	-	0.1	-

(a) RH = relative humidity, (b) DS = dry substance, (c) N = nitrogen content

2.2.3 Physical Properties of Starch

2.2.3.1 Granular size and shape : The size and shape of starch granules are specific to each variety of starch. Table 2.3 shows the size and shape of starches from various origin. Microscopic techniques have been used to characterize starch granules. Photomicrographs of some type of starch granules are shown in Fig. 2.4 [9,16,18,24].

Fig. 2.4 Scanning Electron Micrographs of Starch Granules Prepared from Maize (top), Wheat (centre) and Potato (bottom), All at Magnification x 600 [16].

Table 2.3 Starch Granule Properties [9]

Starch	Type	Size (diameter), range (μm)	Size (diameter), number average(μm)	Shape
Corn	Cereal	3-26	15	Round, polygonal
Potato	Tuber	5-100	33	Oval, spherical
Wheat	Cereal	2-35	15	Round, Lenticular
Tapioca	Root	4-35	20	Oval, truncated
Waxy maize	Cereal	3-26	15	Round, polygonal
Sorghum	Cereal	3-26	15	Round, polygonal
Rice	Cereal	3-8	5	Polygonal, angular
Sago	Pith	5-65	30	Oval, truncated
Arrowroot	Root	5-70	30	Oval, truncated
Amylomaize	Cereal	3-24	12	Round, deformed
Sweet potato	Root	5-25	15	Polygonal

2.2.3.2 Crystalline Structure : The crystallinity in starch granules is studied by x-ray diffractometry. Katz and Itallie examined the common native starches and classified them into three principal categories followed their wide-angle x-ray scattering diffractograms : the cereal starches having an 'A' pattern, the tuber starches and maize starches with more than 30-40% amylose having a 'B' pattern, and bean and other root starches having a 'C' pattern which were believed to be intermediate between 'A' and 'B'. The exact reason for explanation the difference of structure in nature is not entirely clear [16].

Starch granules comprise both crystalline regions and amorphous regions. The areas of crystallinity in native starches are composed about 25-50% of the total volume of the starch granules. The amylopectin component is the important element of the crystalline structure. In tuber and root starches, only the amylopectin molecules constitute the crystalline structure while amylose molecules are present in the amorphous regions. Amylose may be leached out from starch granule under appropriate conditions without seriously damage granules

crystllinity. In common cereal starches, amylose is present as a amylose-fat-complex which forms a weak crystalline structure and could be involved in the structure network of the granules [9].

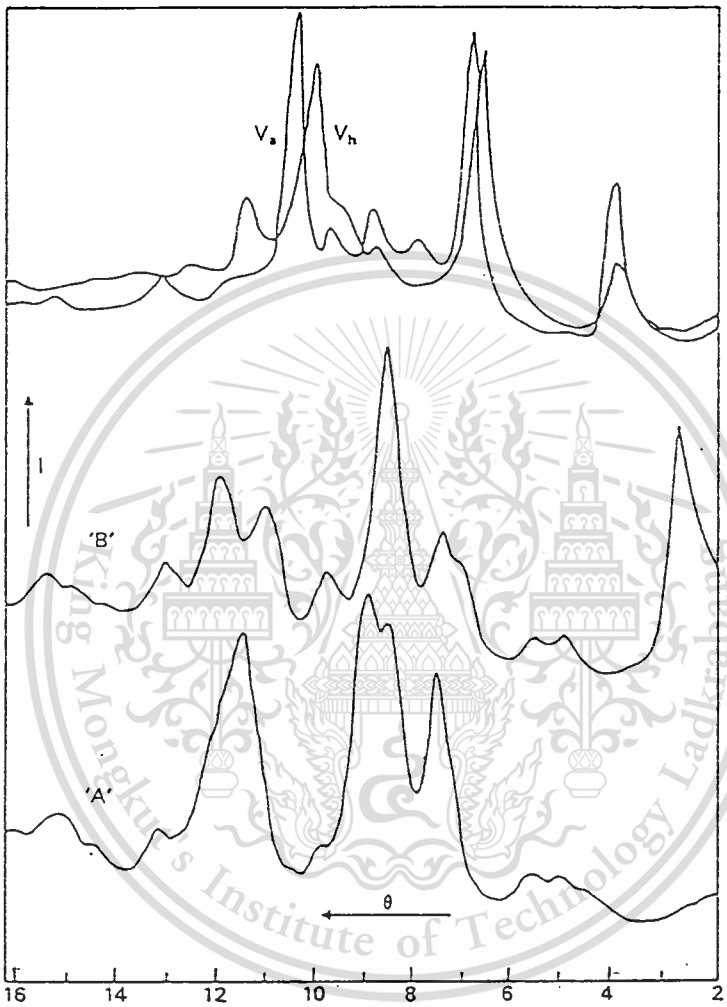


Fig. 2.5 X-ray Diffraction Patterns of Starches : A-type (cereal grains), B-type (tubers), Anhydrous (V_a) and Hydrated (V_h)-type (helical complex) [16].

2.2.3.3 Swelling and gelatinization : Native starches are not soluble in cold water below gelatinization temperature. In cold water, starch granules swell slightly by increasing 10-15% in diameter and can be reversed to their original dimensions by drying. The insoluble properties of starch in cold water are due to the hydrogen bonds. The hydrogen bonds are formed either directly via neighboring alcoholic OH groups of the individual starch molecules or indirectly via water bridges. Although the hydrogen bonding forces are weak,

there are so many hydrogen bonds in a starch granule that is the cause of insoluble in cold water of starch granules.

When starch suspension is heated to progressively higher temperature, starch granules gelatinize. Gelatinization is the disruption of molecular order in starch granules which irreversible changes in properties such as granule swelling, native crystallite melting, loss of birefringence, and leaching out of soluble component (primary amylose).

At initiation of swelling, starch granules are disrupted in amorphous regions by hydrating. As higher temperature, more hydration occurs and hydrogen bonds in crystalline regions begin to be disrupted. Amylose molecules are leached out into the aqueous substrate. The viscosity increases to a maximum depending on the hydrated swelling volume of granules. In the first stage of gelatinization, the shorter micelles dissociate and the longer micelles will persist at higher temperature. The temperature of initial gelatinization and the range over which gelatinization occurs depends on the method that are used for determination and starch granules type. Table 2.4 shows the data of gelatinization temperature from various instruments, and Fig. 2.6 shows endotherms of starches from differential scanning calorimeter, which one of equipment for gelatinization temperature determination [9, 18].

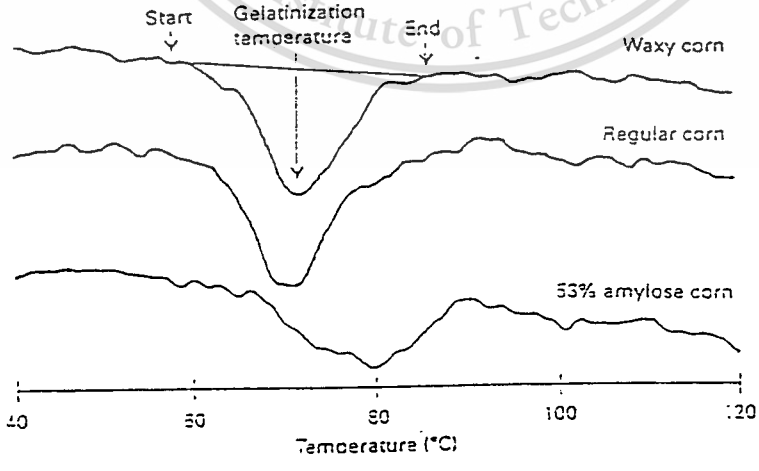


Fig. 2.6 Differential Scanning Calorimeter (DSC) Endotherms of Waxy, Regular and 53% Amylose Maize [24].

Table 2.4 Gelatinization Characteristics of Native Starch [9]

Starch	Kofler hot-stage microscope gelatinization temperature range[a] (°C)	Brabender viscoamylograph[b]		DSC[d] (°C)
		pasting temperature (°C)	peak viscosity (8%, B.U.)[c]	
Corn	62-67-72	75-80	700	70-89
Potato	58-63-68	60-65	3000	57-87
Wheat	58-61-64	80-85	200	50-86
Tapioca	59-64-69	65-70	1200	68-92
Waxy maize	63-68-72	65-70	1100	68-90
Sorghum	68-74-78	75-80	700	-
Rice	68-74-78	70-75	500	-
Sago	60-66-72	65-70	1100	-
Arrowroot	62-66-70	-	-	-
Amylomaize	67-80-92	90-95	-	-
Sweet potato	58-65-72	65-70	-	-

(a) The recorded temperature correspond to the loss of birefringence by 5, 50, and 95% of the granules. (b) Starch concentration, 8%. (c) B.U. = Brabender units.

(d) DSC = Differential scanning calorimetry.

2.2.3.4 Starch paste : Pasting occurs following gelatinization. Starch paste may contain unswollen granules, partly swollen granules, swollen granules, fragments of swollen granules, swollen starch aggregates, dissolved starch molecules, and retrogradated starch precipitates. The important properties of starch paste are viscosity, texture, paste transparency, and resistance to shear as are shown in Table 2.5. The paste properties of starches from different origins differ from each other.

Brabender viscoamylograph is an instrument for determination of starch paste by the resulting curve (Fig. 2.7) shows the pasting temperature, rate of viscosity development, peak

viscosity, rate and extent of viscosity breakdown, and rate extent of viscosity development during paste cooling.

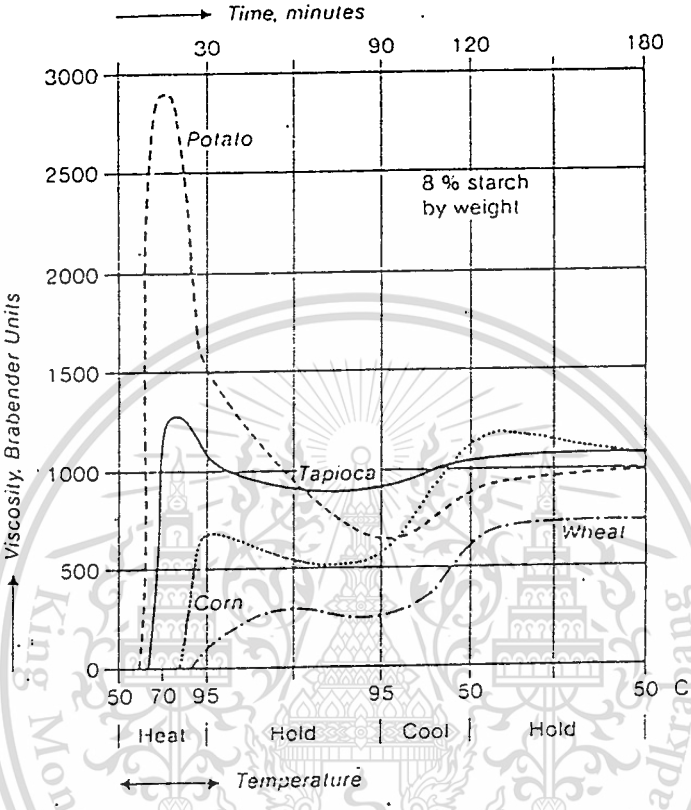


Fig. 2.7 Brabender Viscosity Curve of Common Starch [9].

2.2.3.5 Retrogradation : The term "retrogradation", as applied to starch, means a return from a solvated, dispersed, amorphous state to an insoluble, aggregated, or crystalline condition. The mechanisms of retrogradation are schematically shown in Fig. 2.8.

Retrogradation of starch pastes or starch solutions may have the following effects :

- increase in viscosity
- development of opacity and turbidity
- formation of insoluble " skins" on hot pastes
- precipitation of insoluble starch particles
- formation of gels
- syneresis of water from the paste (weeping).

The process of retrogradation is complex and depends upon many factors : type of starch, starch concentration, cooking procedure, temperature, storing time, pH, cooling procedure, and the presence of other compound. Retrogradation of starch dispersions occurs usually at low temperature and high concentration of starch. The retrogradation rate of various starches are shown in Table 2.5.

Table 2.5 Properties of Starch Pastes [9]

Starch	Paste Viscosity	Paste Texture	Paste Clarity	Resistance to shear	Rate of Retrogradation
Corn	Medium	Short	Opaque	Medium	High
Potato	Very-high	Long	Translucent	Medium-low	Medium
Wheat	Medium-low	Short	Opaque	Medium	High
Tapioca	High	Long	Translucent	Low	Low
Waxy maize	Medium-high	Long	Translucent	Low	Very low
Sorghum	Medium	Short	Opaque	Medium	High
Rice	Medium-low	Short	Opaque	Medium	High
Sago	Medium-high	Long	Translucent	Medium-low	Medium
Sweet potato	High	Long	Translucent	Low	Medium

In retrogradation process, amylose fraction is considered that the primary cause. Dissolved amylose molecules can orient themselves in a parallel alignment and close together though interchain hydrogen bonds. The aggregated chains of amylose are insoluble and then precipitate. In high concentration dispersions, the aggregate amylose entraps the aqueous fluid in a network of partially associated starch molecules, forming gel. Amylopectin fraction is much less prone to retrogradation than amylose fraction because of their branching. The presence of the branched amylopectin fraction has effected on the retrogradation of amylose by slowing down its precipitation and diminishing its gel tendencies. In addition, lipid substances that are inclusion complex form with amylose can be precipitated and then retrogradation occurs.

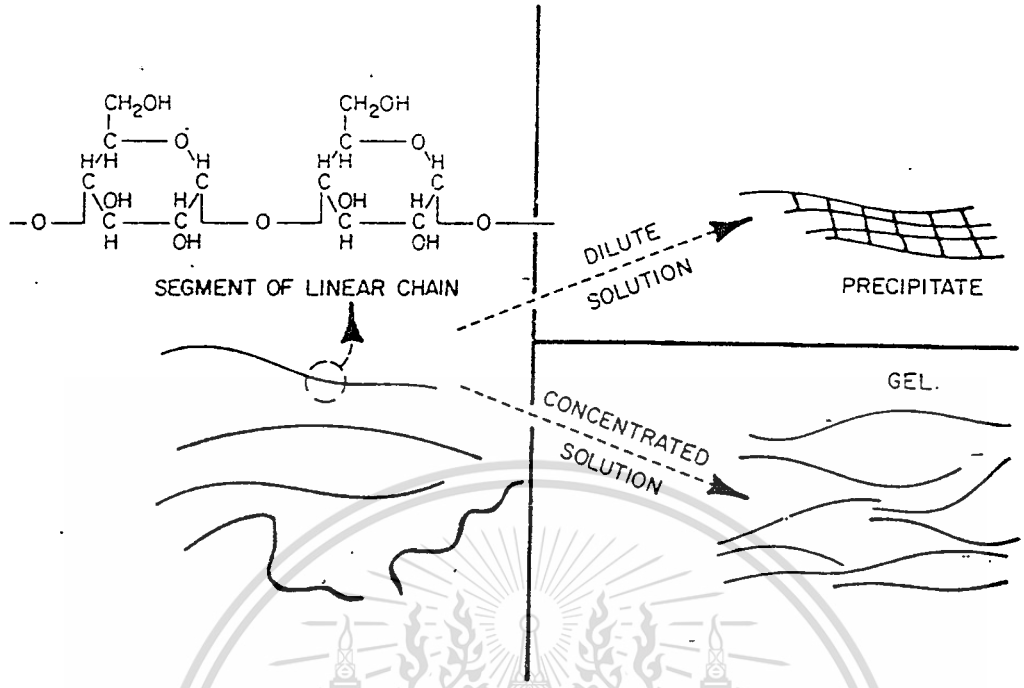


Fig. 2.8 Schematic Representation of Retrogradation Mechanism [39].

2.2.4 Tapioca Starch

Tapioca starch (Tapioca flour, Tapioca, cassava starch, manioc starch) is obtained from the roots of the manioc or cassava plant (*Manihot utilissima*), family Euphorbiaceae, which was originally a native of Central America, but is now grown in Brazil, the Dominican Republic, Nigeria, Florida, Madagascar, Malaysia, the Philippines and Thailand.

Tapioca starch production, the roots of cassava should be processed within 24 hr. after harvesting. Extraction of starch from cassava roots is a simple and straightforward process without the problems associated with the manufacture of corn, wheat or other cereal starches. The cassava roots contain up to 30% of starch. Basically, tapioca starch manufacturing can be divided into the following stages.

1. Washing and peeling of the tubers to remove soil and rind.

2. Rasping or disintegration to destroy the cellular structure and to rupture the cell walls to release the starch as discrete.

3. Screening or extraction to separate starch milk from waste fibrous material.

4. Purification to separate starch granules from their suspension in water by centrifuging or sedimentation.

5. Drying to remove sufficient moisture to a level low enough for long-term storage (about 12-14%).

6. Finishing operations such as pulverizing, sifting, and bagging.

Tapioca starch is very white in color. The starch granular size varies from 5 to 35 μm with an average of about 20 μm. The shape of granules exhibit varying shapes, but mostly are round with a truncated end and possess a smooth outer surface (Fig. 2.9).

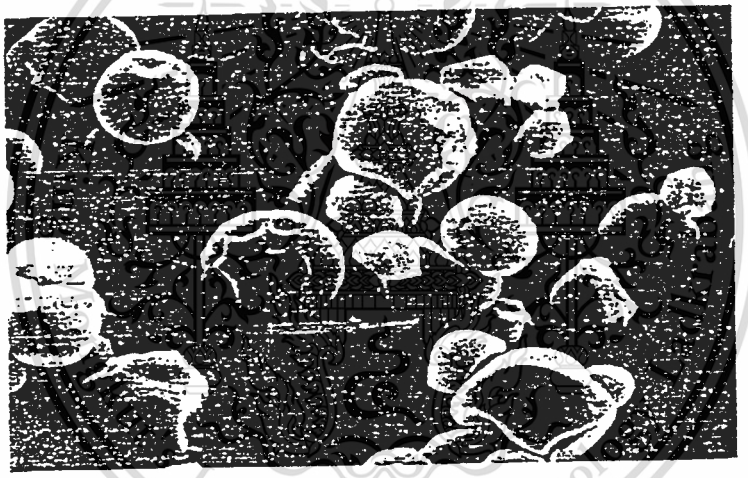


Fig. 2.9 SEM Photograph of Tapioca Starch [8].

The X-ray diffraction pattern of tapioca starch has been found to be 'A' with characteristic peaks at 12, 9, and $8^{\circ}30'$ [8].

Tapioca starch has an amylose content in the range of 16 to 18%. The starch expresses the properties similar to all amylopectin starch, e.g., high viscosity, low retrogradation tendency, and good sol stability [8]. Table 2.6 shows maximum limits of specifications of commercial tapioca starch for a top grade.

Table 2.6 Specifications of a Top Grade of Tapioca Starch [29]

Determination	Maximum limit
Moisture	12.5%
Ash	0.25%
Protein	0.15%
Fibre	0.05%

Tapioca starch is used in many industries. The major industries which using starch as raw material are adhesive industry, paper industry, dextrin industry, sweetener industry, and food industry. In addition, there are a number of other applications of starch which are encounted in many fields, e.g., soap and detergent industry, cosmetic and pharmaceutical uses, biodegradable plastics, etc [8,12,37].

2.2.5 Utilization of Tapioca Starch in Thailand

In Thailand, cassava plant, which is grown in the north-eastern and eastern parts of the country, is an economic plant. Now, the Department of Agriculture, Ministry of Agriculture and Cooperatives has been recommended 7 cassava varieties include Rayong1, Rayong 3, Rayong 5, Rayong 60, Rayong 90, Kasetsart 50, and Sriracha 1 to farmers.

Dominant and Recessive Characteristic of Cassava Varieties in Thailand [2]

Rayong1 (recommended in 1975)

Dominant : - Good ability to grow in all environment and climate.
- Tall linear stem that is easy to eliminate weeds and to correct cuttings.
- Good ability to adjust itself to all conditions.

Recessive : - Low starch content.

Yield (Ton/Rai) : 3.22

Starch content(%) : 18.3 (rainy season) ; 24 (dry season)

Rayong3 (recommended in 1983)

Dominant : - High %starch content

- Response to fertilizer and fertile fields.

Recessive : - Short stem and high branching stems which is difficult to take care of it.

- Cuttings,stem which are cut for growing, are quick deterioration.

Yield (Ton/Rai) : 2.73

Starch content(%) : 23 (rainy season) ; 28 (dry season)

Rayong5 (recommeded in 1994)

Dominant : - High yield and %starch content.

- Good ability to adjust itself to all conditions.
- Cuttings are good growth.

Recessive : - Cassava bacterial blight is found more than the other varieties but no effect to yield.

Yield (Ton/Rai) : 4.02

Starch content(%) : 22.3

Rayong60 (recommended in 1987)

Dominant : - Short harvesting time.

- High yield.

Recessive : - Cream-colored internal tuber.

- Low starch content.

Yield (Ton/Rai) : 3.52

Starch content(%) : 18.5

Rayong90 (recommended in 1989)

Dominant : - High yield and %starch content.
- Response to fertilizer and fertile fields.

Recessive : - Bended stem.
- Cutting dry quickly that cannot keep for long time.

Yield (Ton/Rai) : 3.65

Starch content(%) : 23.7

Kasetsert50 (recommended in 1992)

Dominant : - High yield and %starch content.
- High percentage of growing and living.

Recessive : - High branching stems occure in different condition that is difficult to take care of it.

yield (Ton/Rai) : 3.67

Starch content (%) : 23.3

Srirachal (recommended in 1990)

Dominant : - Ability to grow in low fertile soil.

Recessive : - Low starch content.
- Cream-colored internal tuber.

Starch content(%) : 21.9

Cassava tubers are converted to tapioca chips and pellets about 70-80%, and tapioca starch about 20-30%. Tapioca chips and pellets are almost exported, which mainly in European Union, and tapioca starch which an estimated 60 % volume is exported to markets abroad and another 40 % volume for domestic consumption in forms of modified flour, sweetener, sago, seasoning powder.

The utilization of tapioca starch inside country are used in many industries. Tapioca starch has been applied as the raw material in 6 main industries include paper industry, textile industry, food and beverage industry, plywood industry, glue industry, and alcohol industry.

In order to serve certain purposes of which the native starch fails to do, starch is modified to appropriated properties. In Thailand, the manufacture of modified starch is devided into 3 types as follows :

1. Degradation or conversion : Starch is rendered less sticky through 3 ways:
   - Acid Conversions
   - Oxidization
   - Dextrinization or Pyroconversions

2. Pregelatinization : Modified starch from this method can be dissolved in cold-water and became adhesive property. This modified starch is called "Cold- Water-Soluble-Starch (CWS)" or "Alpha Starch".

3. Derivatives : This method use chemical to adjust molecular structure for modification properties of starch. The modified starch from this method is Starch Ester (Acetated Starch and Phophoric Acid Ester), Starch Ether (Carboxymethyl Ether and Hydroxyethyl Starch), and Cross-Link Starch. The applications are used in food, paper and adhesive industries [1, 32].

The research and development of new products from tapioca starch are investigated in order to increase amount of tapioca utilization in country. For example, High-water absorbing polymer (HWAP) which is synthetic polymer produced from tapioca starch and is used in many applications e.g. sanitary pad. Biodegradable plastics, which is one of new trend to solve or decrease plastic waste problem, can be investigated with tapioca starch.

2.3 STARCH DERIVATIVES

Because of unsuitable properties, starch is modified to new products which having desirable properties. Starch derivatives are new products from starch by definition that is "a chemically modified starch in which the chemical structure of some of the glucose units has been altered" [37]. By this definition, acid-modified starches are excluded from starch derivatives. Examples of starch derivatives are starch esters, starch ethers, oxidized starches, starch graft copolymers, etc. This study was attended to starch graft copolymer, so only a detail of starch graft copolymers is presented.

2.3.1 Starch Graft Copolymers

The technical approach to chemically bonded natural polymer-synthetic polymer compositions is through graft polymerization. Starch is one of natural polymers which can be connected to synthetic polymer by graft copolymerization technique. The nomenclature used for starch graft copolymers is that proposed by Cersa, and earlier reviews on starch graft copolymers have appeared. The structure of a starch graft copolymer is shown schematically in Fig. 2.10, where AGU represents an anhydroglucose unit, and M is the repeating unit of the monomer used in the polymerization reaction.

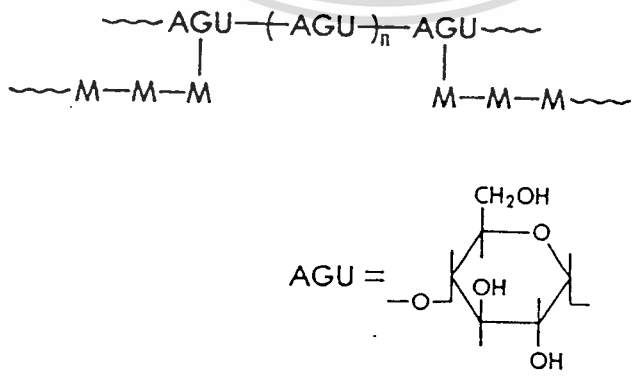


Fig. 2.10 Schematic Representation of a Starch Graft Copolymer [40]

In free radical graft polymerizations of starch, the terms are used to describe reactions as following :

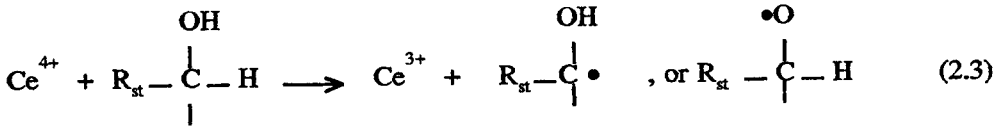
- Grafting frequency or AGUs per chain is defined as the average number of AGUs per molecular weight grafted chain. Grafting frequency will rang from several hundred to several thousand by calculation from the weight percentage of synthetic polymer in the graft copolymer (percent add-on) and the average molecular weight of the grafted branched.

- Grafting efficiency is defined as the percentage of the total synthetic polymer formed that has been grafted to starch.

2.3.2 Synthetic Methods : Basically, the procedure used for synthesizing starch graft polymers is to initiate free radicals on the starch backbone and then allowing these free radicals to serve as macrointiators for the vinyl or a acrylic monomers.

A number of free radical initiating systems have been used to prepare graft copolymers, and these may be divided into two broad categories : chemical initiation and irradiation initiation. The choice depends on the particular monomer to be polymerized. Both chemical and irradiation systems have been employed to graft polymerize onto starch, a wide variety of monomers, both alone and in selected combinations.

3.2.2.1. Chemical initiation : Several chemicals are used for producing free radicals in starch and initiating graft copolymerization. Ceric salts, such as ceric ammonium nitrate, is widely used in graft copolymerization by chemical initiation method. The mechanism of ceric initiated graft copolymerization is shown in Eq.(2.3). Ceric ion produces free radicals exclusively on the starch molecule by a single electron transfer.



$$Ce^{4+} + R_{st}-\underset{|}{\overset{OH}{\overset{|}{C}}}-H \longrightarrow Ce^{3+} + R_{st}-\overset{OH}{\overset{|}{C}}\bullet \text{ , or } R_{st}-\overset{\bullet O}{\overset{|}{C}}-H \qquad (2.3)$$

Redox systems (e.g., hydrogen peroxide-ferrous ion and peroxydisulfate-bisufite systems), and diazonium groups are also capable of producing free radicals in starch and of initiating graft copolymerization. The mechanism of these systems differ from ceric-ion initiated system and show in Eq.(2.3) - (2.11), where $R_{st}-\underset{|}{C}HOH$ represents a secondary hydroxyl group in starch molecule and M_n represents polymeric segment.

$$Fe^{2+} + HOOH \longrightarrow FeOH^{2+} + \bullet OH \qquad (2.4)$$

$$R_{st}-\underset{|}{\overset{|}{C}}(OH)-H + \bullet OH \longrightarrow R_{st}-\underset{|}{\overset{|}{C}}(OH)\bullet + H_2O \qquad (2.5)$$

$$R_{st}-\underset{|}{\overset{|}{C}}(OH)\bullet + M_n \longrightarrow R_{st}-\underset{|}{\overset{|}{C}}(OH)-M_n\bullet \longrightarrow \text{graft copolymer} \qquad (2.6)$$

$$R_{st}-\underset{|}{\overset{|}{C}}(OH)\bullet + FeOH^{2+} \longrightarrow R_{st}-\underset{|}{C}=O + H_2O + Fe^{2+} \qquad (2.7)$$

$$HO\bullet + M_n \longrightarrow HOM_n\bullet \longrightarrow \text{homopolymer} \qquad (2.8)$$

$$S_2O_8^{2-} \longrightarrow 2\,SO_4^{\bullet -} \qquad (2.9)$$

$$SO_4^{\bullet -} + H_2O \longrightarrow HSO_4^{-} + HO^{\bullet} \qquad (2.10)$$

$$R_{st}-\underset{|}{\overset{|}{C}}(OH)-H + SO_4^{\bullet -} \longrightarrow R_{st}-\underset{|}{\overset{|}{C}}(OH)\bullet + HSO_4^{-} \qquad (2.11)$$

In addition to these chemicals, oxidation with ozone can be used for initiation by introduction of functional groups that upon decomposition produce three radicals capable of initiating grafting and homopolymerization such mechanism as shown in Eq. (2.12) and (2.13).

$$R_{st}OOH \xrightarrow{\text{heat}} R_{st}O\bullet + HO\bullet \qquad (2.12)$$

$$R_{st}O\bullet + M_n \longrightarrow R_{st}OM_n\bullet \longrightarrow \text{graft copolymer} \qquad (2.13)$$

Some grafting on starch happens by chain transfer into starch when vinyl monomers are polymerized in starch solution.

A technique frequently used is to allow starch to react with hydrogen peroxide in the presence of a ferrous salts, such as ferrous ammonium sulfate. Homopolymerization is predominant in such systems, and it is uncertain whether a true chain-transfer mechanism is involved. Eq.(2.14), (2.15), and (2.16) show mechanism of chain transfer graft copolymerization. Chain transfer can occur by hydrogen abstraction by a growing polymeric segment, $M_n\bullet$, where $R_{st}-\underset{|}{\overset{OH}{\overset{|}{C}}}-H$ represents a secondary hydroxyl group in the starch molecule.

$$R_{st}-\underset{|}{\overset{OH}{\overset{|}{C}}}-H + M_n\bullet \longrightarrow R_{st}-\underset{|}{\overset{OH}{\overset{|}{C}}}\bullet + M_nH \qquad (2.14)$$

$$R_{st}-\underset{|}{\overset{OH}{\overset{|}{C}}}\bullet + M_n \longrightarrow R_{st}-\underset{|}{\overset{OH}{\overset{|}{C}}}-M_n\bullet \longrightarrow \text{graft copolymer} \qquad (2.15)$$

$$M_n\bullet + M\bullet \longrightarrow \text{homopolymer} \qquad (2.16)$$

3.3.2.2 Radiation Initiation : In addition to chemical initiation methods, ^{60}Co and electron beam irradiation have been used to produce free radicals on the starch backbone. Preirradiation technique, starch is irradiated first and the activated starch is then allowed to react with monomer, often produces less homopolymer than simultaneous irradiation technique, starch and monomers are mixed and irradiated together.

UV irradiation has been used to initiate grafting. Merlin and Fouassier showed that irradiation of starch in either the presence or absence of photosensitizers results in chain scission and the formation of free radicals that can subsequently react with monomer. Several

monomers were graft polymerized on dilute (0.67%) water suspensions of starch by this technique [25,36,40].

2.4 SIZE EXCLUSION CHROMATOGRAPHY (SEC)

Size exclusion chromatography (SEC) is a subgroup of liquid chromatography (LC), which molecular separation depends upon their size. SEC is often referred as gel permeation chromatography (GPC) when using for the separation of organic polymers with organic mobile phase or as gel filtration chromatography (GFC) when using for the separation of water soluble biopolymers with aqueous mobile phase. However, the term size exclusion chromatography (SEC) is the preferred term independent of the type of mobile phase employed [13, 28].

2.4.1 Theory of Chromatographic Retention

Size exclusion chromatography (SEC) is a method to separate molecules by size. Separation in SEC results from the distribution of the sample between the moving mobile phase and the stagnant portion of the mobile phase retained within the porous structure of the stationary phase. Fig. 2.11 illustrates the mechanism of SEC separation. A sample, which consisting of two components of different size, is injected on the top of a column packed with small, rigid porous particles. The smaller molecules can diffuse through the pore structure and spend more time within the pores of the packing than the larger molecules. Therefore, the large molecules elute first from the column where they are monitored by a suitable detector [31].

In conventional liquid chromatography, chromatographic separation of a mixture results from differential migration of solutes through a column. This migration is achieved via preferential interaction between the different components of mixture and the column stationary phase. The mobile phase is the major part to determine that interaction. However, separation

in SEC differ mechanism from other liquid chromatography separation. The stationary phase is preferably inert to the sample, and the mobile phase is chosen to be a good solvent for the sample and should be have low viscosity at the separation condition.

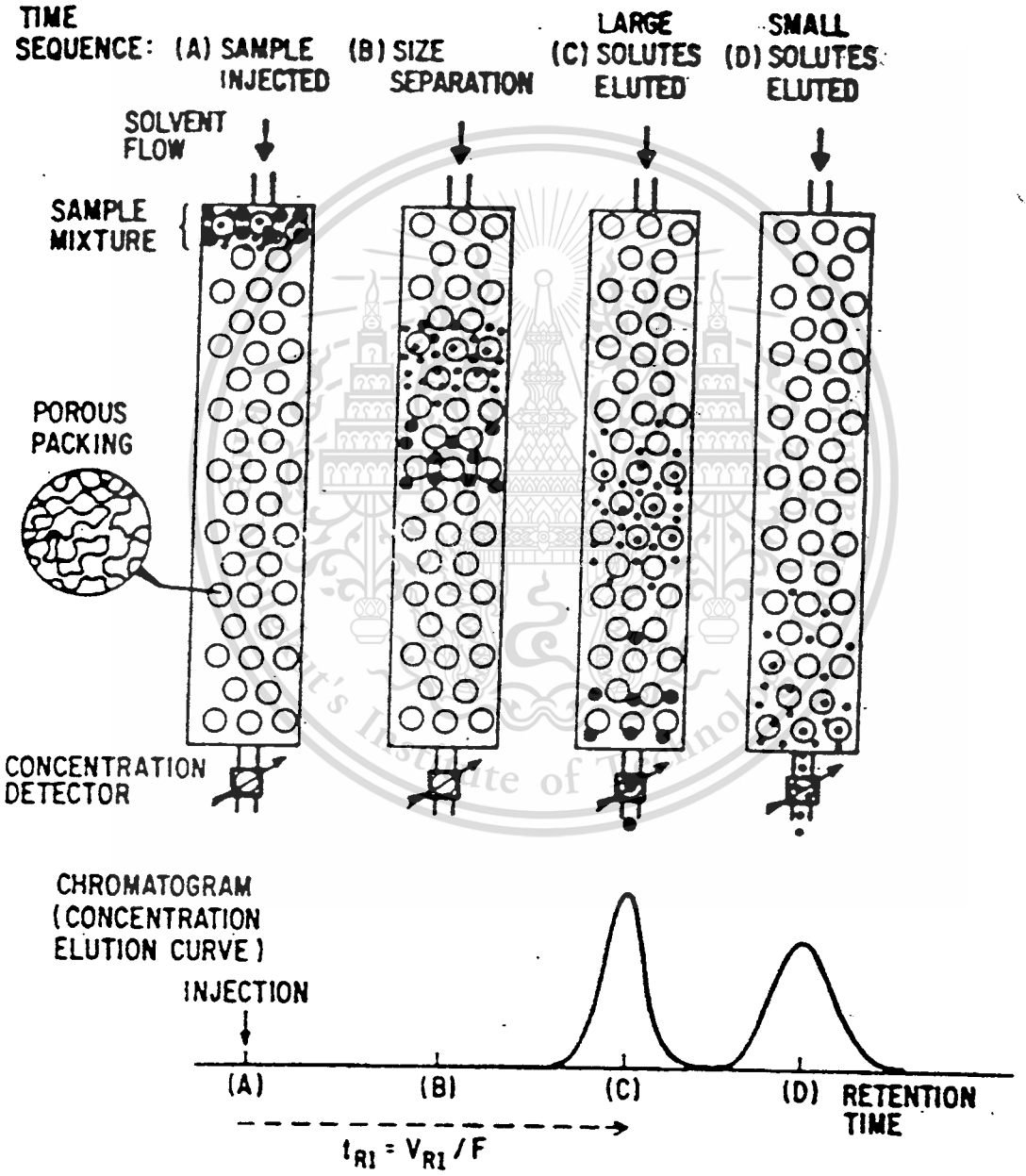


Fig. 2.11 Schematic of SEC Separation Mechanism [31].

Separation in SEC is achieved by the ability of solute molecules to permeate the pores of the stationary phase. Solute retention is determined by the size of the molecules of solute and the size and distribution of stationary phase pores. Retention volume of the solute (V_R) in SEC is defined by the following equation:

$$V_R = V_O + K_{SEC} V_i + K_{LC} V_S \quad (2.17)$$

where : V_O = Exclusion of the column

V_i = Internal pore volume

V_S = Equivalent liquid volume of stationary phase

K_{SEC} = SEC distribution coefficient (i.e., the ratio of the solute concentration in the pores to that outside the pores)

K_{LC} = Ratio of solute concentration in the stationary phase to that in the mobile phase

Ideally, solute adsorption to the packing is minimized by choosing the column packing and mobile phase such that K_{LC} is negligible. The Eq. (2.17) is become to :

$$V_R = V_O + K_{SEC} V_i \quad (2.18)$$

K_{SEC} is constrained to values between 0 and 1 by complete exclusion or complete permeation of solute, respectively. Solute retention volume can be relied upon solute molecular weight (MW). In Fig. 2.12, solute A, the highest MW solute, elutes at the column exclusion volume V_O. As the MW of the solutes decreased, their elution volume increase (peaks B and C) because of increasing of ability to access the pores. Solute D, the smallest MW solute, which is able to access all of the pores of stationary phase, elutes at the column permeation volume.

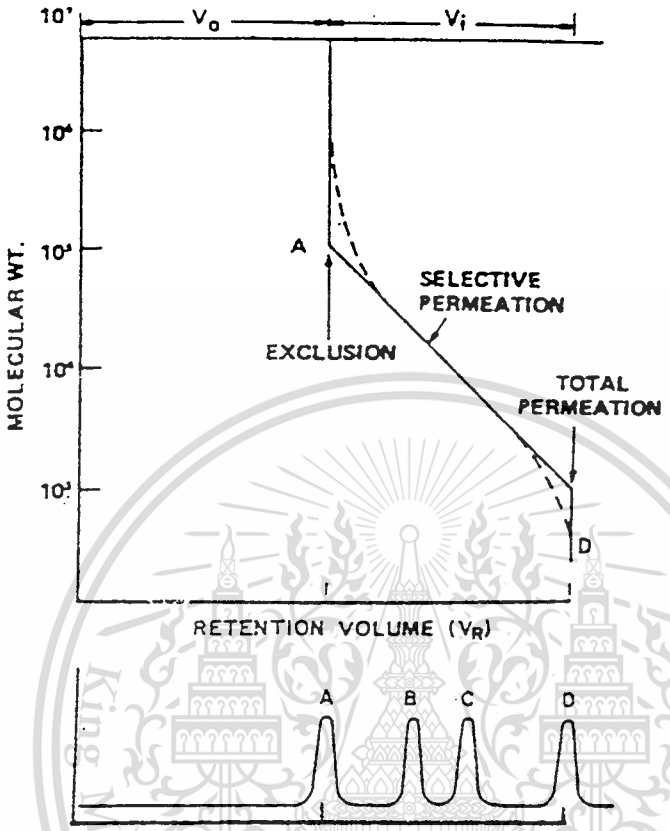


Fig. 2.12 Typical SEC Calibration Curve Showing the Linear Range [31].

2.4.2 Instrumentation

The system of SEC consists of a high-pressure pump, capable of delivering a constant volume with a good pulse damper to reduce pump pulsation, a high precision injector to deliver constant injection volume, a SEC column, a suitable detector, and a data system [31].

Column selection is an essential part of a successful SEC analysis. The major classes of materials for SEC packing column in general use are : (1) carbohydrate-derived gels, (2) polyacrylamide gels, (3) composite gels, (4) polystyrene gels and (5) inorganic gels. The soft, carbohydrate-derived gels are generally used for SEC at atmospheric or slightly elevated

pressure (up to 200 psi). For HPSEC under high pressures, the silica or polystyrene-based gels are used in aqueous or organic solvents, respectively.

The most important parameter to be considered in the selection of the column-packing material is the appropriate pore-size for the particular application. Single-pore-size column materials are used for separation of samples with a narrow molecular weight distribution. To separate a mixture of compounds with widely different molecular weights, coupling two or more columns having nonoverlapping but adjacent fractionation ranges can often be better resolved [13].

Continuous monitoring of solute elution from a SEC column is a achieved with the use of a suitable detector. In SEC , detector belong to one of two categories ; bulk property or solute property detectors. Bulk property detectors, such as refractive index detector, measure the changes in some physical property of the mobile phase because of the presence of solute molecules. Solute property detectors, such as ultraviolet detector, respond to a specific chemical or physical structure of the solute. The differential refractive index (RI) is the most widely used detector in SEC. The connection of RI detector with other detector are used in application to determining absolute molecular weight of polymer, for example, low-angle laser light-scattering (LALLS) detector has been widely used in SEC in conjunction with RI detection for the determination of the absolute molecular weight distribution (MWD) of polymers [31].

2.4.3 Calibration

The SEC experiment alone does not provide any information on either average molecular weight or molecular weight distribution. It is solely a separation technique. In order to obtain molecular weight information from the peak profile, the SEC column must be calibrated against known molecular weight standards. The three most common methods of calibration include peak position, universal, and broad standard calibration.

2.4.3.1 Peak Position Calibration : A series of different molecular weight standards are chromatograghed under constant experimental conditions and their elution volume measured. The calibration curve are constructed by a plot of molecular weight against elution volume as shown in Fig. 2.13. The linear portion of the calibration curve is usually described by an equation of the type.

$$\log M = A - mV_E \qquad (2.19)$$

where M is the molecular weight corresponding to an elution volume V_E and A and m are system constants.

The accuracy of this calibration method depends on whether the solution conformations of the standards and samples are similar, the condition required for the molecular weight and molecular size to be uniquely correlated. Large errors in estimating the sample molecular weight can result when calibration curves prepared from narrow molecular weight standards of one polymer are used to characterize polymers of a different type. However, narrow fraction standards are not available for all synthetic polymers, limiting the use of peak-positioning calibration [28, 31].

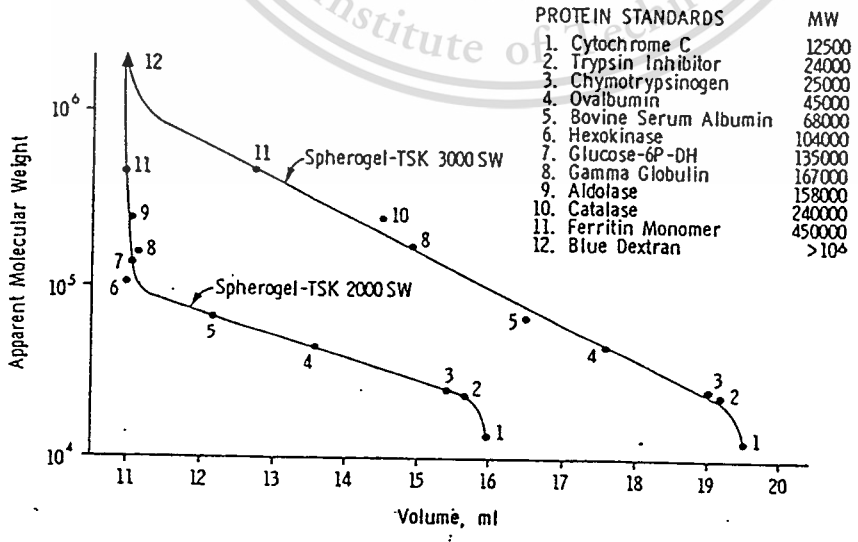


Fig. 2.13 Protein Calibration Curves for Spherol TSK-SW 2000 and TSK-SW 3000 Column. Mobile Phase : Phosphate Buffer 0.2 M., pH 6.8, Flow Rate 1.0 ml./min. [28].

2.4.3.2 Universal Calibration : Because of limiting the use of peak position calibration, the universal calibration parameter is used to characterize the effective dimensions of macromolecules and various polymers. The hydrodynamic volume is one such parameter that has been widely applied in this calibration. The hydrodynamic volume, which is independent of the chemical nature of the polymer, is equal to the product of the intrinsic viscosity [η] and the molecular weight of the polymer (M). A plot of the logarithm of the hydrodynamic volume against the elution volume provides a calibration curve that is approximately valid for all polymers. Fig. 2.14 shows single curve from a plot of the logarithm of the product [η]M against the elution volume of variety of polymers with tetrahydrofuran used as a solvent.

The experimental finding of the functional relationship between the molecular weight of monodisperse standard samples and the elution volume of the solution must be determined for a given solvent and column under fixed condition. The universal calibration method is based on the Benoit relationship between solute relation and hydrodynamic volume as the following relationship exists :

$$[\eta]_{Ai}M_{Ai} = [\eta]_{Bi}M_{Bi} \quad (2.20)$$

where $[\eta]_i$ and M_i represent the intrinsic viscosity and molecular weight (MW), respectively, for polymers A and B eluting at retention increment (i). The value $[\eta]_i$ for a linear polymer in a particular solvent is a function of MW as described by the Mark-Houwink equation.

$$[\eta] = KM^{\alpha} \quad (2.21)$$

where the constant K and α vary with polymer composition, solvent, and temperature. These constants are available in the literature for a variety of solvents or they can easily be determined by measurement of the intrinsic viscosity. The resulting expression is solved for M_{Bi} by substitution of [η] and rearrangement yield the following equation :

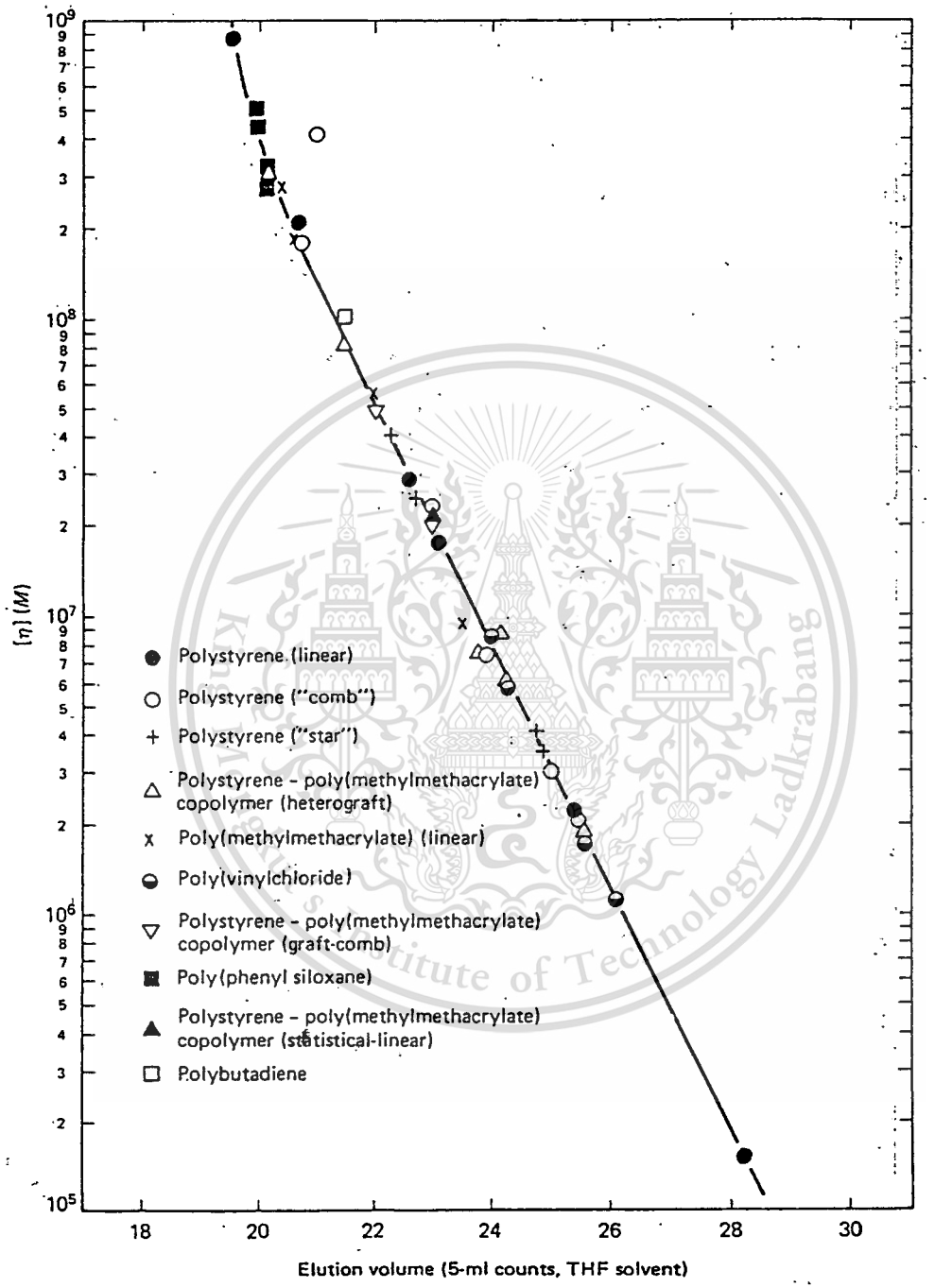


Fig.2.14 Universal Calibration in Gel Permeation Chromatography for a Variety of Polymers in Tetrahydrofuran [3].

$$M_{Bi} = (K_A / K_B)^{k1} (M_{A1})^{k2} \quad (2.22)$$

where

$$k1 = \frac{1}{(\alpha_B + 1)}$$

$$k2 = \frac{(\alpha_A + 1)}{(\alpha_B + 1)}$$

Eq. (2.22) describes the relationship between the MW of two polymers eluting at the same retetion-time increment. The constants relation the two MWs are dependent on solvent type and temperature, but independent of MW and type of SEC column. If the Mark-Houwink constants for standard samples and unknown polymers are known, the molecular weight of unknown polymer will be determined in comparison with universal calibration curve. Fig. 2.15 shows molecular weight of monodisperse polystyrene standards as a function of elution volume in tetrahydrofuran [3, 31].

2.4.3.3 Broad Standard Calibration : There are two methods to achieve calibration on using broad molecular weight distribution (MWD) standards. First, the integral MWD method utilizes the complete MWD curve of the standard. Second, the linear calibration method utilizes a broad MWD polymer standard with known M_w and M_n and experimental condition to construct a linear log M vs. retention volume calibration curve. The method consists of a search for an effective linear calibration of the following equation :

$$\log M_i = C_1 + C_2 t_i \quad (2.21)$$

where t_i corresponds to retention time, C_1 and C_2 are constants, and M_i is the molecular weight. When effective calibration constants are calculated such that the computed molecular weight and polydispersity values agree with known values for the specific polymer. M_w and M_n are defined as the following form :

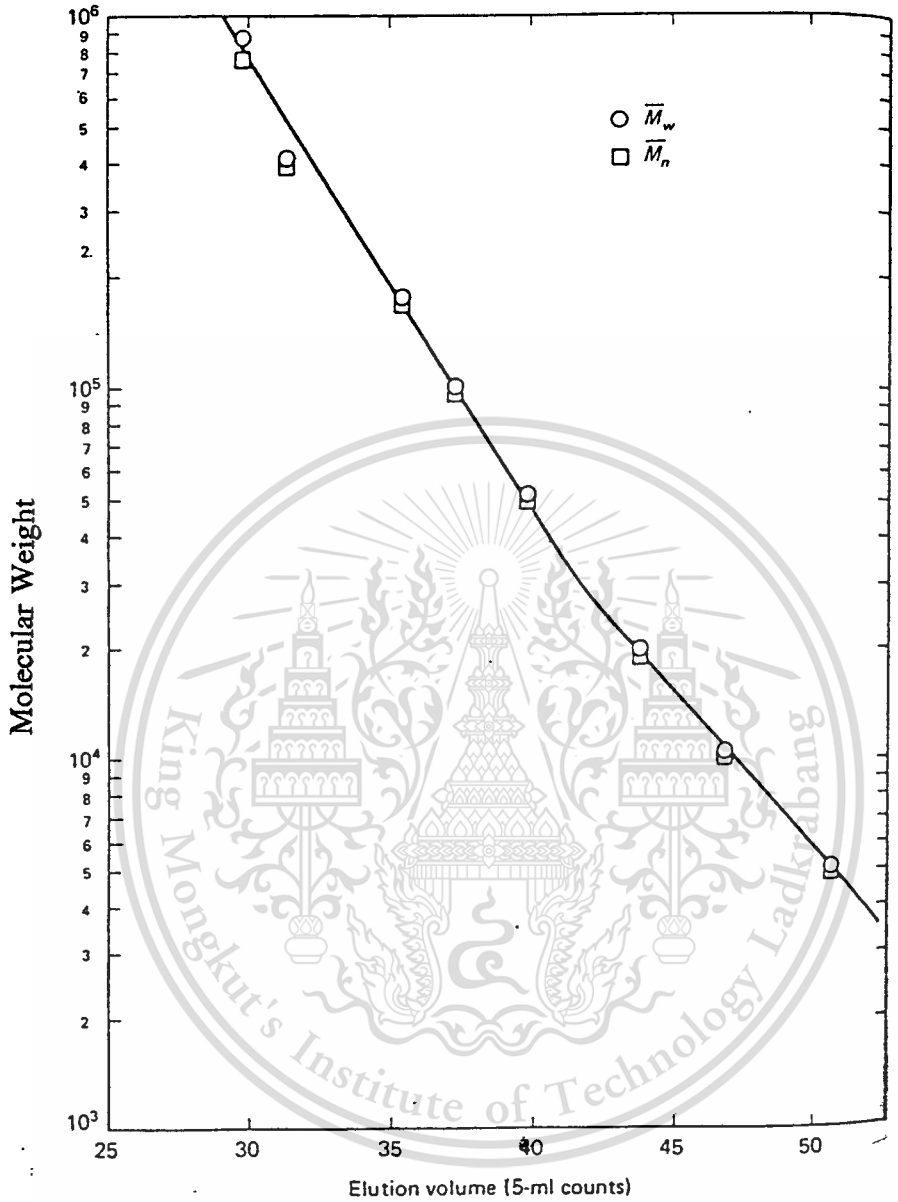


Fig. 2.15 Molecular Weight of Monodisperse Polystyrene Standards as a Function of Elution Volume in Tetrahydrofuran [3].

$$M_w = \frac{\sum C_i M_i}{\sum C_i} \quad (2.24)$$

$$M_n = \frac{\sum C_i}{\sum C_i / M_i} \quad (2.25)$$

where C_i and M_i represent the concentration and molecular weight values at the $_i$th elution increment, respectively. The method requires the availability of a well-characterized standard of known M_w and M_n [31].

2.4.4 Applications of Size Exclusion Chromatography

Applications of SEC are numerous. There may be classified into two main categories ; gel filtration of biomolecules and other water-soluble polymers; and gel permeation chromatography of organic-solvent-soluble synthetic polymers. The utility of SEC is in the area of molecular fractionation according to the size of the molecules for determination of their molecular weight and molecular-weight distribution, removal from small molecules, study of molecular associations, and several others [13].

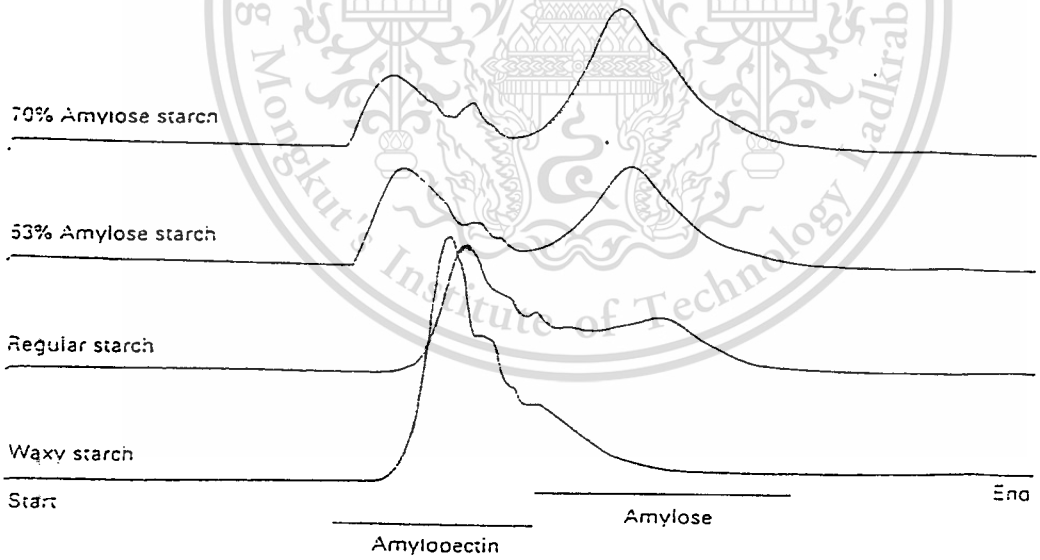


Fig. 2.16 High-Performance Size Exclusion Chromatograms of Various Starches [24].

Starch is one of biopolymers. Starch and its derivatives are used in many industries. Starch can be hydrolyzed by acid or enzyme or modified by various chemical reagents. It is important to know the molecular weight of the starch after the treatment. SEC is a valuable

tool to study these chemical modifications [39]. Fig. 2.16 shows the high-performance chromatograms of various starches.

2.5 GRAFT COPOLYMERIZATION PROCESS

Copolymerization is the polymerization of two or more monomers which is the method to produce the new materials. The copolymers have different properties from homopolymers made from single monomers. The properties of copolymers depend on the composition and type of feed monomers.

Graft copolymerization results from the formation of an active site at a point on a polymer molecule other than its end, and exposure to a second monomer. Most graft comonomers are formed by radical polymerization. The major activation is chain transfer to polymer. In addition, ultraviolet or ionizing radiation, or redox initiation, among other methods, can also be used to produce the polymer radicals leading to graft copolymer [11].

2.5.1 Kinetics of Copolymerization

The first mechnism of copolymerization was purposed by Dostal (1936). There are four possible ways in which monomers can add to a growing free radical depends only on the nature of the end group on the radical chain.

Reaction	Rate	
$M_1\bullet + M_1 \longrightarrow M_1\bullet$	$k_{11}\,[M_1\bullet]\,[M_1]$	
$M_1\bullet + M_2 \longrightarrow M_2\bullet$	$k_{12}\,[M_1\bullet]\,[M_2]$	
$M_2\bullet + M_1 \longrightarrow M_1\bullet$	$k_{21}\,[M_2\bullet]\,[M_1]$	
$M_2\bullet + M_2 \longrightarrow M_2\bullet$	$k_{22}\,[M_2\bullet]\,[M_2]$	(2.26)

The M_1, M_2, $M_1\bullet$, and $M_2\bullet$ represent monomer 1, monomer 2, radical of monomer 1, and radical of monomer 2 , respectively.

In 1944, Alfrey, Mayo, Simha, and Wall added the assumption of the steady state applied to each radical type separately, thus, the concentrations of $M_1\bullet$ and $M_2\bullet$ must each remain constant. The rate of conversion of $M_1\bullet$ to $M_2\bullet$ must equal that of conversion of $M_2\bullet$ to $M_1\bullet$.

$$k_{12}\,[M_1\bullet]\,[M_2] \quad = \quad k_{21}\,[M_2\bullet]\,[M_1] \tag{2.27}$$

The rate of disappearance of the two types of monomers are given by

$$\frac{-d\,[M_1]}{dt} = k_{11}\,[M_1\bullet]\,[M_1] + k_{21}\,[M_2\bullet]\,[M_1]$$

$$\frac{-d\,[M_2]}{dt} = k_{12}\,[M_1\bullet]\,[M_2] + k_{22}\,[M_2\bullet]\,[M_2] \tag{2.28}$$

To define $r_1 = k_{11}/k_{12}$ and $r_2 = k_{22}/k_{21}$ and to combine Eq. (2.27) and (2.28), the composition of copolymer being formed at any instant can be shown as following:

$$\frac{d\,[M_1]}{d\,[M_2]} = \frac{[M_1]}{[M_2]}\,\frac{r_1\,[M_1] + [M_2]}{[M_1] + r_2\,[M_2]} \tag{2.29}$$

The Eq. (2.29) is known as the copolymer equation. The r_1 and r_2 represent monomer reactivity ratios which are the ratios of the rate constant for a given radical adding its own monomer to the rate constant for its adding the other monomer. Therefore, $r_1>1$ indicates that the radical $M_1\bullet$ prefers to add M_1 rather than M_2 or $r_1<1$ indicates that it prefers to add M_2 rather than M_1 [11].

2.5.2 Emulsion Polymerization

Emulsion polymerization is one of important process of polymerization. There are two immiscible liquid phase in system. An aqueous continuous phase and a nonaqueous discontinuous consisting of monomer and polymer. The initiator is located in the aqueous phase, and the monomer-polymer particles are quite small, 0.05 to 5 μm in diameter.

The soap or emulsifier plays an important role in emulsion polymerization. Initially, it exists in the form of micelles, spherical or rodlike aggregates of 50-100 soap molecules with their hydrophobic "tails" oriented inward and their hydrophilic "heads" outward that shown in Fig. 2.17. Some of monomer dissolves in the micelles, but most of it exists as droplets a micrometer or more in diameter. Consequently, when free radicals are generated in the aqueous phase, the micelles capture most of them.

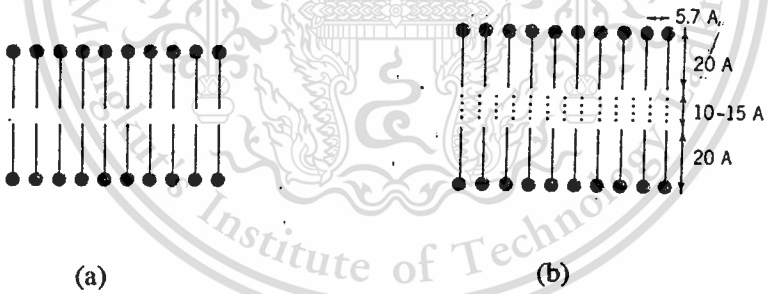


Fig. 2.17 Idealized Structure of a Soap Micelle (a) without and (b) with Solubilized Monomer [11].

After 2-3 % polymerization, the system contains of (1) stabilized, monomer-swollen polymer particles rather than micelles and (2) monomer, which remains mainly in droplets, although it constantly diffuses to refill the swollen particles where polymerization continues. The result is, when monomer is quit water-insoluble,

$$R_p = k_p [M] [M\bullet] \qquad (2.30)$$

where [M] now is the concentration of monomer in the swollen polymer particles, R_p is rate of polymerization, and k_p is rate constant of propagation reaction. Since this concentration, mole per liter of swollen particles, may remain constant from low concentration up to 70 or 80 % conversion, R_p should be independent of the total concentration of monomer, i.e., mole per liter of emulsion. Rate increases with increasing soap (can initial micelle) concentration.

2.5.3 Smith-Ewart Kinetics

The nucleation stage constitutes the so-called Interval I in an emulsion polymerization, The beginning period in which the particle number is changing. In Interval II and III the particle number is believed to be essentially constant. All discussions of particle nucleation start with The Smith-Ewart theory in which Smith and Ewart (1948) in a quantitative treatment of Harkins' micellar theory conducted to obtain an equation for the particle number as a function of emulsifier concentration and initiation and polymerization rates. This equation was mainly advanced for systems of monomers with low water solubility (e.g., styrene), partly solubilized in micelles of an emulsifier with low critical micelle concentration (CMC) and seemed to work well for such systems.

The main components and phases in an emulsion polymerization system is illustrated by Fig. 2.18. The arrows indicate the possible distribution of the components between the phases.

Monomer (i) will usually exist as monomer droplets; (ii) some monomer depending on water solubility, will be dissolved in the continuous phase; and (iii) some monomer will be solubilized in micelles.

Emulsifier (i) will be partly dissolved in the continuous phase; (ii) if concentration is above the CMC, the excess will form emulsifier micelles; and (iii) some emulsifier will be absorbed on monomer droplets, and may even be dissolved into the droplets.

Initiator (i) will mostly be dissolved in the continuous phase as water-soluble initiators are usually applied. For special applications partly or completely oil soluble initiators may be used. There will be distributed similarity to the monomer.

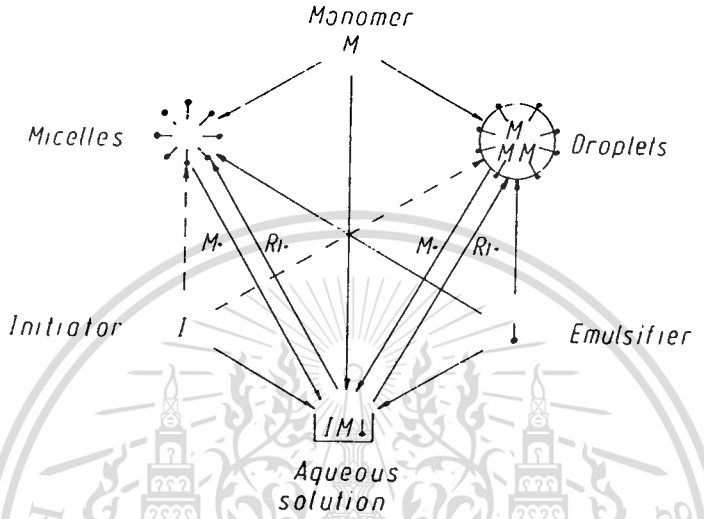


Fig. 2.18 Schematic Illustrations of the Components and Phases Usually Present in an Emulsion Polymerization system. The Arrows Indicate the Possible of Components Among Phases [27].

In an ideal emulsion system, free radical generation is in the aqueous phase at a rate of about 10^{13} per cubic centimeter per second. There are about 10^{14} polymer particles per cubic centimeter. Simple calculations exhibit that termination of the free radicals in the aqueous phase is negligible and that diffusion current are sufficient for the rapid diffusion of free radicals into the polymer particles-on the average, about one per particle every 10 sec.

It can also be calculated from the known termination rate constant that two free radicals within the same polymer particle would mutually terminate rapidly. Therefore, on the average half the particles will contain radicals at any moment. The rate of polymerization per cubic centimeter of emulsion is

$$R_p = k_p[M]\, N_p \qquad (2.31)$$

where N_p is the number of polymer particles per unit volume of emulsion. Since the monomer concentration is nearly constant, the rate relies principally on the number of particles present and not on the rate of generation of radicals.

The number-average degree of polymerization ($\overline{x}_n$), disregarding chain transfer, and assuming termination by coupling, also depends upon the number of particles:

$$\overline{x}_n = \frac{k_p N_p [M]}{d [M\bullet]/dt} \qquad (2.32)$$

where d [M•]/dt is the rate of radical formation (not the net rate) and may be proportional to the square root of initiator concentration in the aqueous phase.

The number of particles per unit volume, N_p, can be allied to the concentrations of emulsifier and initiator. The Smith-Ewart kinetics require that

$$\begin{aligned} R_p &\alpha N_p [I]^{0.4}, [E]^{0.6} \\ N_p &\alpha [I]^{0.4}, [E]^{0.6} \\ \overline{x}_n &\alpha N, [E]^{0.6}, [I]^{-0.6} \end{aligned} \qquad (2.33)$$

where [E] is the soap or emulsifier concentration and [I] is the initiator concentration.

An increase in emulsifier concentration increase N_p and, therefore, increases R_p and $\overline{x}_n$ (Fig. 2.19). High molecular weights are possible in emulsion polymerization because initiation is in one phase, water, and termination is in another phase, monomer-polymer. Because of high molecular weight with high rate, this method is popular. Seeded polymerizations can be useful for produce large-particle-size latexes. A completed "seed" latex is diluted to give the desirable value of N_p particles per liter of emulsion. No additional emulsifier is added, so no new particles are formed. Monomer is fed in and initiator is added. Polymerization occurs in the prior formed particles, so that each one grows as monomer

diffuses to it and is converted. When the seed monomer and added monomer are different, graft copolymers may be formed provided the dead polymer in the seed can react by means of residual unsaturation or chain transfer.

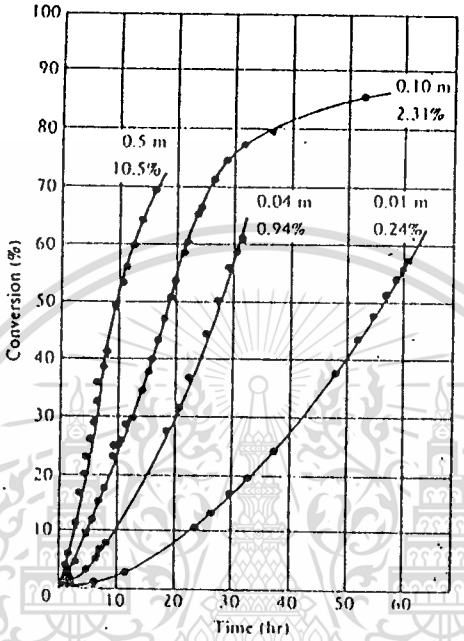


Fig. 2.19 Polymerization of Isoprene at 50°C for Four Concentrations of Emulsifier (Potassium Laurate) [30].

The result of emulsion polymerization in latex form may be the desired form for the purposed end use. Some adhesives and paints can directly use latexes. However, if massive polymer is desired, recovery may involve coagulation by heating, freezing, salt or acid addition, spray drying, or mechanical turbulence. Emulsifiers, coagulants, and initiator fragments often remain as impurities in the final product under these circumstance [30].

CHAPTER 3

EXPERIMENTAL

3.1 MATERIALS

The tapioca starches, which are recommended varieties : Rayong 1 (R1), Rayong 3 (R3), Rayong 5 (R5), Rayong 60 (R60), Rayong 90 (R90), Kasetsart 50 (KU 50), Sriracha 1 (SR), were given from W. Oui Pathipanawat, Department of Agriculture, Ministry of Agriculture and Cooperatives. The cassava tubers were harvested from Rayong Field Crops Research Centre, Rayong Province located in the eastern part of Thailand. The starch preparation was procedured as follows: The cassava tubers were cleaned with water, peeled and chopped into pieces. Starches were extracted by water and filtered with cheese cloth to separate waste fibrous material and starch milk. Starches were recovered by sedimentation of the solid starch granules and then discarded supernatant. The sedimented starch granules were dried by hot-air oven at 50-60 $^{\circ}$C overnight. The starch agglomerated were ground to fine particles and sieved through the sieve No. 106 (140 mesh).

The commercial tapioca starch, Rose brand was given from Tai Wah Company, Ltd. and Four Star brand was available from market. All monomers were commercial grade and used without inhibitor separation. The other chemicals were AR grade.

3.2 APPARATUS

1. Gel Permeation Chromatography	: GPC 150, Waters
2. Spectrophotometer	: UV -160, Shimadzu
3. Spectronic 21	: Bauch & Lomb
4. Infrared Spectrophotometer	: IR 810, Jasco
5. Differential Scanning Calorimeter	: DSC-50, Shimadzu
6. Particle Size Analyzer	: Mavern 1.1a, Mastersizer X
7. Brabender Viscoamylograph	: PT 100, Brabender OHG Duisburg
8. X-ray Diffractometer	: TW 1710, Philips
9. Scanning Electron Microscope	: JSM-T 220 A, JEOL
10. Kjeldhar Apparatus	: UDK 126A, VELP Scientifica
11. Hot-air Oven	: 1375, Shel-lab
12. Furnace	: CFS 1200, Carbolite Furnaces
13. Centrifuge	: ZK 380, Hermle
14. Autoclave	: HA-3D, Hirayama
15. Freeze dryer	: F1, Eyela

3.3 PART I : TAPIOCA STARCH CHARACTERIZATION

3.3.1 Chemical Properties Analysis

3.3.1.1 Moisture Content : Moisture content in starches were determined by oven drying at 40 °C for 1 hr. and then for 4 hr. at 120 °C [29].

3.3.1.2 Ash Content : Ash contents were determined according to A.O.A.C. methods [5]. Accurately weighed ca. 3 g. of starch in porcelain crucible was ignited in furnace at 550 °C until light gray ash results. Ash contents were reported on dry-starch basis (dsb).

3.3.1.3 Protein Content : Protein contents were determined by Kjeldhar method. The procedure was as follows :

Starch samples were weighed about 1.0 g. into the digestion tubes. Added 10.0 g. of catalyst mixture (100.0 g. of sodium sulfate and 7.0 g. of cupper sulfate) and 20 ml. of concentrated sulfuric acid (sp.gr. 1.84). Heated in the digestion apparatus at 280 °C for 15 min. and then to raise the temperature to 380 °C for 60 min. or until the solution became clear.

Added approximately 40 ml. of distilled water to the digestion tube. The receiving flask should contained 50 ml. of 4% boric acid solution a ten drops of methyl red-bromocresol green indicator (20 ml. of 0.1 % bromocresol green and 4 ml. of 0.1 % methyl red in 95% ethanol). The delivery tube of the distillation apparatus should be below the surface of the boric acid solution.

Added approximately 100 ml. of 35% sodium hydroxide to make the mixture basic and distilled the ammonia into the boric acid solution in receiving flask, collected at least 200

ml. of distillate by using automatic Kjeldhar apparatus (UDK 126A, VELP Scientifica). Titrated the distillate with 0.01N hydrochloric acid.

The percentage nitrogen in sample was calculated from the formula as follow:

$$\%N = \frac{\text{(ml. acid x normality acid) x 1.4}}{\text{g. sample}} \quad (3.1)$$

The weight of nitrogen in samples were converted to protein by multiplied by 6.25. The results were reported on dry-starch basis [7].

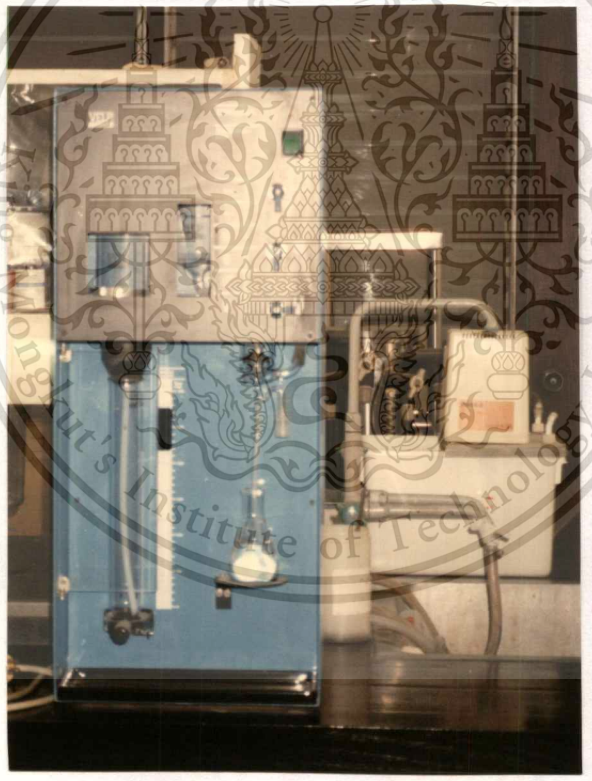


Fig. 3.1 Stream Distillation Unit

3.3.1.4 Lipid Content : Total lipids in starches were estimated by combinating two procedures. The partial hydrolysis of starch and precipitation of the free fatty substances and the solvent extraction of lipids [34].

Starch sample (10 g.) was suspended in 40 ml. of distilled water. Boiling 4 N hydrochloric acid (120 ml.) was added to starch suspension. Acidified starch sample was heated to boiling and boiled for about 5 min. All precipitate was recovered by gravity filtration through Whatman No. 1 filter paper and washed residue with distilled water at room temperature until the filtrate was neutral to methyl orange indicator. Adhering fat from inside of beaker was wiped with a clean filter paper and combined with main residue, and then placed on a watch glass and dried for 3 hr. in hot-air oven at 50 °C. The residues were extracted (6 times, 20 ml. each) with a mixture of chloroform and methanol (2:1, v/v). The combined extracts were filtered and evaporated to weighing. Lipid contents were reported on a dry-starch basis.

3.3.1.5 Phosphorus Content : Phosphorus contents were analyzed by using molybdivanado-phosphoric acid spectrophotometric analysis, following the method of Corn Industries Research Foundation [29].

Starch samples were weighed accurately 5.0 g. into porcelain crucible, added 5 ml. of 2 % calcium acetate solution. Placed the crucible on a hot plate and carefully evaporated to dryness, then increased heat and carbonized the sample on the hot plate. The crucible was heated in a furnace at 600-650 °C until the ash was free of carbon (1-2 hr.). After cooling to room temperature, the ash was wet with 7.5 ml. of water. Slowly washed down the sides of the crucible with 2.5 ml. of 29 % nitric acid. Transferred to a 100 ml. volumetric flask, rinsed the crucible with three 10 ml. portions of distilled water. Diluted to volume with distilled water and mixed thoroughly. Pipetted 10 ml. of sample solution into 50 ml. volumetric flask and added 25 ml. of water to another flask to serve as a blank. To each flask added, in order, 5 ml. of 29% nitric acid, 5 ml. of 0.25% ammonium vanadate, and 5 ml. of 5% ammonium molybdate, mixed thoroughly after addition of each reagent. Diluted to volume with distilled water, mixed thoroughly and allowed to stand for 10 min. Phosphorus content of samples were determined by spectrophotometer (UV-160 spectrophotometer, Shimadzu) at 460 nm, using the blank as a reference solution, and read mg. of phosphorus in the aliquot from the standard

curve which prepared from standard phosphorus solution with concentration between 0.2-2.0 mg. of phosphorus contents.

3.3.1.6 Amylose Content : The method of amylose determination is based on the absorption of iodine by amylose to produce a blue color.

A. Reagents

1. 1N Sodium hydroxide
2. 1N and 0.1N Hydrochloric acid
3. Iodine solution, 0.2% in 2% KI

B. Procedure

1. Stock standard solution : Amylose standard (No. A-9262 Type II, Sigma) were weighed acculately 100 mg. and dissoved in 50 ml. of 1N sodium hydroxide by shaking, diluted to approximately 50 ml. with distilled water, added approximately three fourths the calculated amount of 1N HCl required to neutralize the solution, and made up to 100 ml. and stored in refrigerator.

2. Stock sample solution : Defatted starch sample was weighed exactly 100 mg. into a 100 ml. volumetric flask. Added 1 ml. methyl alcohol, mixed throughly, and then 10 ml. 1N sodium hydroxide was added, left overnight. There should be no lumps or clots ofter this period and then diluted to 100 ml. with distilled water and left overnight to dissove completely.

3. Sample solution : Stock sample solution was pipetted 5 ml. into a 100 ml. volumetric flask. Added 3 drops of phenolphthalin indicator solution and 50 ml. distilled water. Added the 0.1 N hydrochloric acid in drops and shook until the pink color disappeared. Added 2 ml. iodine solution, shook, and diluted to 100 ml.

4. Stand amylose solution : Pipetted the stock amylose standard solution 1 ml. and treated as sample solution preparation.

Finally, read absorption at 630 nm. using a standard amylose solution, sample and iodine blank. Amylose content was calculated as following :

$$\text{Amylose content} = \frac{\text{Unknown reading}}{\text{Standard reading}} \times \frac{\text{mg. dry amylose}}{5 \times \text{dry solid in sample}} \times 100 \quad (3.2)$$

3.3.2 Physical Properties Analysis

3.3.2.1 Percent Transmittance (%T) : The percent transmittance of starch solutions were determined according to Wang and White (1994). The starch solutions (1%, w/v in water on a dry-starch basis) were heated in a boiling water bath and stirred for 30 min. After temperature equilibration at room temperature, the %T at 650 nm was measured using the spectronic 21 [34].

3.3.2.2 Thermal Properties : The thermal properties were measured by using DSC-50 differential scanning calorimeter (Shimadzu, Japan) according to Jane, et al. (1992). Starch sample (about 2 mg.,dsb) was weighed in the aluminium sample pan, mixed with distilled water (about 8 µl), and sealed. The sealed pans were allowed to stand from 1 to 3 hr at room temperature before heating. The reference material was 8 µl distilled water. The samples were heated from 30 to 120 $^{\circ}$C at a heating rate of 10 $^{\circ}$C/min. The enthalpies were calculated on a dry-starch basis [21].

3.3.2.3 Scanning Electron Microscope (SEM) : The starch samples were examined with a JEOL scanning electron microscope (SEM), model JSM-T220A (JEOL Ltd., Japan). Starch samples were sprinkled on adhesive tapes, attached to specimen studs, and coated with gold-palladium. Representative micrographs were taken of each starch type at magnification of 1,000x.

Fig. 3.2 DSC-50 Differential Scanning Calorimeter

3.3.2.4 The Size and Distribution of Starch Granules : The average of starch granules size and size distribution were studied by particle size analyzer, model MaterSizer X (Malvern Instruments Ltd., U.K.) and calculated with MAVERN Ver. 1.1a. The starch granules were suspended in methanol (3 times distillation and filtered though 0.45 μm nylon filter).

Fig. 3.3 The Particle Size Analyzer, Model MasterSizer X.

3.3.2.5 X-ray Diffraction : The starch samples were prepared according to Wootton and Mahdar (1993). The starch sample were allowed to reach equilibrium moisture content by storing over saturated sodium chloride for three days before measuring with X-ray diffractometer. The X-ray diffraction patterns were obtained with an X-ray diffractometer (Philips model TW 1710) which generated Cu-K_α radiation. Operating condition was at 40 KV, 30 mA, and scanning region 4°-60° of 2θ [38].

3.3.2.6 Pasting Properties : Pasting characteristic of starch suspensions (6%, w/w, dsb), were measured using the Brabender Viscoamylograph (BRABENDER OHG Duisburg, model PT 100), and operating at a bowl speed of 75 rpm. The temperature was raised from 30°C to 95°C at rate of 1.5 $^\circ$C/min, maintained at 95 $^\circ$C for 30 min., and decreased to 50 $^\circ$C at the same rate.

3.3.3 The Structure and Molecular Weight Determination

3.3.3.1 The Structure and Molecular Weight Determination of Starch : The starch sample were defatted by extraction with 85% ethanol in Soxhlet extractor for 24 hr. Sample preparation was prepared as described by Jackson (1991). The defatted starches (100 mg.) were solubilized in 90% aqueous dimethyl sulfoxide (10 ml.) by heating the dispersion at 96 $^\circ$C with continuous stirred for 24 hr. in a water bath with magnetic stirrer. The solubilized starches were subsequently centrifuged at 3,000xg for 10 min. and filtered through a 0.8-8.0 μm glass filtered (millipore) before chromatographic analysis.

3.3.3.2 Fractionations of Starch : Fractionations of starch were prepared by following the procedure of Jane and Chen (1992), which modified from Schoch (1942). Defatted starch suspension (1.33%, w/v, in water) was heated in a water bath at 96 $^\circ$C until starch gelatinization. The pH of the starch solutions were adjusted to 5.9-6.3 with 1.0 M phosphate buffered and autoclaved at 121 $^\circ$C for 3 hr.

The starch solution was heated at 96 °C with continuous stirring for 2 hr. to disperse starch molecules. Added 20% by volume of *n*-butanol and then the solution was heated with stirring at 96 °C for 1 hr. The mixture was transferred to a prewarmed dewar flask, sealed, and allowed to cool down to room temperature over 24-36 hr. An amylose-butyl-alcohol complex was formed during cool.

The solution was centrifuged (5 °C, 10,000 rpm., 30 min.) for separated the precipitate, which was amylose-butyl-alcohol complex, and the supernatant which remained the amylopectin. The precipitate, amylose fraction, was further purified by two recrystallizations and was dried with freeze dryer overnight. The supernatant was concentrated with a rotor evaporator and then treated twice with *n*-butanol to remove amylose residues. The procedure of amylose and amylopectin sample preparation for injection was carried out by following the procedure of starch solubilization as described above [24].

3.3.3.3 Debranching of Starch : Debranched starch were prepared according to Wang and White (1994). Defatted starch (50 mg.) was suspended in 9 ml. of distilled water and heated with stirring in a boiling water for 1 hr. After cooling, 1 ml. of 0.1 M acetate buffer (pH 3.5) and ~ 1200 U of *Pseudomonas* isoamylase (EC. 3.2.1.68, Sigma Chemical Co., St. Louis, MO) were added. The mixture was incubated at 40 °C in a water bath with slight stirring for 48 hr. to complete the debranching reaction. The digested starch sample was heated to inactivate the enzyme and evaporated from 10 to 1 ml. After inactivating, the digested starch sample was pipetted 0.5 ml. and 4.5 ml. of dimethyl sulfoxide (DMSO) was added to make a 90% DMSO solution. The 90% DMSO solution was heated and stirred in boiling water for 1 hr. and then for another 24 hr. at room temperature and filtration through a 0.8-8.0 μm glass filter before injection [35].

3.3.3.4 Gel Permeation Chromatography Analysis : All the samples (amylose, amylopectin, starch and debranched starch sample) were prepared by solubilizing in 90% aqueous DMSO and filtrating through 0.8-8.0 μm glass filter before injection. The instrument was GPC-150C Gel Permeation Chromatography with a refractive index detector (Waters,

Milford, MA and results were recorded and calculated by millinium software version 2.0. A guard column, ultrahydrogel 120 (7.8x300 mm), and ultrahydrogel 250 (7.8x300 mm.) were connected in series, respectively. The mobile phase was deionized water with filtered through a 0.45 μm cellulose acetate filter and degassed by boiling for 15 min. and gentle heating with hot plate while the system was running with flow rate 0.4 ml./min. The column, injection, and solvent and pump compartments were maintained at 45°C, 45°C, and 35 °C, respectively.

A calibration curve for the chromatograms was constructed by using pullulan standards (Shodex P-82 standard, Waters, milford, MA).

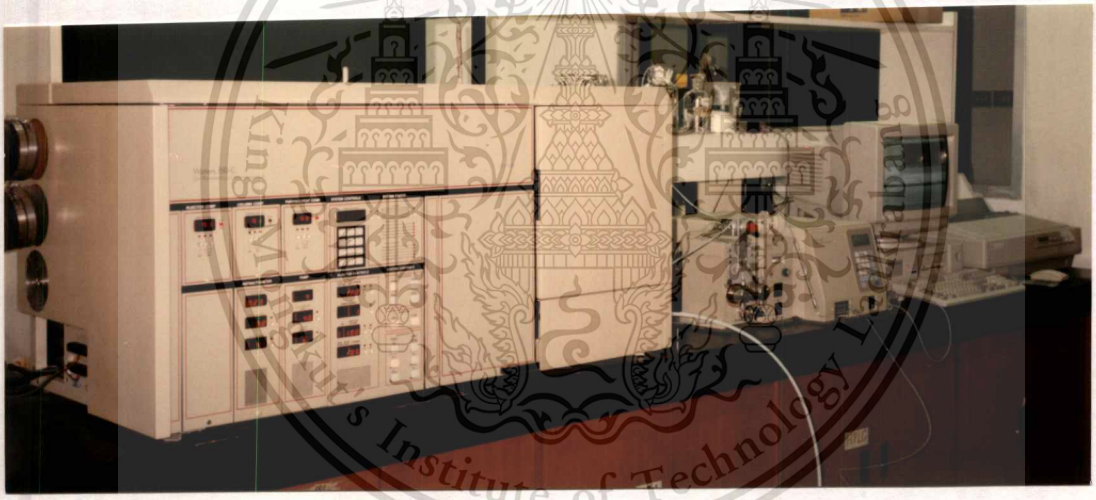


Fig. 3.4 GPC 150C Gel Permeation Chromatrograpy

3.4 PART II : DERIVATIVES CHARACTERIZATION

3.4.1 Starch Graft Copolymerization and Purification

3.4.1.1 Graft Copolymerization : In graft copolymerization reaction, starch (150 g.) was stirred in water (1800 ml.) in 2 liter four-necked flask glass reactor under nitrogen atmosphere for 1 hr. at 90°C. After cooling down to 60 °C, NP-40 (10% by weight of

monomer) was added and stirred continuously until starch slurries became homogeneous, followed by the required amount of monomers. The mixture was stirred for 10 min. and then initiator solution was added. After the mixture had stirred for 3 hr. at 60 °C, 1 N NaOH was added to neutralize the solution. The graft copolymer and ungrafted polymer products were precipitated with excess methanol and isolated by filtration, washed with water and methanol. The products were dried with hot air oven at 50-55 °C until constant weight.

3.4.1.2 Ungrafted Polymer Separation : Ungrafted polymers were extracted with Soxhlet's extraction (Fig. 3.5). The dried crude product was weighted accurately 2 g. and extracted in Soxhlet's extractor with acetone for 72 hr. The ungrafted polymers were solubilized in acetone-extracted solvent in round bottom flask, and then acetone was evaporated. The weight of this fraction was used to calculate the weight of ungrafted polymers in the starch graft copolymer.

3.4.1.3 Side Chain Grafted Polymer Separation : The side chain grafted polymers were separated from starch graft copolymer by hydrolysis of starch. The purified starch graft copolymer, insoluble from acetone extraction, was weighed accurately 1 g. and added to 100 ml. of glacial acetic acid. The mixture was heated and stirred at 90-100 °C for 1 hr., followed by adding dropwise 2 ml. of 60% perchloric acid within 1-2 min. The mixture became clear and then poured into cooling water immediately. The side chain grafted polymers were precipitated, and isolated by filtration, washed with water. The side chain grafted polymers were dried at 60 °C to constant weight. These fractions were used to analyze by IR spectrophotometer for checking the separation.

Fig. 3.5 The Soxhlet's extraction equipment.

3.4.2 Starch Graft Copolymers Characterization

3.4.2.1 Molecular Weight Determination : The sample preparation for molecular weight determination were followed: side chain grafted polymers and ungrafted polymers (about 15 mg.) were stirred with 10 ml. of THF for 3 days at room temperature and separated insoluble material by centrifugation. The supernatant was filtered through a 0.45 μm nylon filter before injection. The instrument was Gel Permeation Chromatography, model GPC 150C (Waters, Milford, MA), with RI detector and autosampler installation. The linear Styrogel column (MW range from $2x10^3$ to $4 x10^6$) was calibrated with polystyrene standards. The mobile phase was HPLC-grade tetrahydrofuran (THF) with flow rate 1 ml./ min. The injection and column compartments were carried out at 35 $^{\circ}$C. The molecular weight of samples were calculated by MAXIMA 820 GPC Analysis program.

3.4.2.2 Gravimetric Characterization : Graft polymerization parameters were calculated as following:

$$\text{percent grafting (G)} = \frac{W_1 - W_0 \times 100}{W_1} \quad (3.2)$$

$$\text{percent grafting efficiency (GE)} = \frac{W_3 \times 100}{W_2 + W_3} \quad (3.3)$$

$$\text{grafting frequency (GF)} = \frac{W_0 / 162}{W_3 / MW} \quad (3.4)$$

where W_0 = starch weight

W_1 = starch graft copolymer

W_2 = ungrafted polymer

W_3 = side chain grafted polymer

MW = molecular weight of side chain grafted polymer

3.4.2.3 Total Solid Content and Conversion : The procedure for determination of the total solid content of starch graft copolymer was followed by weighing 2 g. of the resulting product (after complete polymerization) into small tray and drying with hot-air oven at 110°C for 2 hr. After cooling down to room temperature in desiccator, the sample tray was weighed accurately and repeated procedure until the sample tray was constant weight.

The percentage of total solid content (TSC) and percentage of conversion (%C) were calculated as the formulae :

$$\text{TSC (\%)} = \frac{C - A \times 100}{B - A} \quad (3.5)$$

A = weight of tray (g)

B = weight of tray with sample before drying (g)

C = weight of tray with sample after drying (g)

$$\%C = \frac{X}{Y} \times 100 \quad (3.6)$$

X = total solid content from experiment

Y = total solid content from the calculation

3.4.2.4 IR Spectrophotometer : For checking the purity of products after separation by Soxhlet's extraction and hydrolysis of starch, tapioca starch, starch graft copolymer, side chain grafted polymer, and ungrafted polymer were measured by infrared spectrophotometer (IR 810 spectrophotometer, Jasco). Sample preparation was prepared by KBr disc technique.

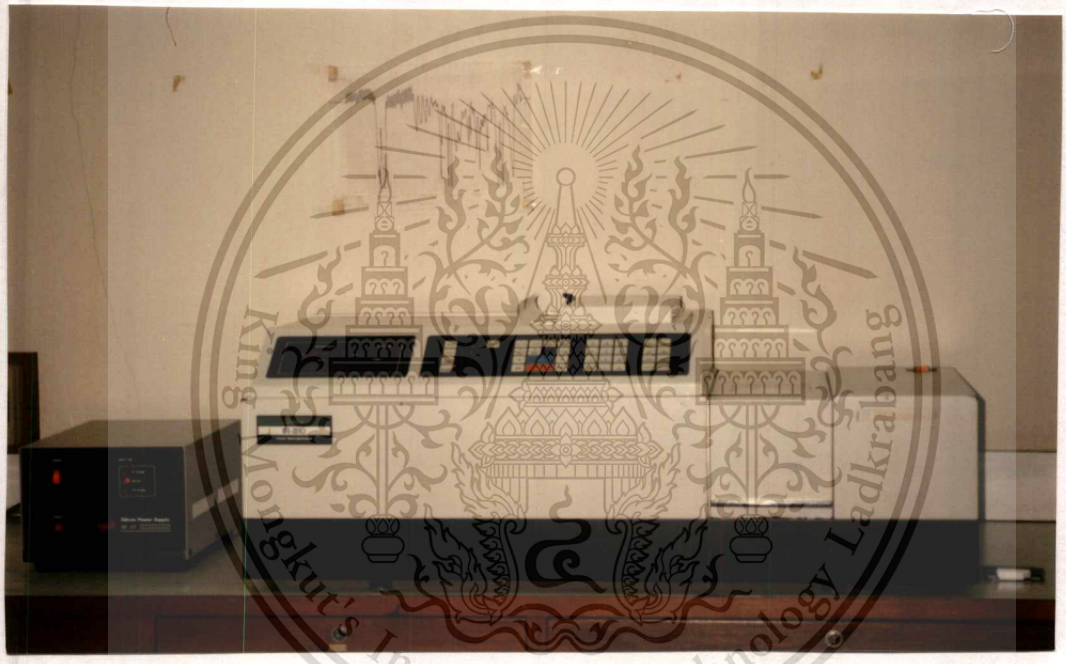


Fig. 3.6 IR 810 Spectrophotometer

3.4.2.5 Thermal Properties Determination : The glass transition temperature (T_g) of starch graft copolymer was determined with differential scanning calorimeter (DSC 50, Shimadzu, Japan). The purified starch graft copolymer was ground by vibration grinder in liquid nitrogen. The fined powder of purified starch graft copolymer was weighed approximately 10 mg. into aluminium pan and sealed. An empty aluminium pan was used as reference pan. The sample pan was run from 30 °C to 250 °C at heating rate 10 °C/min.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 PART I : TAPIOCA STARCH CHARACTERIZATION

To this research, the tapioca starch samples were divided into two groups following their preparations. All recomemded-variety tapioca starch samples were prepared following the procedure of laboratory (describes in Chapter 3) and both comercial tapioca starch samples were prepared following the procedure of industrial manufacture.

4.1.1 Chemical Properties of Tapioca Starches

Results of minor chemical compositions showing in Table 4.1 indicated that the preparation effected to chemical compositions. The percentage of moisture contents of commercial tapioca starches (10.93 and 12.24%) were higher than all recommended-variety tapioca starches (7.02-9.12%). This may be the effect of drying : the laboratory process used hot-air oven for drying ; the industrial process used cyclone pulp drying.

The phosphorus contents also indicated the difference between two groups of samples. The phosphorus contents of commercial tapioca starches (0.007 %) were less than all tapioca starch from recommended varieties (0.014-0.017 %). This suggested that the purified stage by sulfuric acid and centrifugation, only industrial manufacture, might reduce phosphorus content in commercial tapioca starch samples.

Ash contents of recommended-variety tapioca starches were 0.220-0.277% and commercial tapioca starches were 0.263 and 0.223%. Sriracha 1 was the higest and Rayong 60 was the lowest ash content among tapioca starches from recommended-variety group.

Crude fat contents of recommended-variety tapioca starches were 0.019-0.104% and commercial tapioca starches were 0.036 and 0.041%. Rayong 60 was the lowest and Rayong 5 was the highest crude fat content among the recommended-variety starches.

Crude protein contents of recommended-variety tapioca starches were 0.097-0.190% and commercial tapioca starches were 0.048 and 0.152% as showed in Table 4.1. Rayong 1 was the lowest and Rayong 3 was the highest crude protein content among the recommended varieties tested.

As above, three minor chemical compositions contained in commercial tapioca starches, which were ash, crude protein and crude fat, were in range obtained from recommended-variety tapioca starches. These results indicated that starch preparation did not effect to these minor chemical compositions.

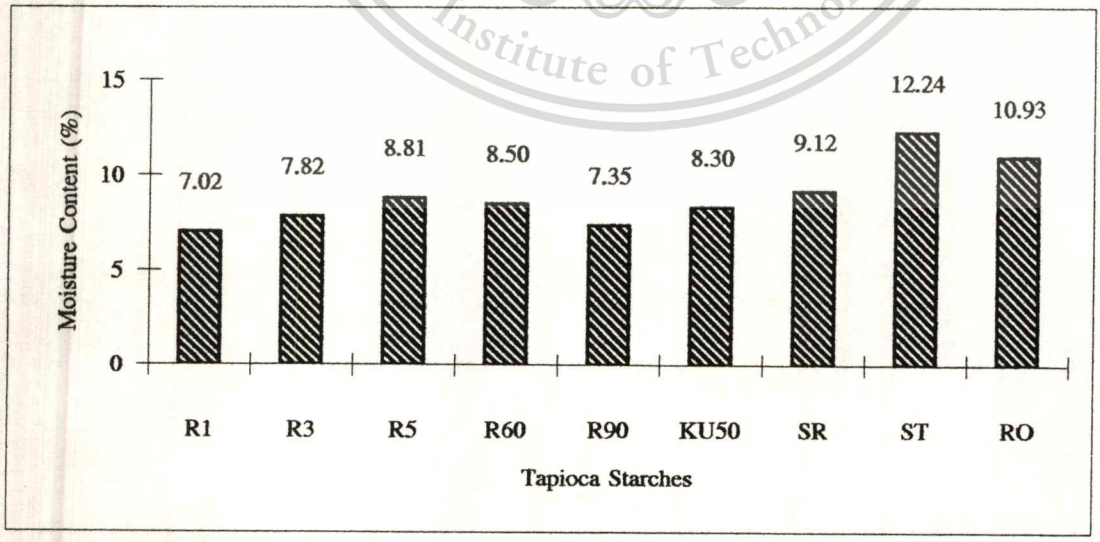


Fig. 4.1 Moisture Contents of Tapioca Starches

Table 4.1 Amylose Content and Minor Components of Tapioca Starches

Sample	Moisture[C] (%)	Ash[C] (%)	Phosphorus[C] (%)	Crude Fat[C] (%)	Crude Protein[C] (%)	Amylose Content[C] (%)
R1[A]	7.02 ±0.24	0.240 ± 0.014	0.016 ± 0.0003	0.057 ± 0.002	0.097 ± 0.003	15.09 ± 0.42
R3[A]	7.82 ± 0.11	0.273 ± 0.005	0.016 ± 0.0003	0.044 ± 0.005	0.190 ± 0.008	14.85 ± 0.17
R5[A]	8.81 ± 0.29	0.270 ± 0.014	0.016 ± 0.0001	0.104 ± 0.013	0.146 ± 0.009	13.97 ± 0.35
R60[A]	8.50 ± 0.29	0.277 ± 0.005	0.015 ± 0.0004	0.019 ± 0.003	0.136 ± 0.002	16.74 ± 1.73
R90[A]	7.35 ± 0.18	0.253 ± 0.005	0.014 ± 0.0002	0.044 ± 0.005	0.120 ± 0.006	13.12 ± 0.19
KU50[A]	8.30 ± 0.58	0.220 ± 0.000	0.017 ± 0.0002	0.097 ± 0.001	0.133 ± 0.012	12.66 ± 0.14
SR[A]	9.12 ± 0.17	0.223 ± 0.012	0.016 ± 0.0002	0.050 ± 0.004	0.124 ± 0.005	13.44 ± 0.19
ST[B]	12.24 ± 0.67	0.263 ± 0.009	0.007 ± 0.0002	0.036 ± 0.003	0.048 ± 0.002	13.42 ± 0.54
RO[B]	10.93 ± 0.66	0.220 ± 0.008	0.007 ± 0.0004	0.041 ± 0.009	0.152 ± 0.010	13.97 ± 0.34

[A] recommended-variety tapioca starch, [B] commercial tapioca starch , [C] All results were culculated on dry-starch basis (dsb).

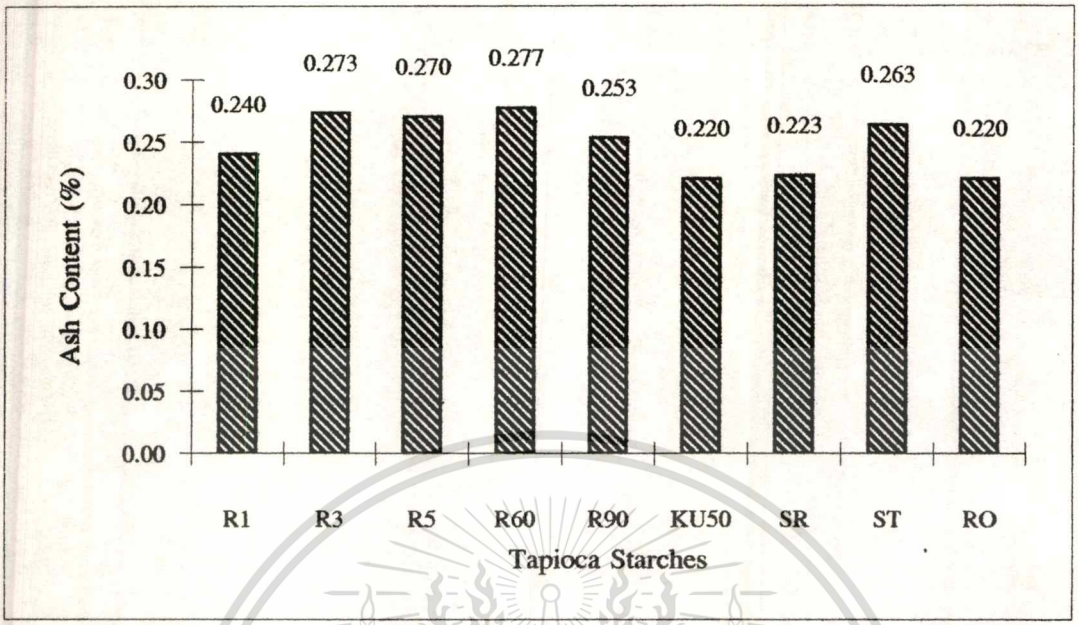


Fig. 4.2 Ash Contents of Tapioca Starches

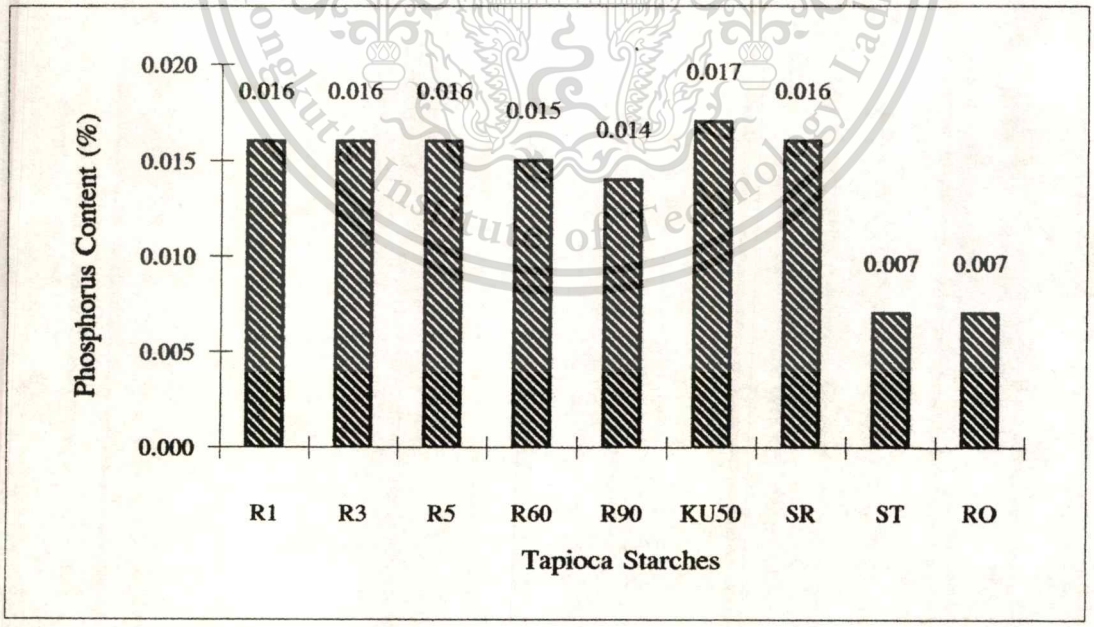


Fig. 4.3 Phosphorus Contents of Tapioca Starches

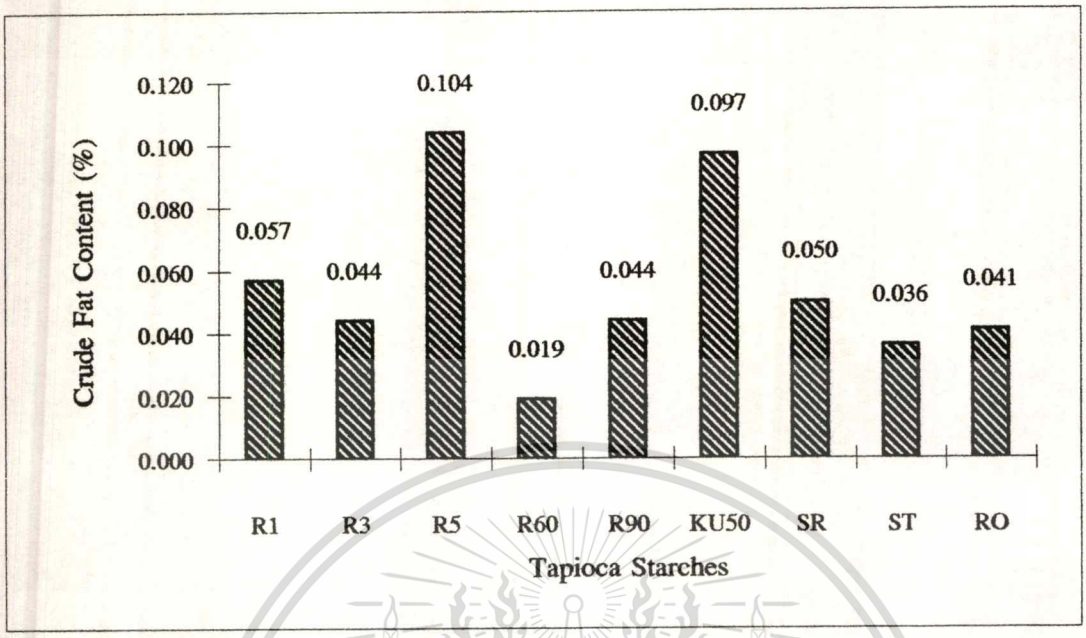


Fig. 4.4 Crude Fat Contents of Tapioca Starches

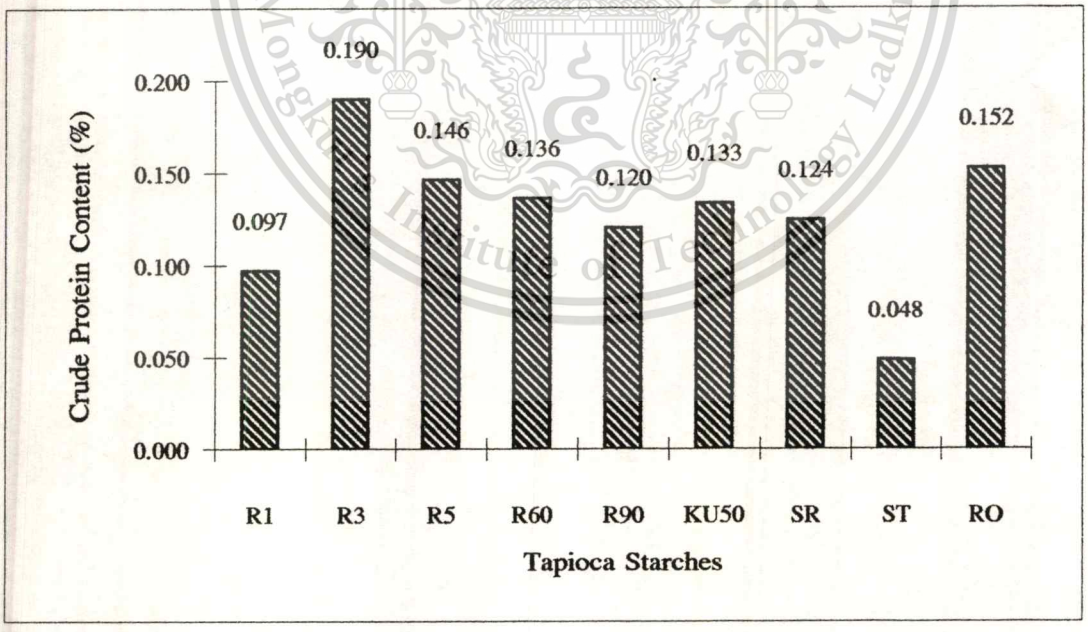


Fig. 4.5 Crude Protein Contents of Tapioca Starches

To recommended-variety tapioca starches, the minor chemical compositions, which were moisture contents (7.02-9.12%), ash contents (0.220-0.277%), phosphorus contents (0.014-0.017%), crude fat contents (0.019-0.104%), and crude protein contents (0.097-0.190%), fell within the range of values obtained by other researchers (<13%, 0.02-0.33%, 0.008-0.040%, 0.08-1.54%, 0.03-0.60%, respectively) [6]. Similarly, the minor chemical compositions contained in commercial tapioca starches (Table 4.1) also fell within the range of values obtained by other researchers.

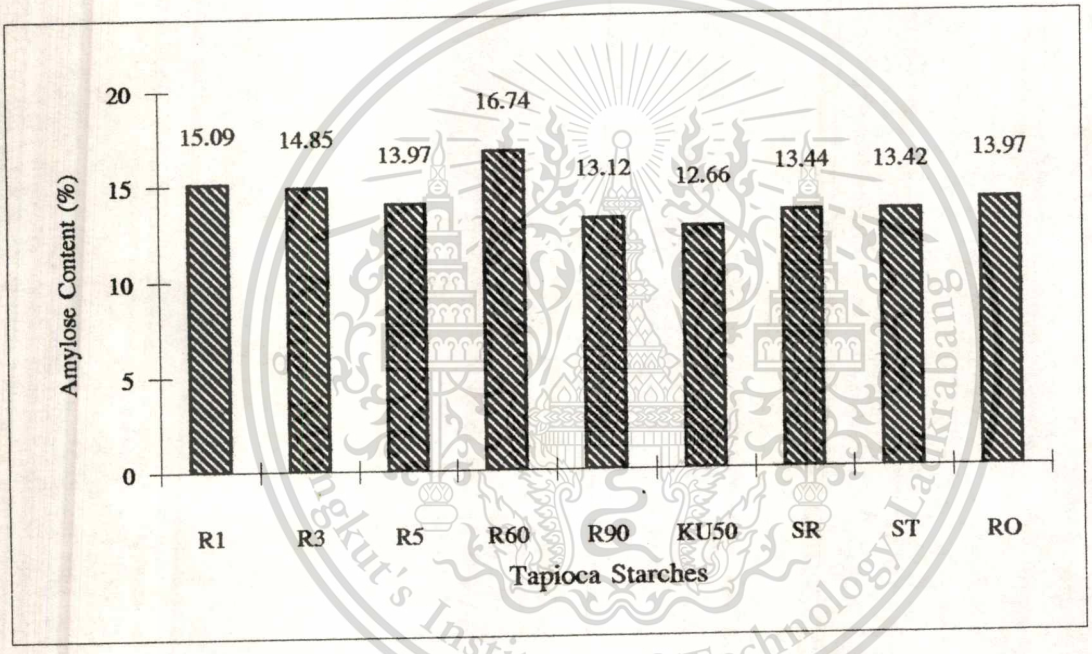


Fig. 4.6 Amylose Contents of Tapioca Starches

Amylose contents of recommended-variety tapioca starches (Table 4.1) were 12.66-16.74% and commercial tapioca starches were 13.42 and 13.97%. Kasetsart50 starch was the lowest amylose content among the recommended-variety tapioca starches tested while Rayong60 starch was the greatest.

4.1.2 Physical Properties of Tapioca Starches

Most scanning electron micrographs of the tapioca starches displayed round with a truncated end (Fig. 4.8-4.16). The average of granular sizes of tapioca starches (Table 4.2) from recommended varieties were 12.64-15.67 μm, which Sriracha1 starch was the lowest while Rayong5 starch was the largest average granular sizes. However, all recommended-variety starches had smaller average granular sizes than commercial tapioca starches. Scanning electron micrographs also showed that commercial tapioca starches were bigger and more agglomerate than recommended variety starches. All X-ray diffraction patterns of tapioca starches showed 'A' pattern.

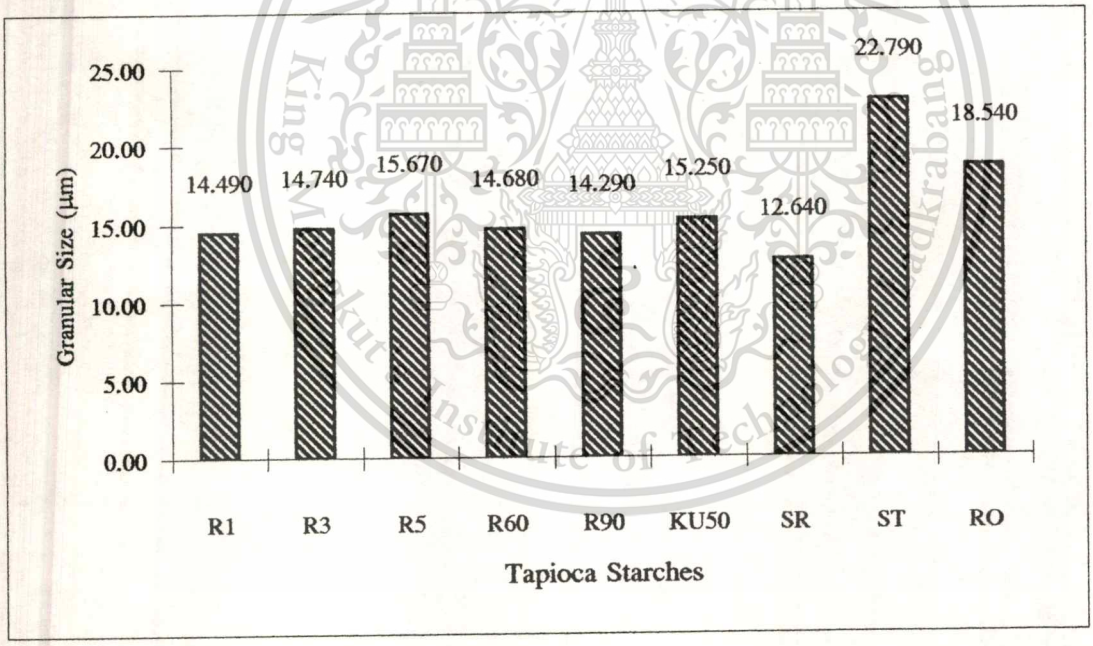


Fig. 4.7 Average Granular Sizes of Tapioca Starches

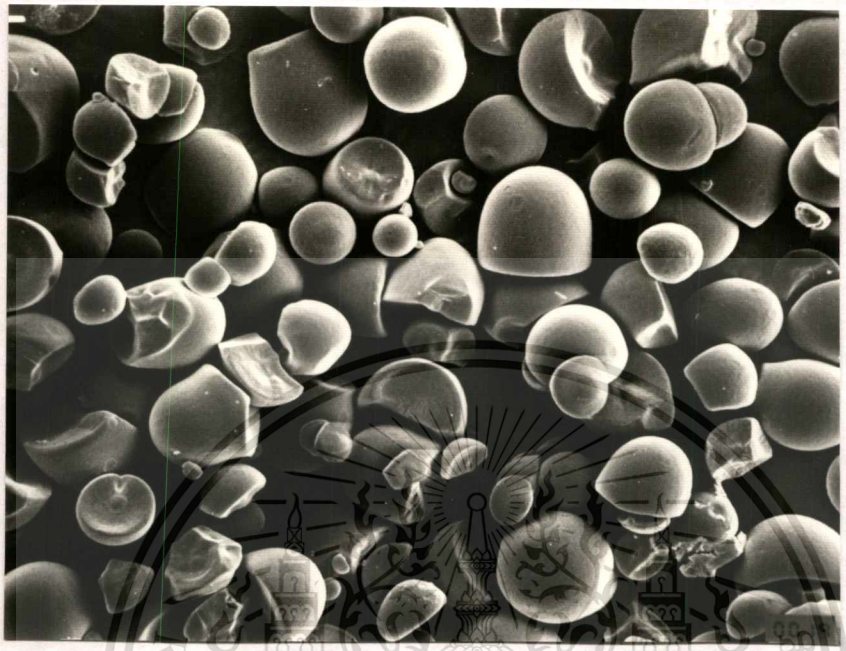


Fig. 4.8 SEM Photo of R1 Starch (Magnification x 1000)

Fig. 4.9 SEM Photo of R3 Starch (Magnification x 1000)

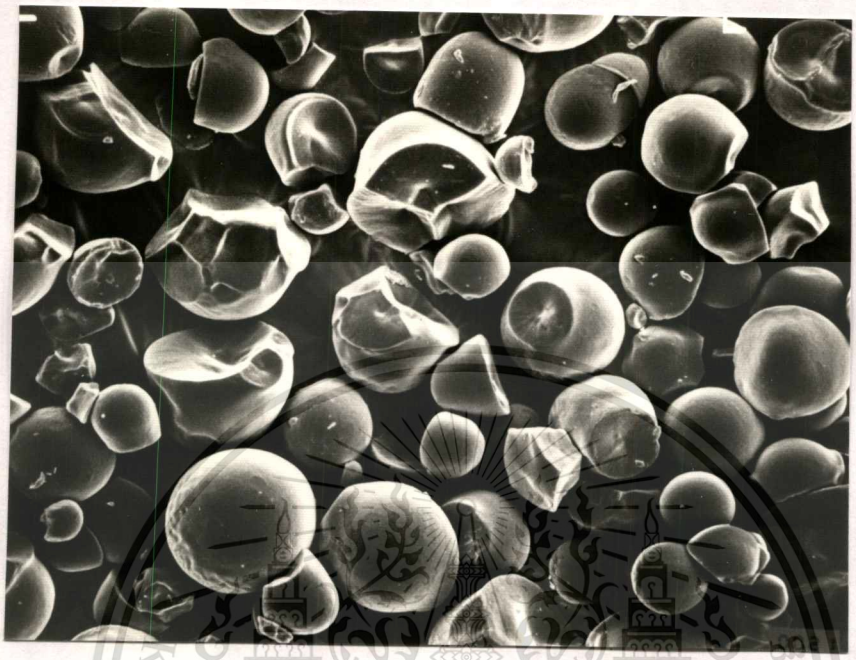


Fig. 4.10 SEM Photo of R5 Starch (Magnification x 1000)

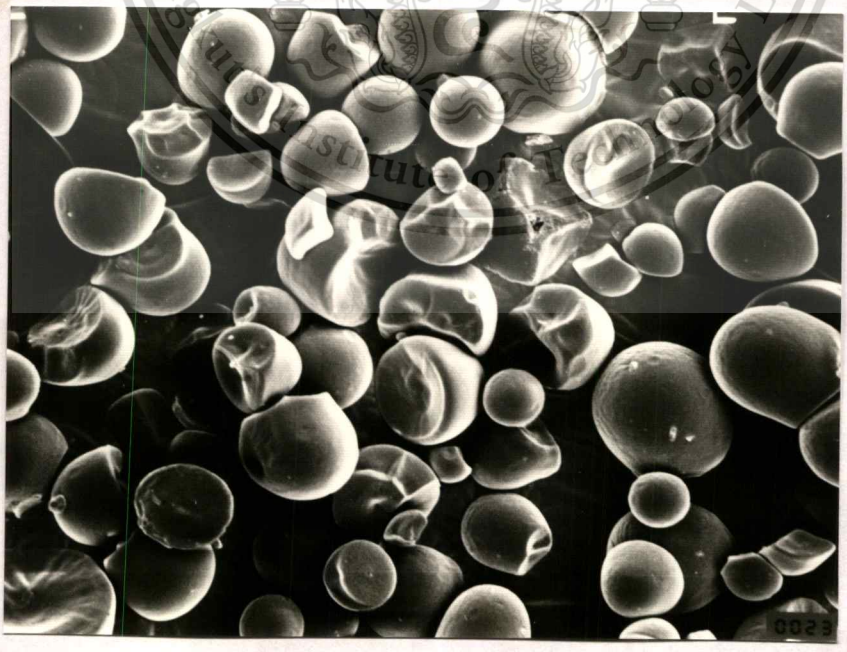


Fig. 4.11 SEM Photo of R60 Starch (Magnification x 1000)

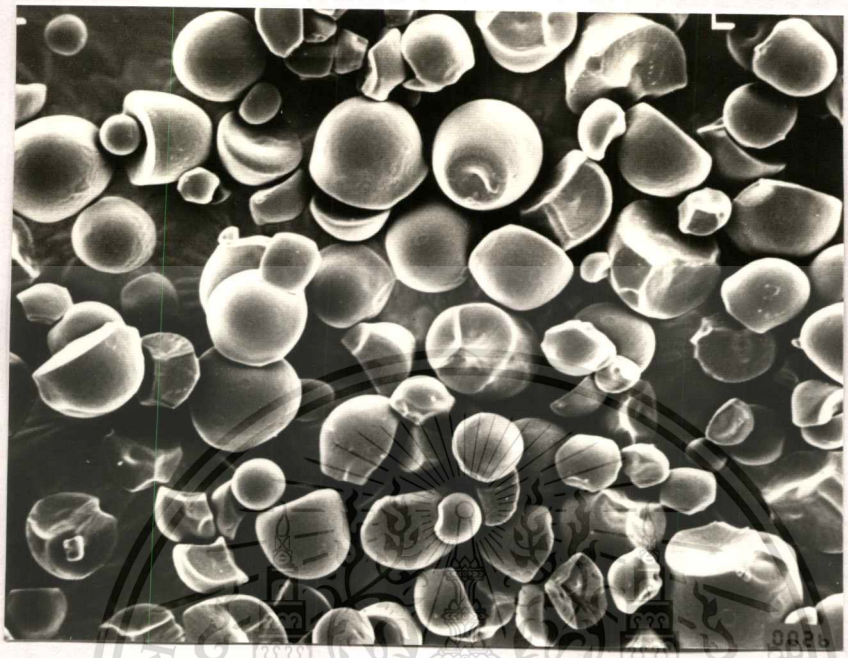


Fig. 4.12 SEM Photo of R90 Starch (Magnification x 1000)

Fig. 4.13 SEM Photo of KU50 Starch (Magnification x 1000)

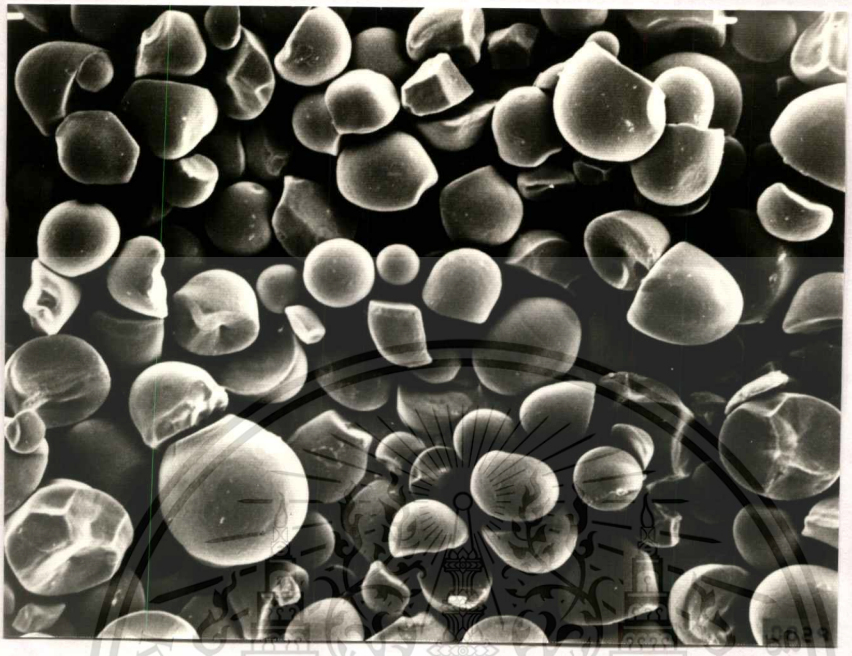


Fig. 4.14 SEM Photo of SR Starch (Magnification x 1000)

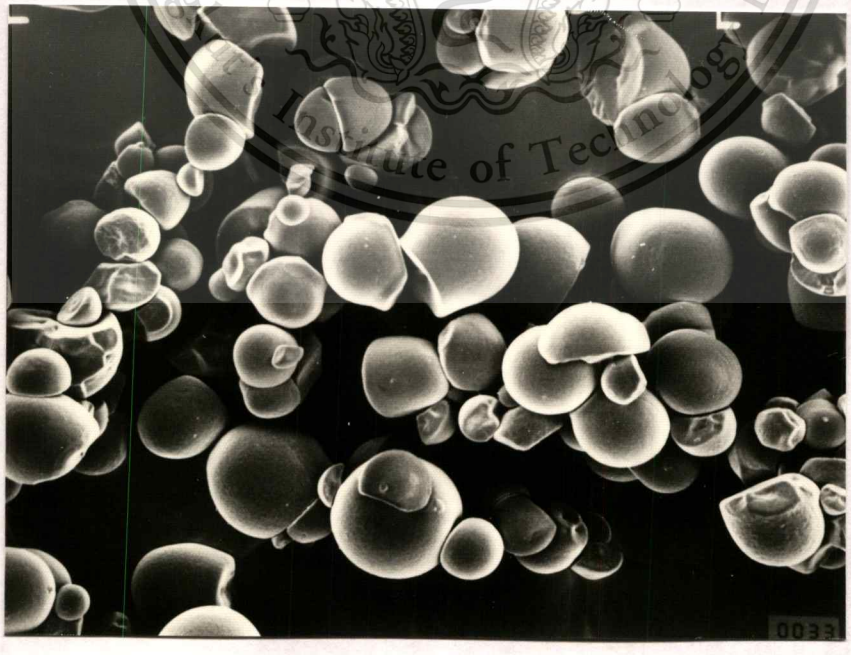


Fig. 4.15 SEM Photo of ST Starch (Magnification x 1000)

Fig. 4.16 SEM Photo of RO Starch (Magnification x 1000)

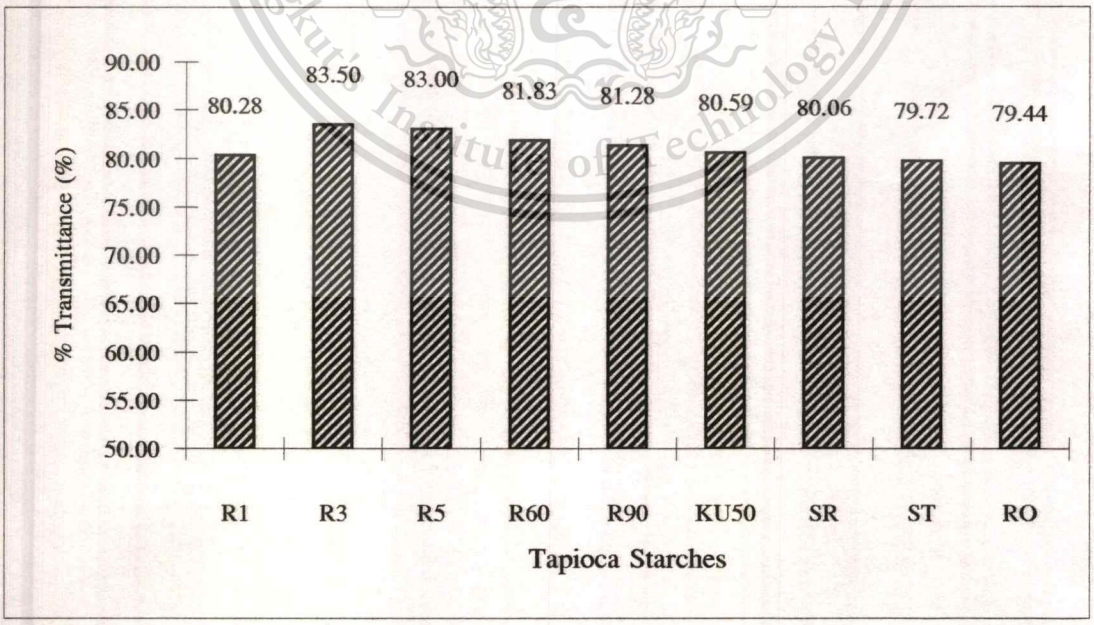


Fig. 4.17 Percent Transmittance of Tapioca Starches

Table 4.2 Physical Properties of Tapioca Starches

Sample	Pasting Properties[C]		%Transmittance[D] (%)	Granular Size (μm)[E]			
	Gelatinization Temperature (°C)	Maximum Viscosity (B.U.)		d(0.1)	d(0.5)	d(0.9)	mode
R1[A]	69.75	315	80.28 ± 0.28	5.49	13.66	25.71	14.49
R3[A]	67.50	480	83.50 ± 0.76	2.82	13.31	22.75	14.74
R5[A]	66.00	460	83.00 ± 1.08	2.72	14.07	23.39	15.67
R60[A]	60.45	390	81.83 ± 0.62	2.41	13.00	22.70	14.68
R90[A]	66.00	495	81.28 ± 1.17	2.43	12.79	22.69	14.29
KU50[A]	68.25	370	80.59 ± 0.59	2.59	13.36	22.60	15.25
SR[A]	69.60	320	80.06 ± 0.91	2.27	11.48	19.93	12.64
ST[B]	71.25	355	79.72 ± 0.80	6.29	19.16	38.42	22.79
RO[B]	64.50	585	79.44 ± 1.23	5.11	17.07	35.54	18.54

[A] recommended -variety tapioca, [B] commercial tapioca starch, [C] 6% starch (dsb.) concentration, [D] 1% starch (dsb.)concentration,

[E] calculated by MasterSizer-X Version 1.1

Percent transmittance of tapioca starches from recommended varieties were 80.06-83.50 %, which Sriracha1 starch was the lowest while Rayong 3 was the higest percent transmittance. Pecent transmittance of tapioca starches from commercial brands were 79.44 and 79.72%. Commercial tapioca starches were lower percent transmittances than recommended-variety tapioca starches. This suggested that granular sizes and agglomerations of commercial tapioca starches were more than all tapioca starches from recommended varieties, so that clarity of starch pastes were less than recommended-vareity tapioca starches.

Pasting properties analyzed by Brabender Viscoamylograph was shown in Table 4.2 and Fig. 4.18. Gelatinization temperature of recommended-variety tapioca starches were 60.45-69.75 °C and maximum viscosity were 315-495 B.U. Each starches had different viscoamylograph patterns. Rayong 60 starch was the lowest gelatinization temperature and Rayong 1 starch was the highest gelatinization temperature but Rayong 1 paste had lowest viscosity and Rayong 90 paste was the highest viscosity among recommended variety tapioca starches. These results were the same as other researchers (58.5-70.0 °C) [8].

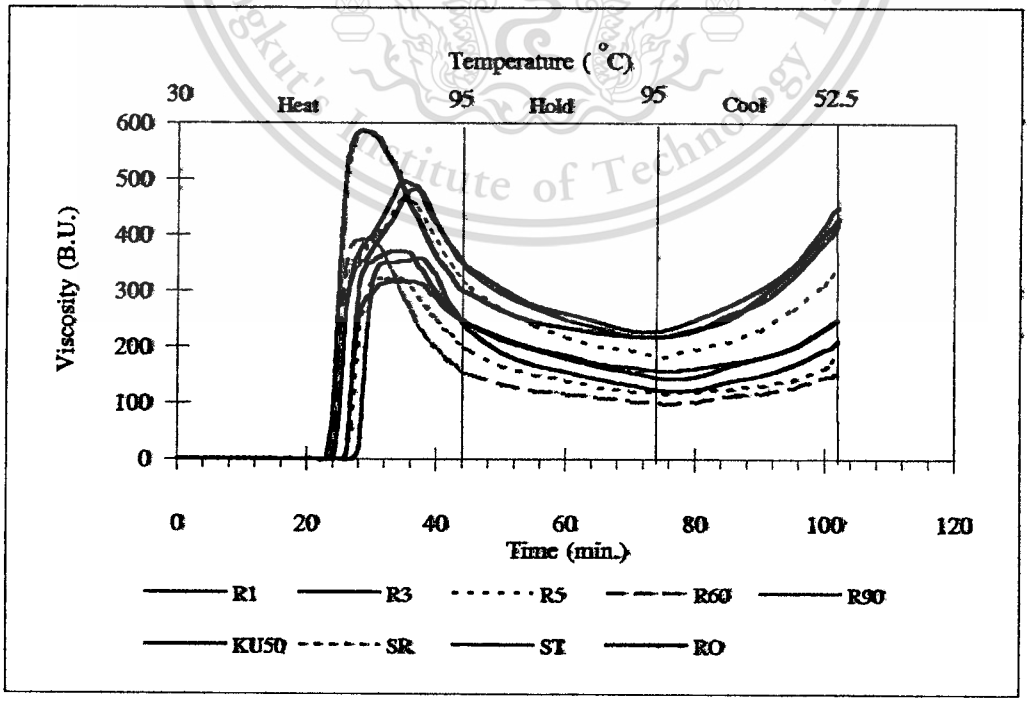


Fig. 4.18 Brabender Viscosity Curve of Tapioca Starches

Table 4.3 Thermal Properties of Tapioca Starches[C]

Sample	T_o(°C)	T_p(°C)	T_c(°C)	ΔH (mJ)
R1[A]	67.23 ± 0.09	73.90 ± 0.29	82.67 ± 0.49	-27.97 ± 1.90
R3[A]	65.45 ± 0.05	70.70 ± 0.00	81.20 ± 1.00	-25.95 ± 1.58
R5[A]	64.83 ± 0.21	70.60 ± 0.28	81.90 ± 1.56	-22.54 ± 1.80
R60[A]	65.20 ± 0.28	70.63 ± 0.09	82.20 ± 0.64	-27.27 ± 2.01
R90[A]	66.65 ± 0.35	73.30 ± 0.00	83.15 ± 0.35	-27.61 ± 0.17
KU50[A]	68.05 ± 0.95	72.65 ± 0.65	83.20 ± 0.70	-34.11 ± 5.33
SR[A]	65.50 ± 0.10	71.20 ± 0.30	84.40 ± 4.00	-30.51 ± 7.21
ST[B]	63.97 ± 1.45	73.87 ± 0.17	83.17 ± 0.58	-31.86 ± 5.22
RO[B]	64.13 ± 0.69	72.30 ± 0.29	83.07 ± 0.31	-25.37 ± 3.33

[A] recommended-variety tapioca starch, [B] commercial tapioca starch,

[C] starch 2 mg. (dsb) : water 8 µl.

Thermal properties (e.g. onset [T_o], peak [T_p], and complete [T_c] gelatinization temperatures and enthalpy changes [ΔH]), indicated by DSC analysis (Table 4.3) were slightly different. There was no significant relation between these values and preparation process. Rayong 5 was the lowest T_o and T_p (64.83 and 70.60 °C, respectively) and Kasetsart50 was the highest T_o and T_c (68.05 and 83.20 °C, respectively) in among of recommended-variety tapioca starches.

4.1.3 Molecular Structure of Tapioca Starches

Molecular structures of starches were characterized by gel permeation chromatography (GPC) and were calculated from the calibration curve generated using pollulan standards. All starches chromatograms (Fig. 4.20-4.27) were separated into 3 peak fractions. Fraction (Fr.) I, II, III were regarded as representing amylopectin, large amylose, and small amylose, respectively. Average molecular weight of Fr.I or amylopectins (Table 4.4) were 2.70-3.40 x10^7, Rayong 1 and Sriracha 1 starches were the lowest and the higest average

molecular weights among the recommended-variety tapioca starches, respectively. Both average molecular weights of amylopectins of commercial tapioca starches were 2.95 x 10^7, which fell within range of amylopectin average molecular weights of recommended-variety tapioca starches. Average molecular weights of Fr.II or large amyloses were 3.50-4.20 x10^6, which Rayong 1 and Rayong 90 starches were the lowest and the highest molecular weights among the recommended-variety tapioca starches, respectively. Average molecular weights of large amyloses of commercial tapioca starches were 3.75-4.00 x10^6, which fell within range of large amylose average molecular weights of recommended-variety tapioca starches. Average molecular weights of Fr.III or small amyloses of recommended-variety tapioca starches (Table4.4) were 0.62-1.45 x 10^6, which Kasetsart 50 and Sriracha 1 starches were the lowest and the highest average molecular weights among the recommended-variety tapioca starches, respectively.

Table 4.4 Average Molecular Weights and Distributions of Components of Tapioca Starches

Sample	Fr. I	Fr. II	Fr. III	Area Ratio Fr. I : Fr. II : Fr. III
R1	2.70 x 10^7	3.50 x 10^6	1.25 x 10^6	58.66 : 19.59 : 21.75
R3	2.95 x 10^7	3.55 x 10^6	1.23 x 10^6	61.15 : 20.74 : 18.10
R5	3.10 x 10^7	3.85 x 10^6	0.70 x 10^6	61.29 : 17.69 : 21.04
R60	3.05 x 10^7	3.85 x 10^6	1.41 x 10^6	68.05 : 15.28 : 16.68
R90	3.15 x 10^7	4.20 x 10^6	1.27 x 10^6	68.63 : 18.01 : 13.36
KU50	3.10 x 10^7	4.05 x 10^6	0.62 x 10^6	62.96 : 16.92 : 21.12
SR	3.40 x 10^7	4.00 x 10^6	1.45 x 10^6	60.43 : 22.11 : 17.46
ST	2.95x 10^7	3.75 x 10^6	0.48 x 10^6	74.42 : 9.25 : 16.33
RO	2.95 x 10^7	4.00 x 10^6	0.49 x 10^6	69.75 : 10.72 : 19.53

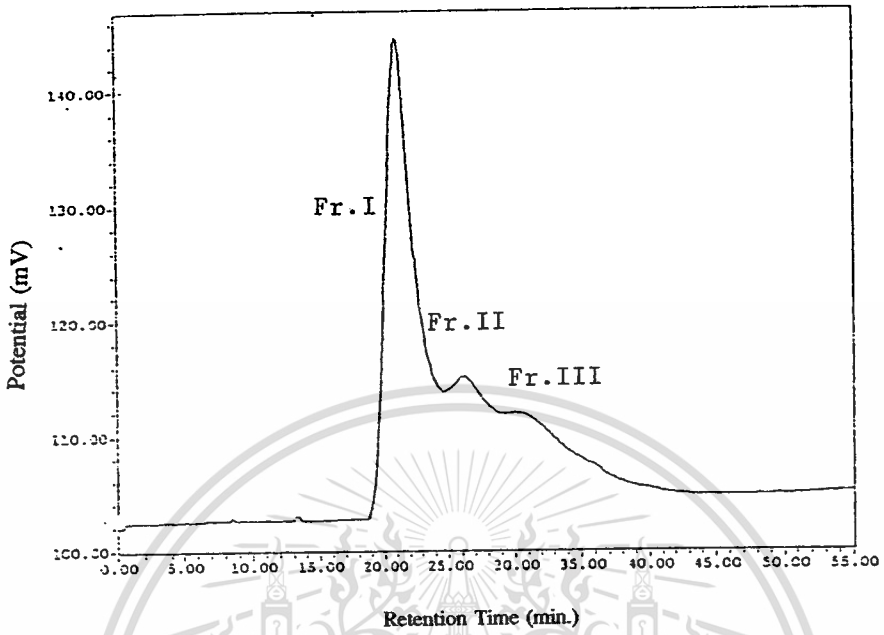


Fig. 4.19 Gel Permeation Chromatogram of R1 Starch

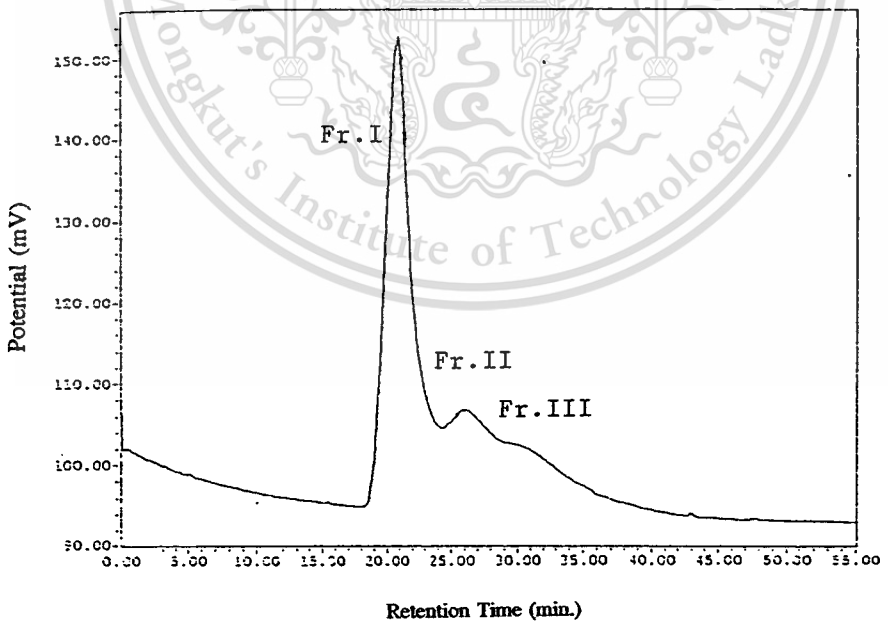


Fig. 4.20 Gel Permeation Chromatogram of R3 Starch

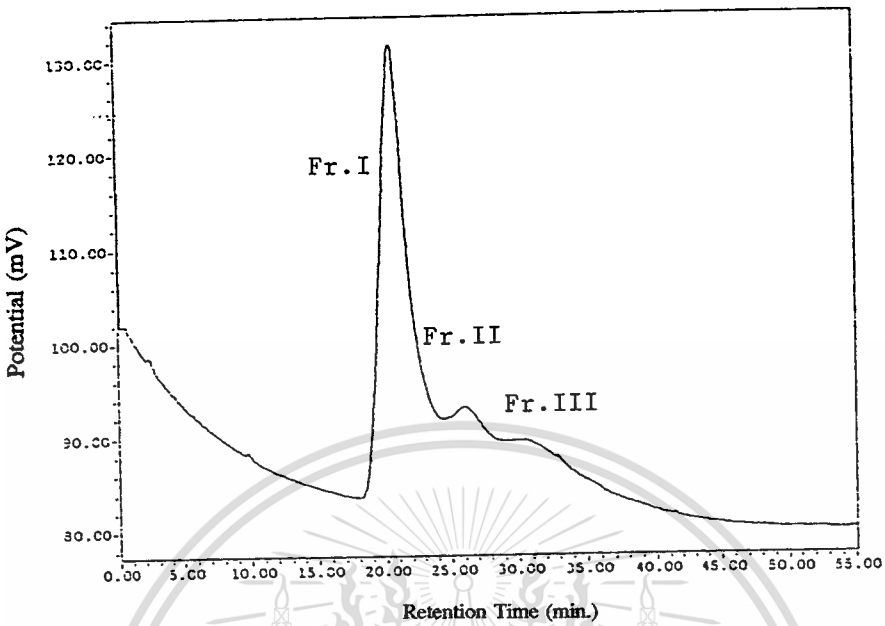


Fig. 4.21 Gel Permeation Chromatogram of R5 Starch

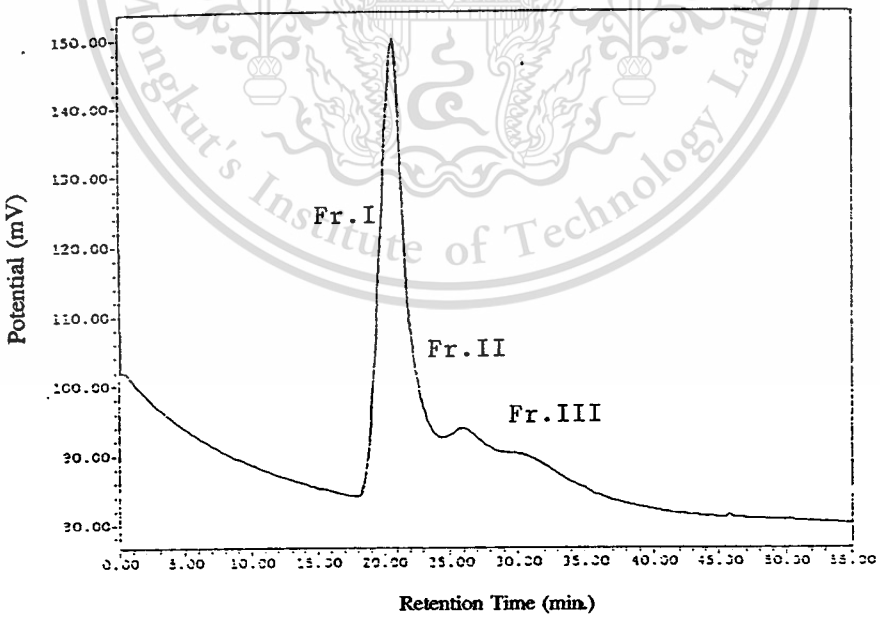


Fig. 4.22 Gel Permeation Chromatogram of R60 Starch

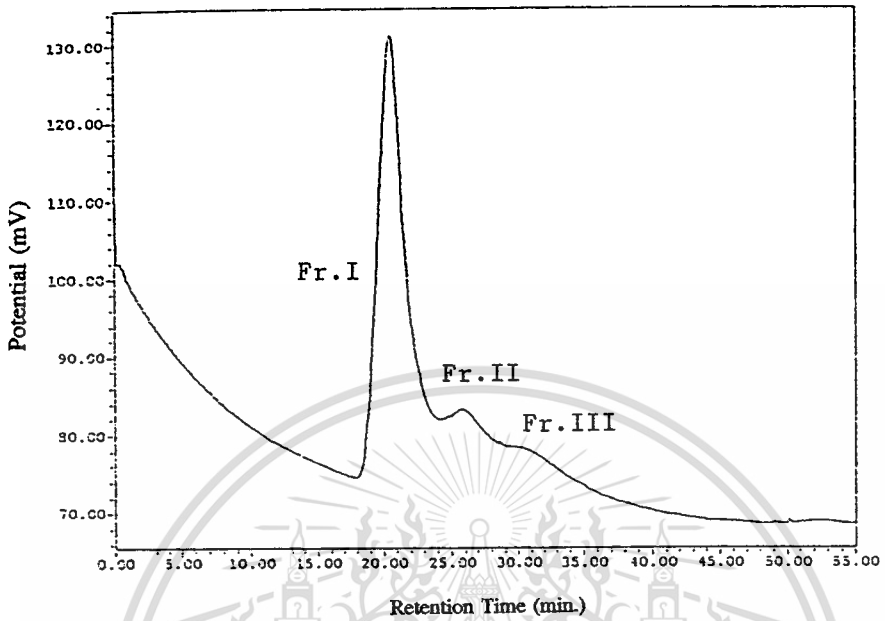


Fig. 4.23 Gel Permeation Chromatogram of R90 Starch

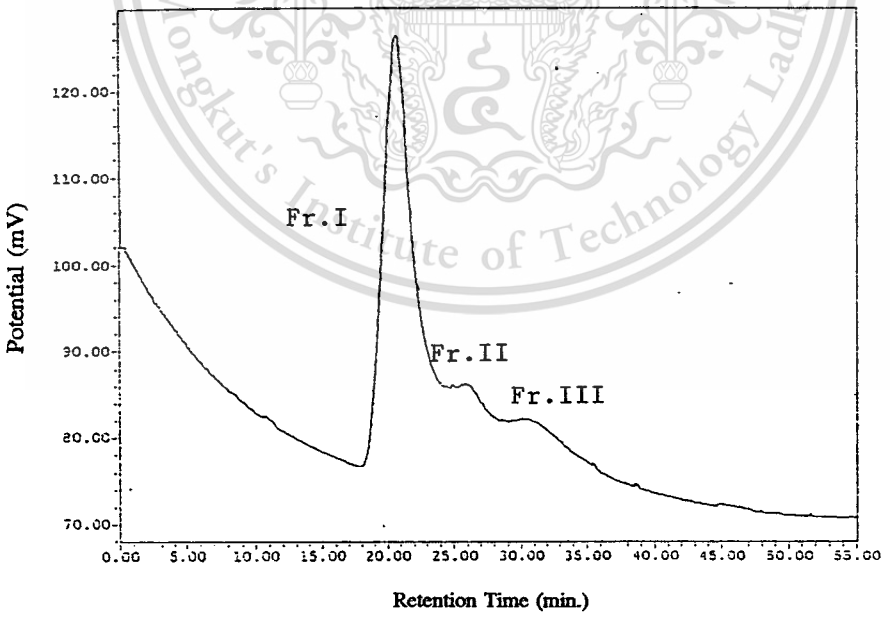


Fig. 4.24 Gel Permeation Chromatogram of KU50 Starch

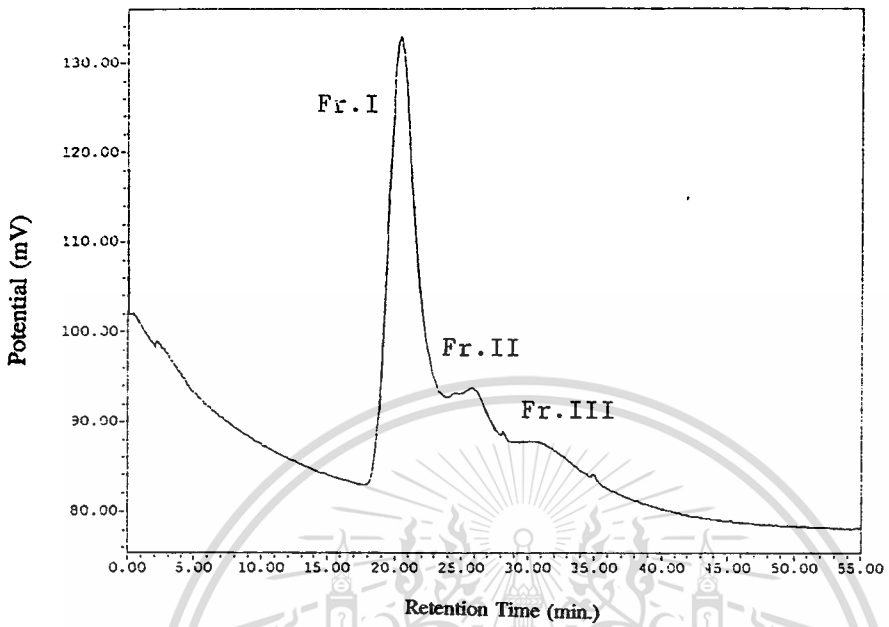


Fig. 4.25 Gel Permeation Chromatogram of SR Starch

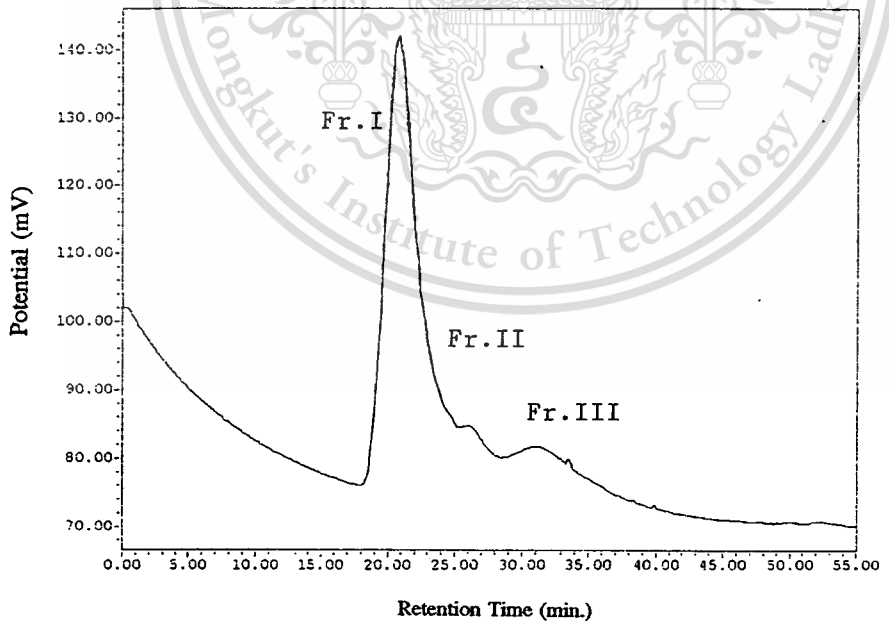


Fig. 4.26 Gel Permeation Chromatogram of ST Starch

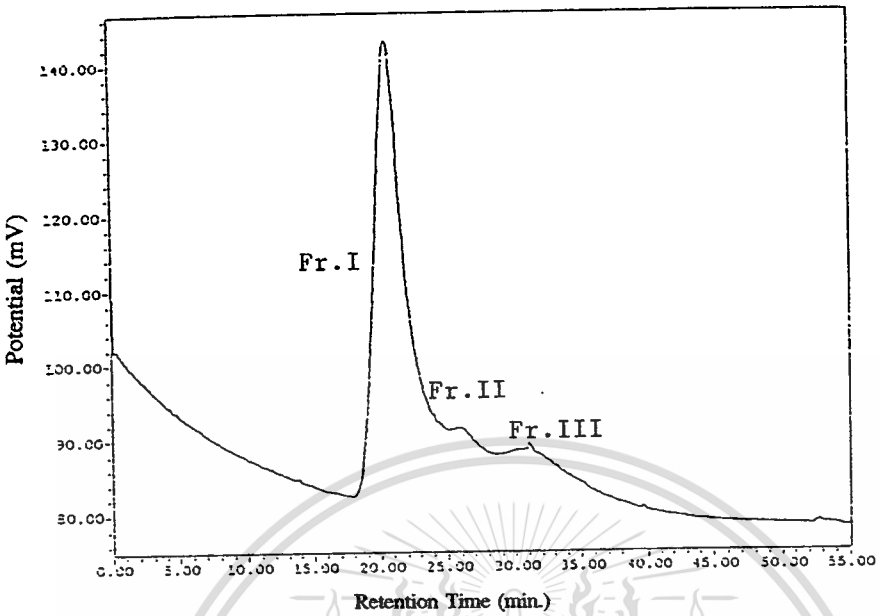


Fig. 4.27 Gel Permeation Chromatogram of RO Starch

After separation, amylopectin fractions and amylose fractions were analyzed by GPC. All amylopectin fractions chromatograms (Fig. 4.28-4.36) showed single peak. The average molecular weights of amylopectins from recommended-variety tapioca starches (Table 4.5) were 1.70-2.50 x 10^7, which Rayong1 and Rayong3 starches were the lowest and the highest average molecular weights, respectively. The average molecular weights of amylopectins from commercial tapioca starches were 2.35 x 10^7 and 2.40 x 10^7. The average molecular weights of amylopectin fractions after separation were lower than average molecular weights of Fr.I before separation. This suggested that the separation process may be effect to molecular weights by fragmentation between process or destroyed the formation of hydrogen bonds between molecules which would mean a decrease in molecular size.

Chromatograms of amylose fractions after separation showed 3 peak fractions (Fig.4.37-4.45). Fr.I was amylopectin residue, and Fr. II and Fr. III (Table 4.5) were large and small amyloses, respectively. The average molecular weights of large and small amyloses were 1.30-3.60 x10^4 and 1.53-7.10 x10^5, respectively. The average molecular weights of large and small amyloses after separation were lower than large and small amyloses before

separation. The reason was same as the suggestion of amylopectin fractions.

Table 4.5 Average Molecular Weights of Amylopectin and Amylose Fractions After Separation

Sample	Amylopectin Fraction	Amylose Fraction		
		Fr. II	Fr. III	Area Ratio Fr. II : Fr. III[A]
R1	1.70×10^7	1.60×10^6	4.70×10^5	16.76 : 83.24
R3	2.50×10^7	2.40×10^6	7.10×10^5	11.56 : 88.44
R5	2.15×10^7	1.30×10^6	3.25×10^5	12.34 : 87.66
R60	2.00×10^7	1.40×10^6	2.35×10^5	7.29 : 92.71
R90	2.15×10^7	2.30×10^6	4.30×10^5	10.54 : 89.46
KU50	2.40×10^7	3.60×10^6	1.53×10^5	7.85 : 92.15
SR	2.05×10^7	2.20×10^6	3.10×10^5	10.60 : 89.40
ST	2.35×10^7	2.70×10^6	2.80×10^5	8.95 : 91.05
RO	2.40×10^7	2.55×10^6	3.05×10^5	8.94 : 91.06

[A] Area ratio Fr.II+Fr.III = 100%

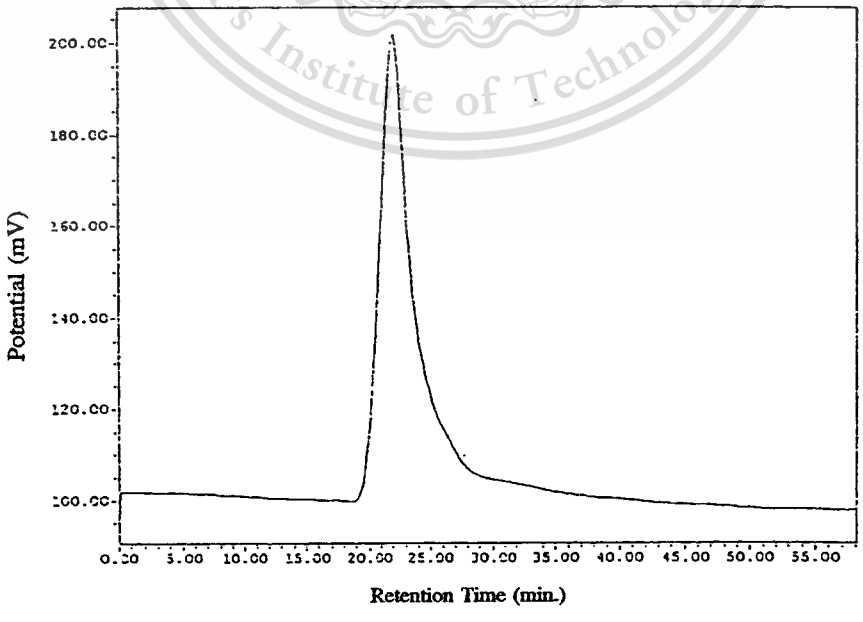


Fig. 4.28 Gel Permeation Chromatogram of Amylopectin Fraction of R1 Starch

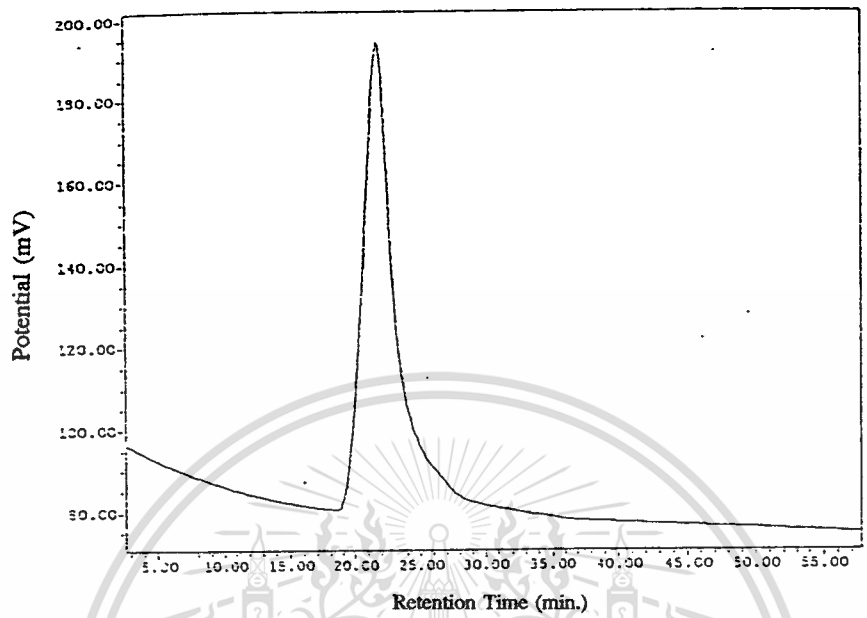


Fig. 4.29 Gel Permeation Chromatogram of Amylopectin Fraction of R3 Starch

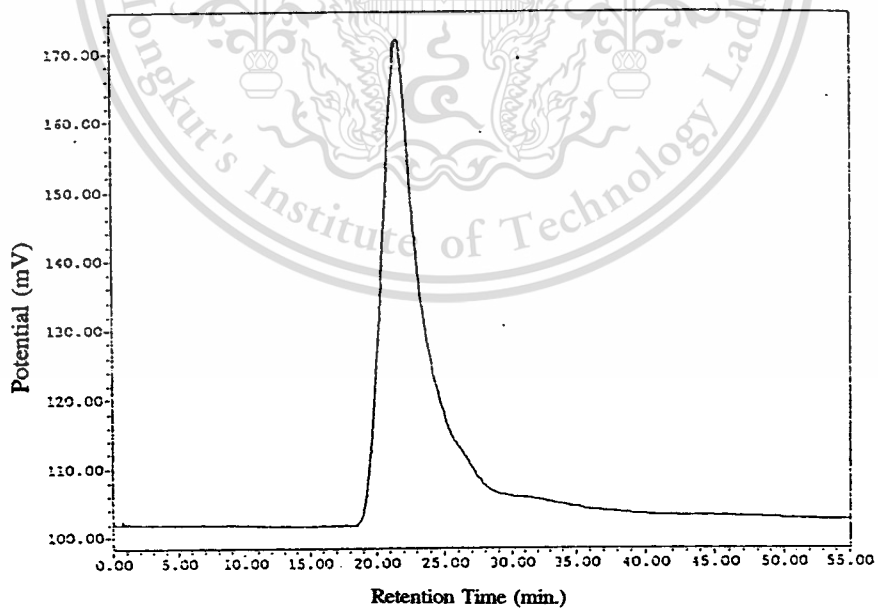


Fig. 4.30 Gel Permeation Chromatogram of Amylopectin Fraction of R5 Starch

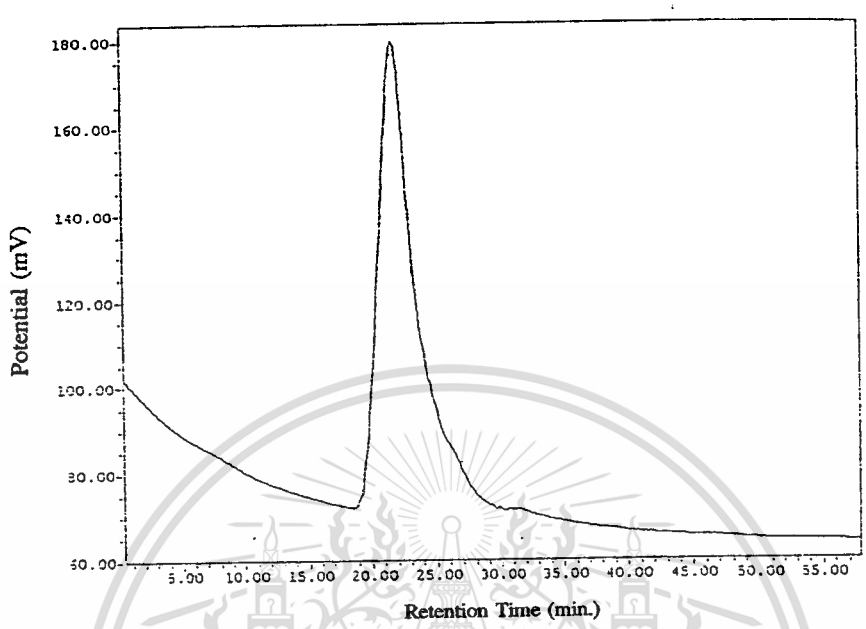


Fig. 4.31 Gel Permeation Chromatogram of Amylopectin Fraction of R60 Starch

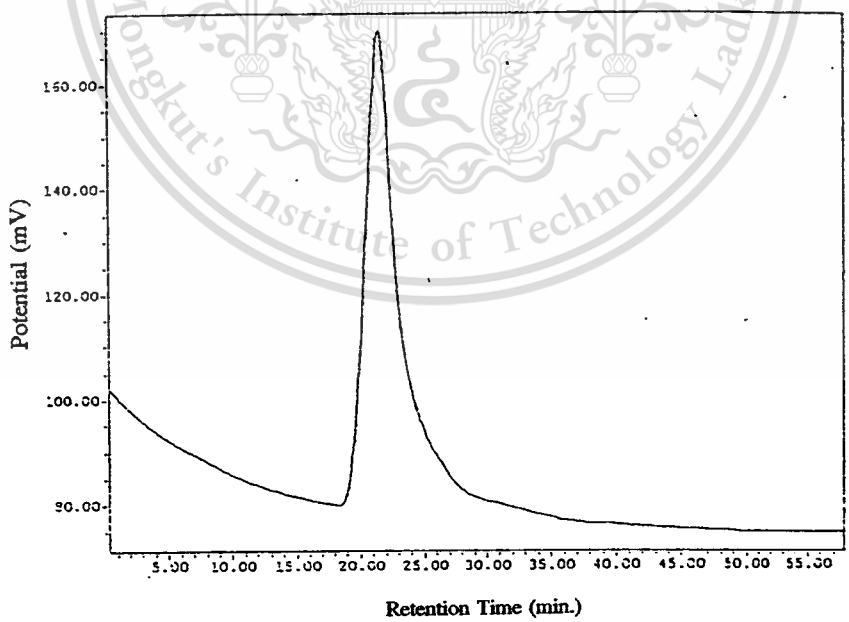


Fig. 4.32 Gel Permeation Chromatogram of Amylopectin Fraction of R90 Starch

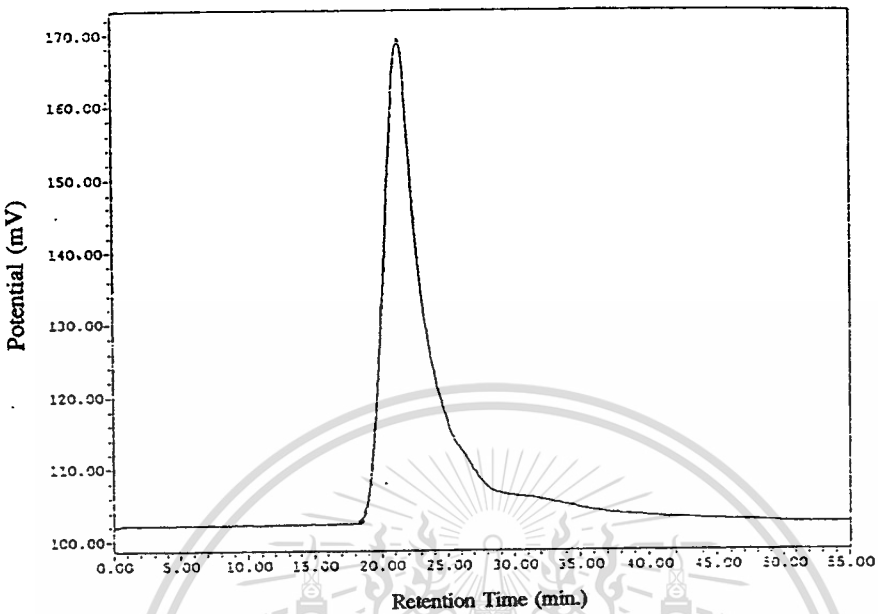


Fig. 4.33 Gel Permeation Chromatogram of Amylopectin Fraction of KU50 Starch

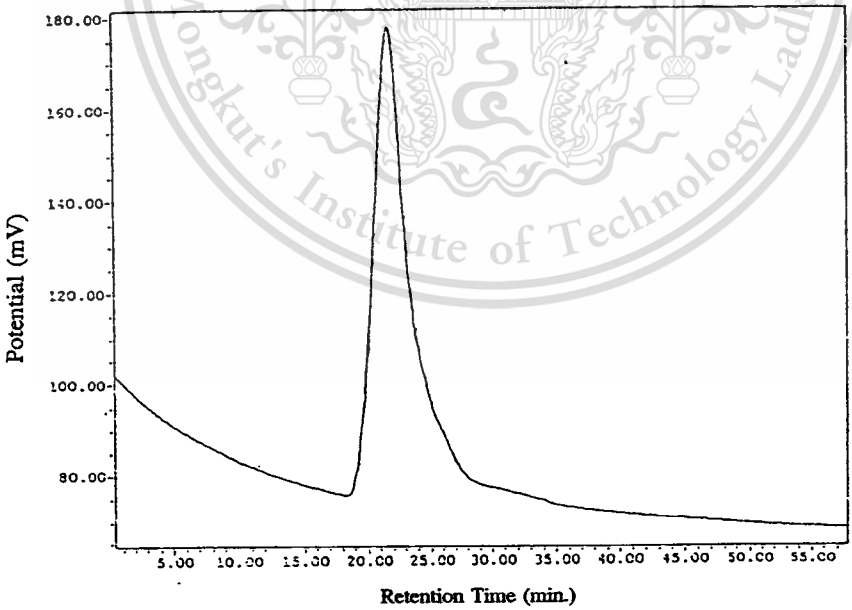


Fig. 4.34 Gel Permeation Chromatogram of Amylopectin Fraction of SR Starch

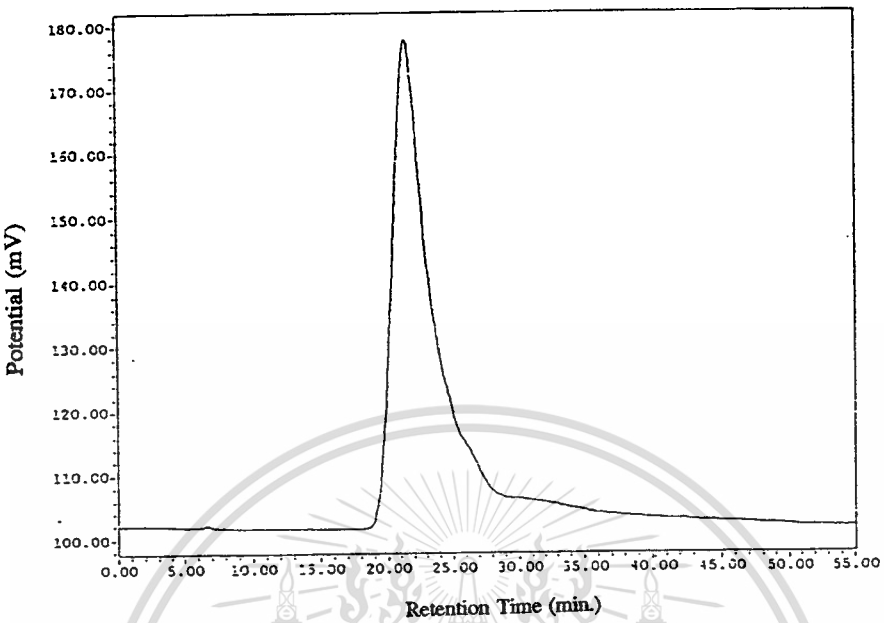


Fig. 4.35 Gel Permeation Chromatogram of Amylopectin Fraction of ST Starch

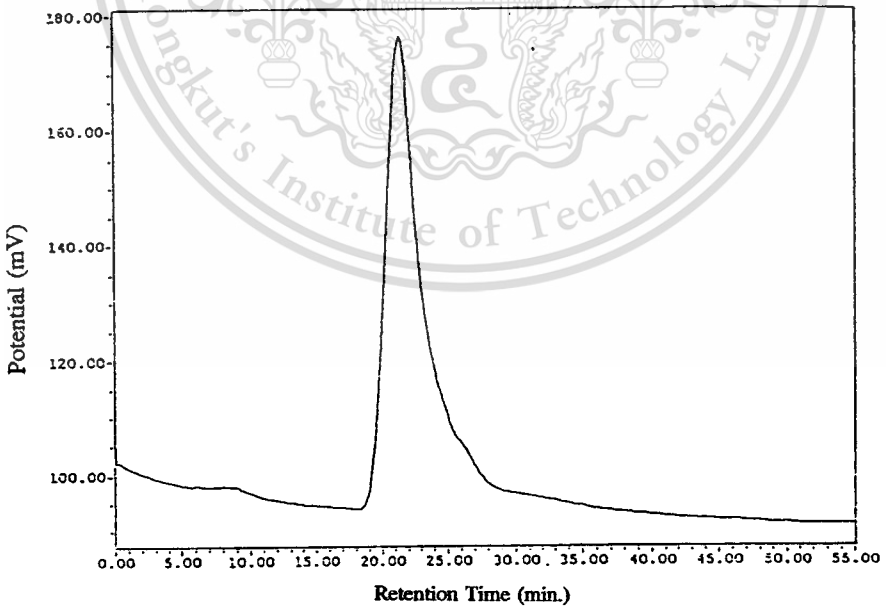


Fig. 4.36 Gel Permeation Chromatogram of Amylopectin Fraction of RO Starch

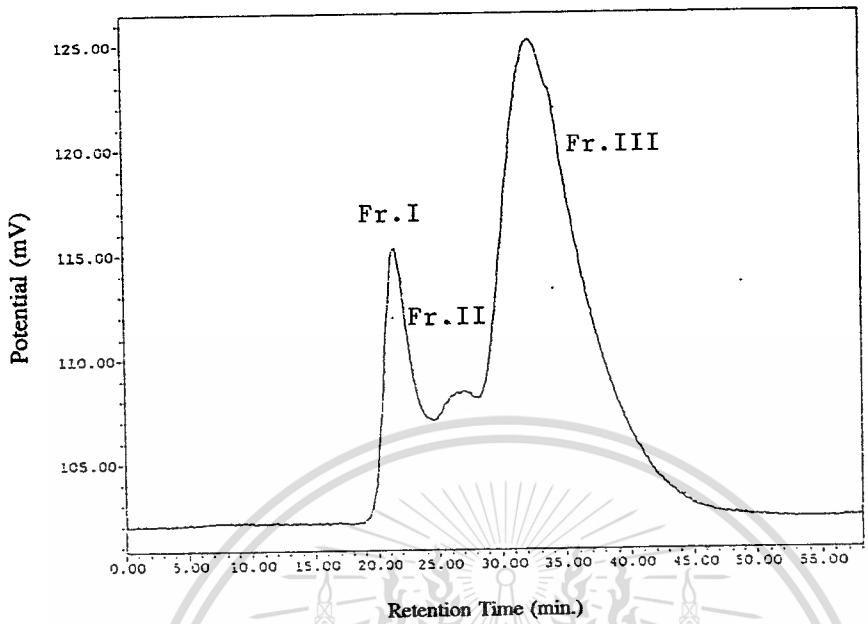


Fig. 4.37 Gel Permeation Chromatogram of Amylose Fraction of R1 Starch

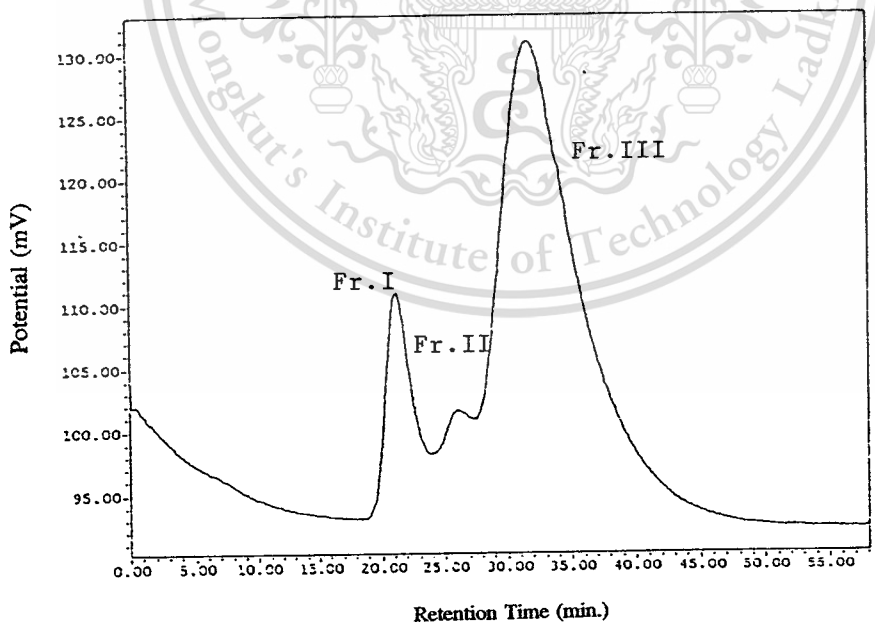


Fig. 4.38 Gel Permeation Chromatogram of Amylose Fraction of R3 Starch

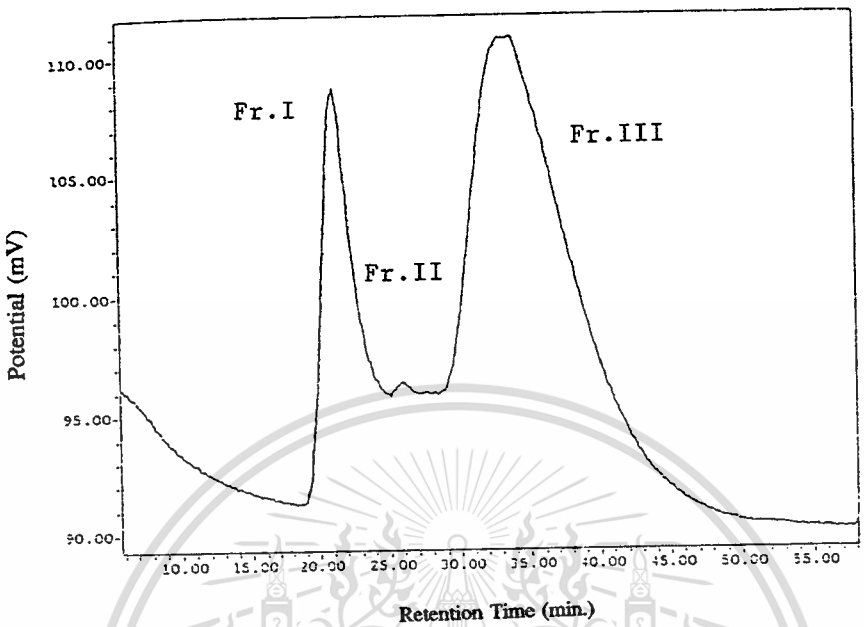


Fig. 4.39 Gel Permeation Chromatogram of Amylose Fraction of R5 Starch

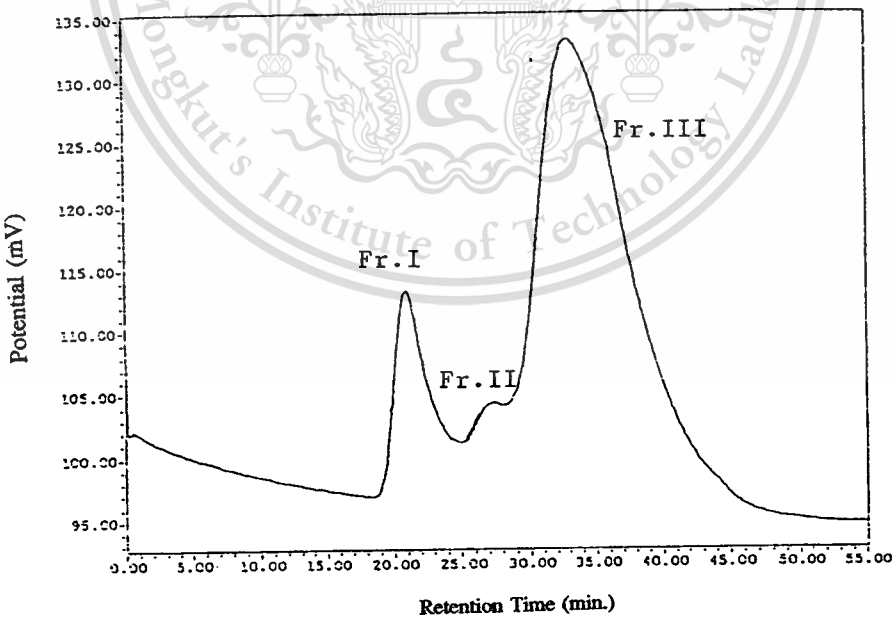


Fig. 4.40 Gel Permeation Chromatogram of Amylose Fraction of R60 Starch

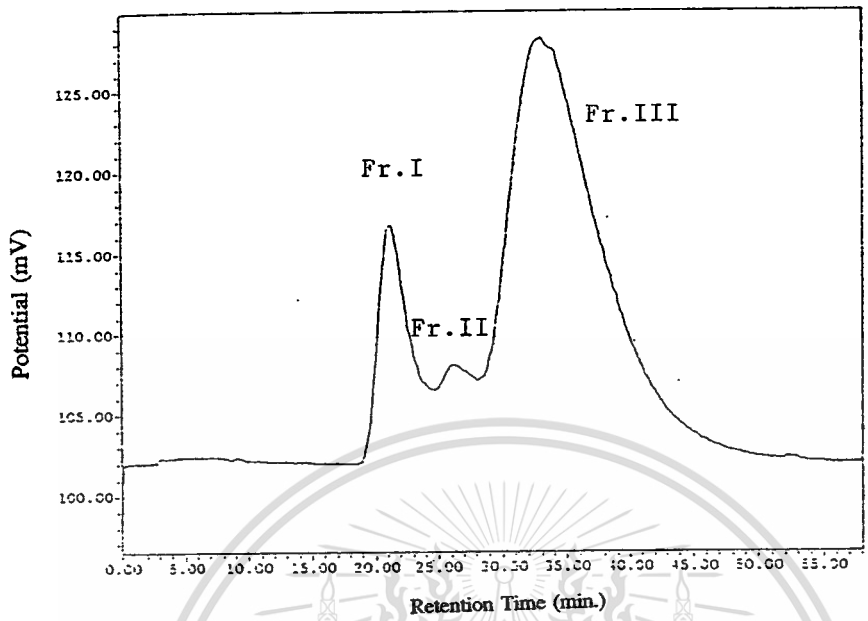


Fig. 4.41 Gel Permeation Chromatogram of Amylose Fraction of R90 Starch

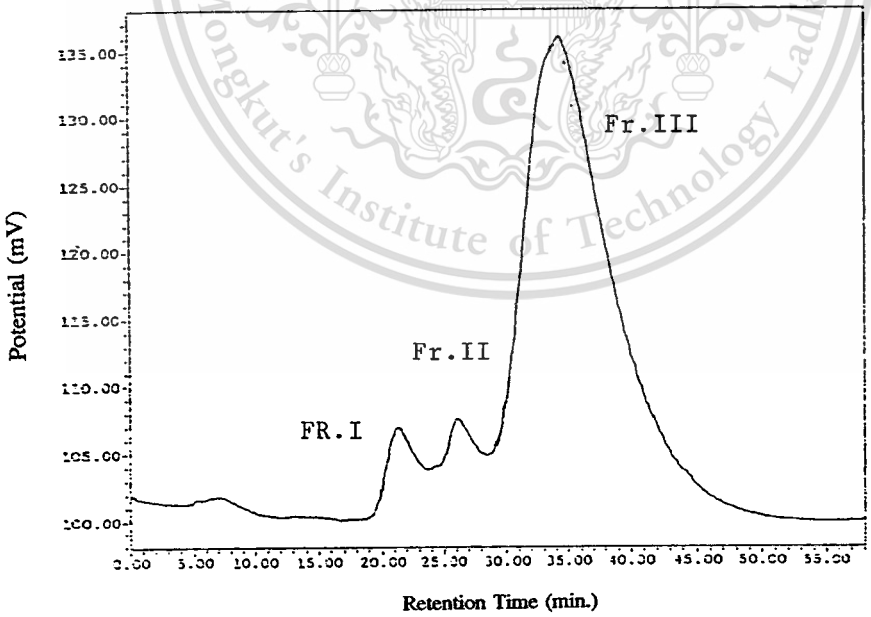


Fig. 4.42 Gel Permeation Chromatogram of Amylose Fraction of KU50 Starch

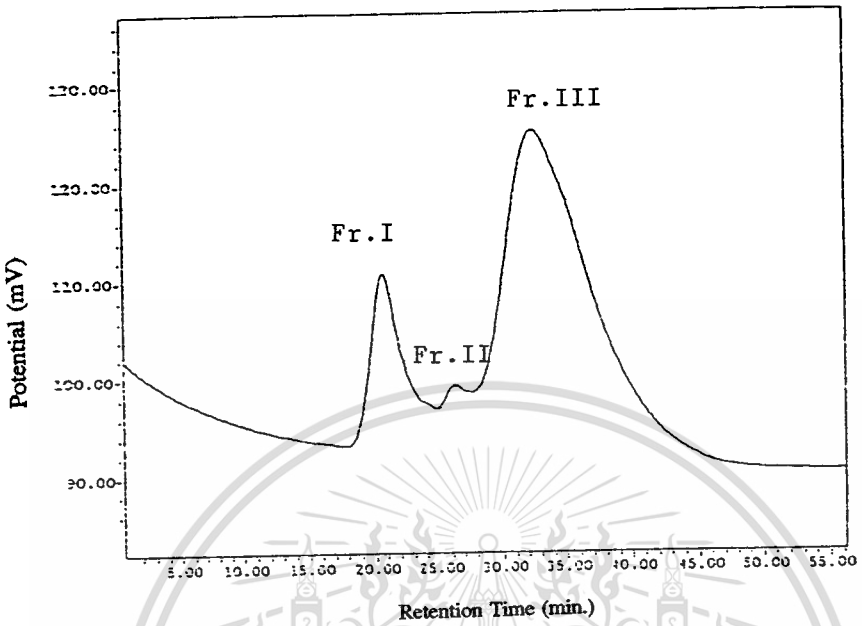


Fig. 4.43 Gel Permeation Chromatogram of Amylose Fraction of SR Starch

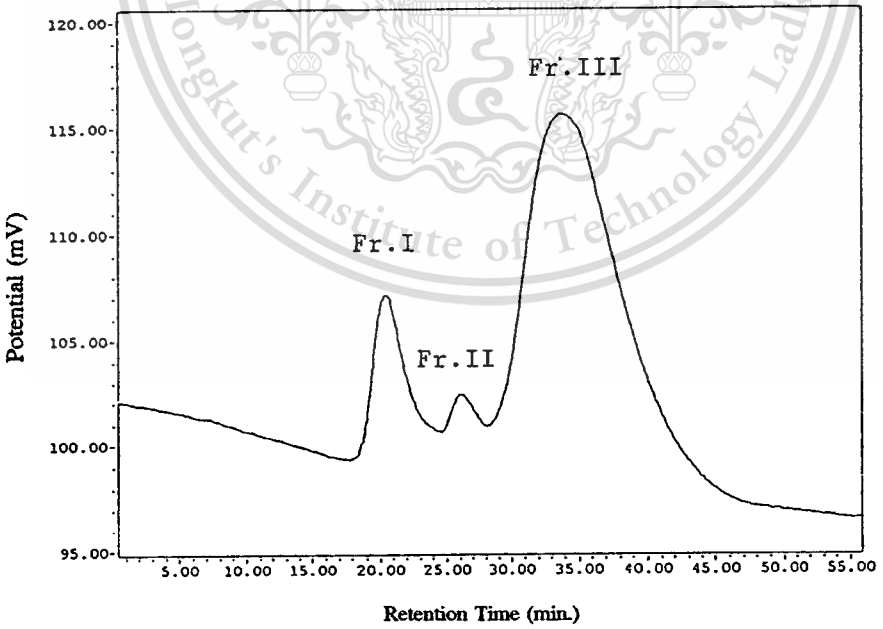


Fig. 4.44 Gel Permeation Chromatogram of Amylose Fraction of ST Starch

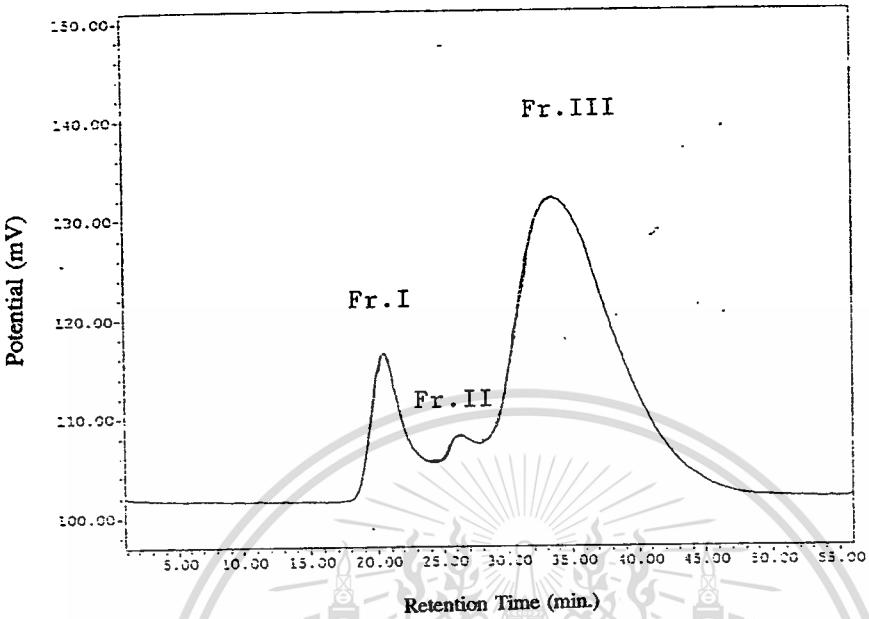


Fig. 4.45 Gel Permeation Chromatogram of Amylose Fraction of RO Starch

All tapioca starch samples were debranched with isoamylase, which all the α (1-6) linkages were hydrolyzed, and then debranched starches were analyzed by GPC. Table 4.6 showed average molecular weights and distributions of debranched starches. All debranched starch chromatograms (Fig. 4.46-4.54) showed 4 peaks, but these chromatograms were not same. They were different average molecular weights and distributions. However, they might be divided into 2 groups following chromatogram patterns, first group was Rayong1; Rayong3; and Rayong 5 starches, second group was Rayong60; Rayong90; Kasetsart50 and Sriracha1 starches. In first group, all debranched starch chromatograms showed 4 fractions, and average molecular weights and distributions were closed range. In second group, average molecular weights of Fr.I, II and IV closed with first group but different distributions, and average molecular weights of Fr.III were less than average molecular weights of Fr.III of debranched starches in first group. This due to peaks of Fr.III of debranched starch in second group inclined to right more than first group. However, distributions of Fr.III of debranched starch in second group closed with first group.

Table 4.6 Average Molecular Weights and Distributions of Debranched Tapioca Starches Components

Sample	Fr. I	Fr. II	Fr. III	Fr. IV	Area Ratio Fr. I : Fr. II : Fr. III : Fr. IV
R1	4.00×10^6	1.90×10^6	9.00×10^4	1.30×10^3	5.85 : 12.69 : 66.54 : 14.92
R3	4.50×10^6	2.50×10^6	9.30×10^4	1.30×10^3	6.68 : 13.09 : 65.76 : 14.47
R5	4.35×10^6	2.00×10^6	8.60×10^4	1.30×10^3	6.21 : 12.11 : 64.50 : 17.18
R60	3.70×10^6	2.40×10^6	2.40×10^4	1.32×10^3	1.16 : 6.21 : 67.17 : 25.46
R90	4.80×10^6	2.25×10^6	3.70×10^4	1.32×10^3	1.75 : 7.20 : 68.60 : 22.45
KU50	4.80×10^6	2.40×10^6	2.45×10^4	1.30×10^3	0.76 : 6.16 : 67.40 : 25.68
SR	4.80×10^6	2.45×10^6	1.55×10^4	1.32×10^3	0.41 : 5.53 : 64.07 : 29.99
ST	5.60×10^6	2.80×10^6	9.70×10^4	1.25×10^3	10.66 : 14.57 : 63.20 : 11.58
RO	5.30×10^6	3.50×10^6	8.80×10^4	1.18×10^3	11.19 : 14.55 : 62.53 : 11.78

Both debranched commercial starches chromatograms (Fig.4.53-4.54) showed 4 peaks, which same as debranched starches chromatograms from recommended varieties. Both chromatograms of debranched starches from commercial starches were similar with debranched starches in first group. This due to Rayong1 and Rayong5 varieties of cassava, which were in first group, were grown more than the other varieties. The average molecular weights and distributions of debranched starches from commercial starches were different from first group because of different cultivation time.

Fr.I and Fr.II of debranched starch were large and small amylose, respective, while Fr.III was A chain of amylopectin and Fr.IV were B chain of amylopectin and components hydrolyzed from amylose. Although amylose was purposed that linear chain, really, it had little branched chains. Amount of Fr.I and II of debranched starches from first group were higher than amount of Fr.I and II of debranched starches from first group were less than amount of Fr.III and IV debranched starches from second group. This indicated that amylose

of starches in first group were less amount of branched chain than starches in second group. To both debranched commercial starch, amylose would had less branched chain which seeing in amount of Fr.IV lower than debranched starch from recommended varieties. In addition, average molecular weights of Fr.III of debranched commercial starches were higher than Fr.III of all debranched starches from recommended varieties, but average molecular weights of Fr.IV were slightly less than Fr.IV of the others. Although the average molecular weights of each fractions were different, the range of molecular weights of each fractions were close values. However, the area ratios of starches in each groups were different, so that molecular structures of these starches were not similar structures.

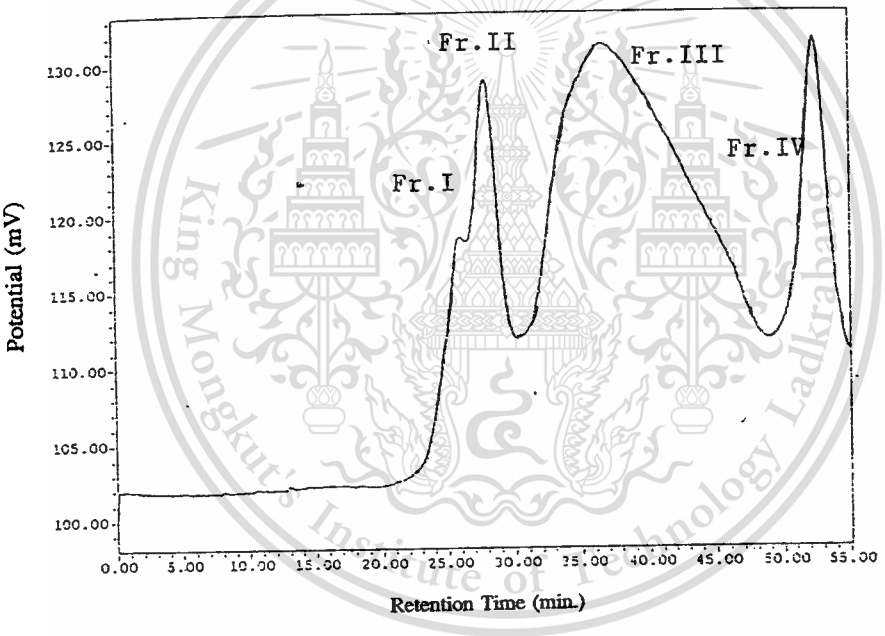


Fig. 4.46 Gel Permeation Chromatogram of Debranched Starch of R1 Starch

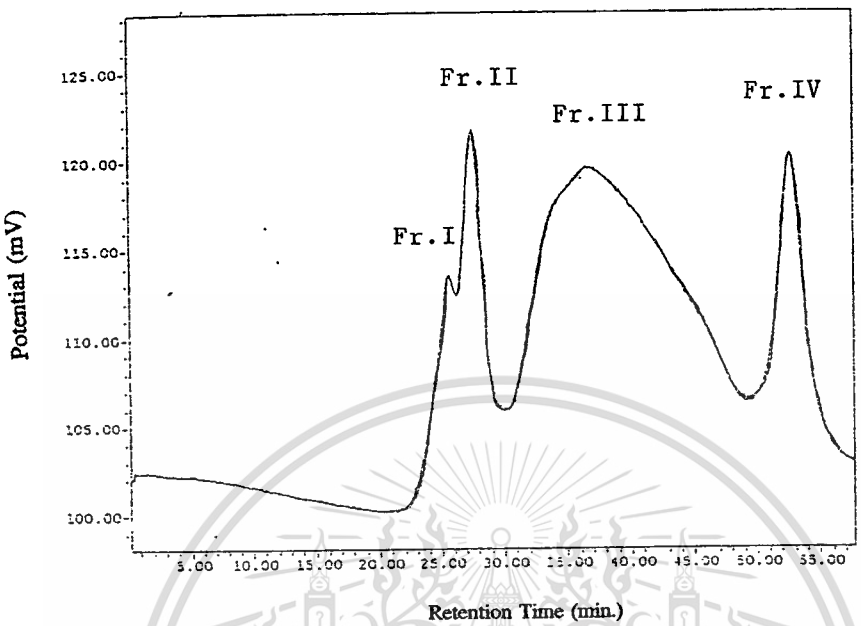


Fig. 4.47 Gel Permeation Chromatogram of Debranched Starch of R3 Starch

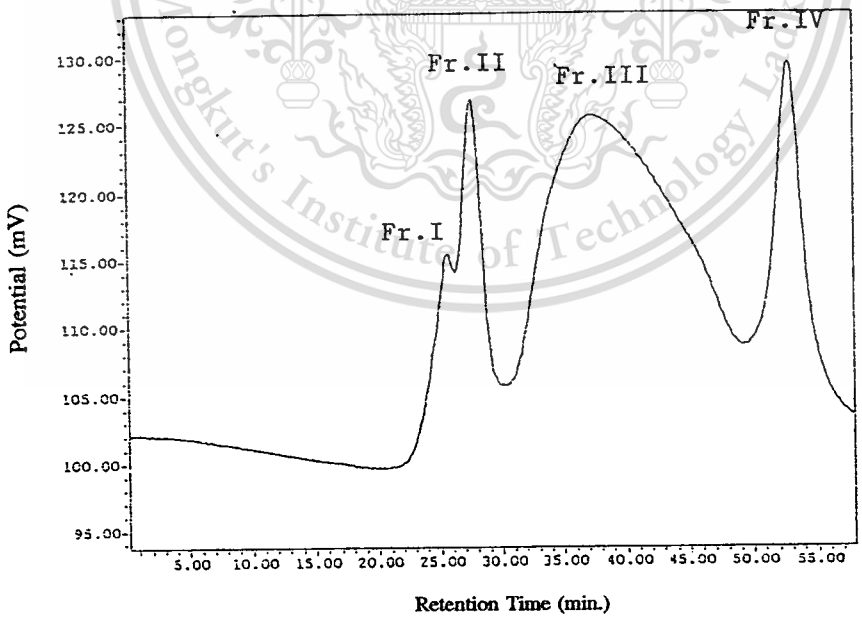


Fig. 4.48 Gel Permeation Chromatogram of Debranched Starch of R5 Starch

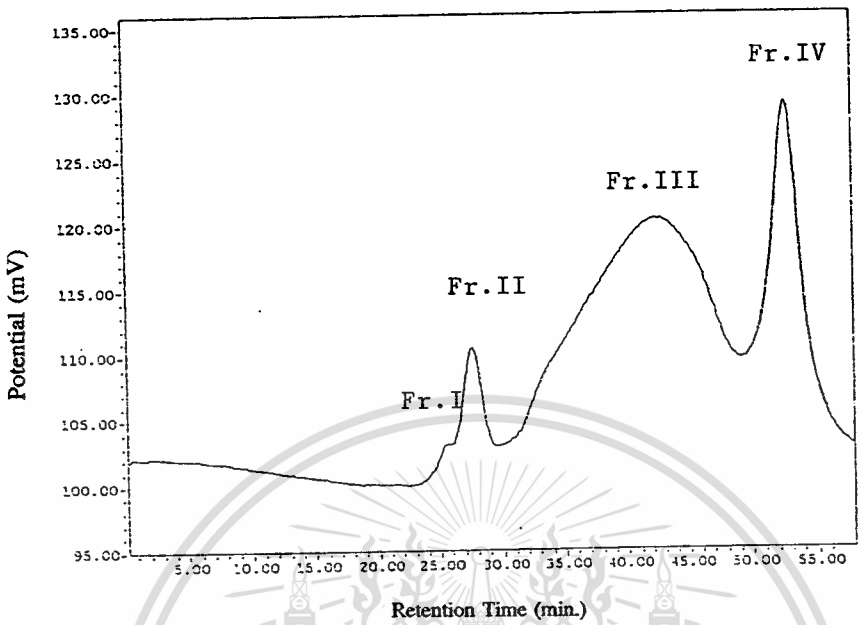


Fig. 4.49 Gel Permeation Chromatogram of Debranched Starch of R60 Starch

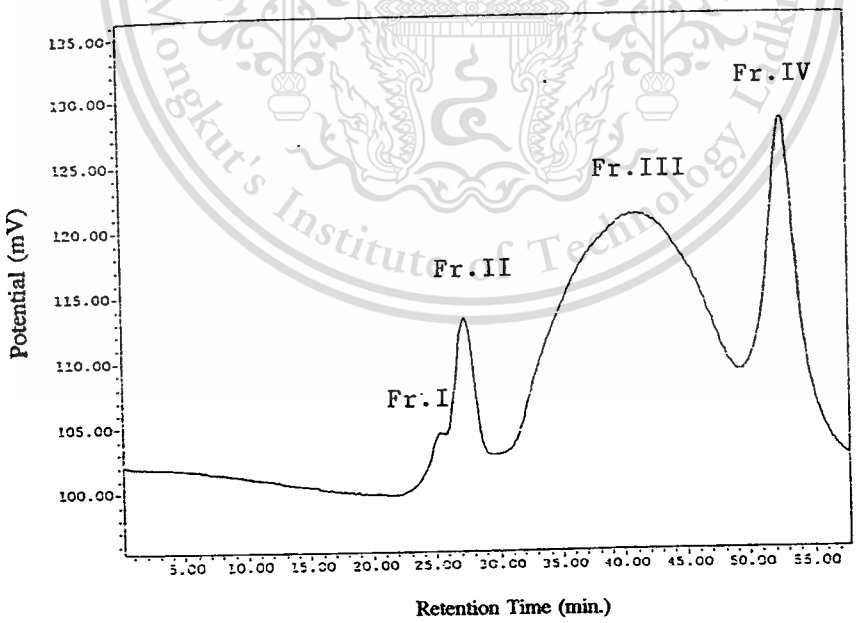


Fig. 4.50 Gel Permeation Chromatogram of Debranched Starch of R90 Starch

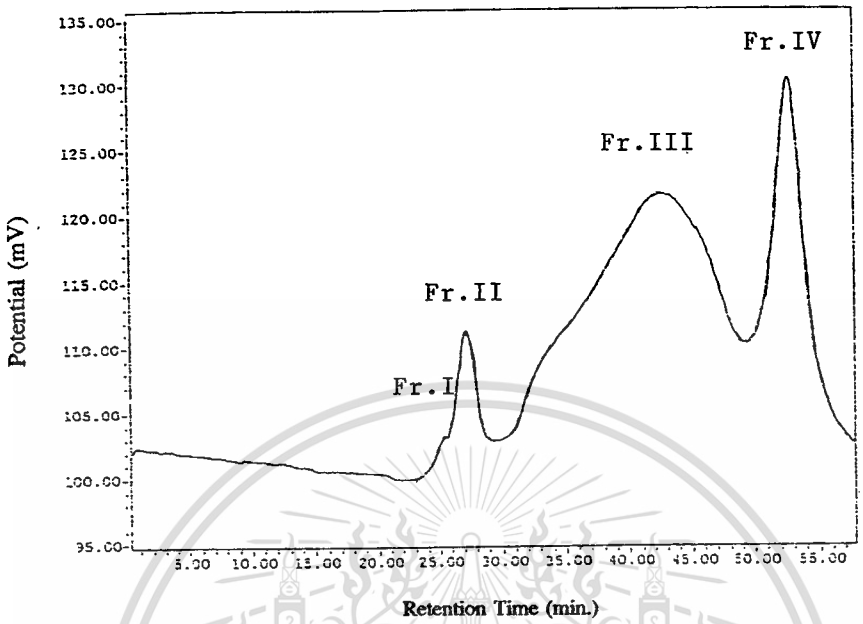


Fig. 4.51 Gel Permeation Chromatogram of Debranched Starch of KU50 Starch

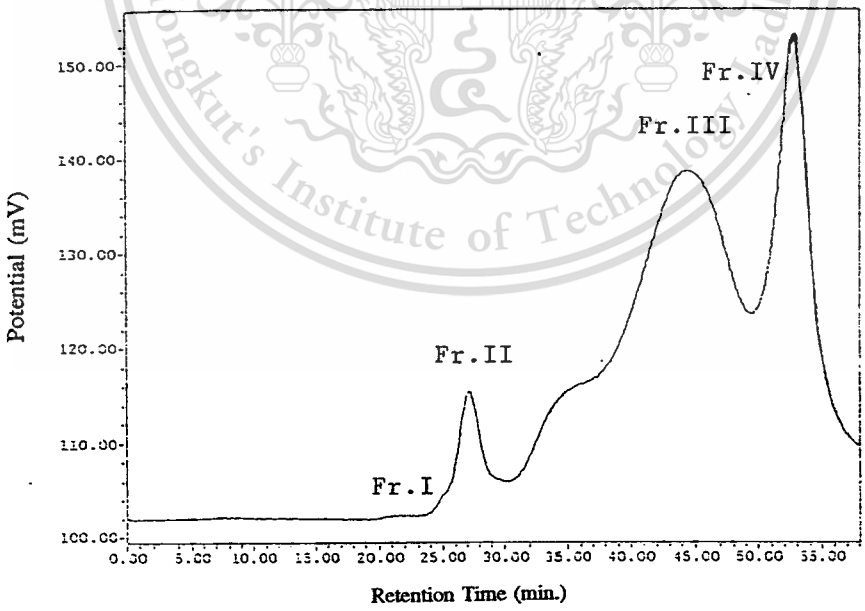


Fig. 4.52 Gel Permeation Chromatogram of Debranched Starch of SR Starch

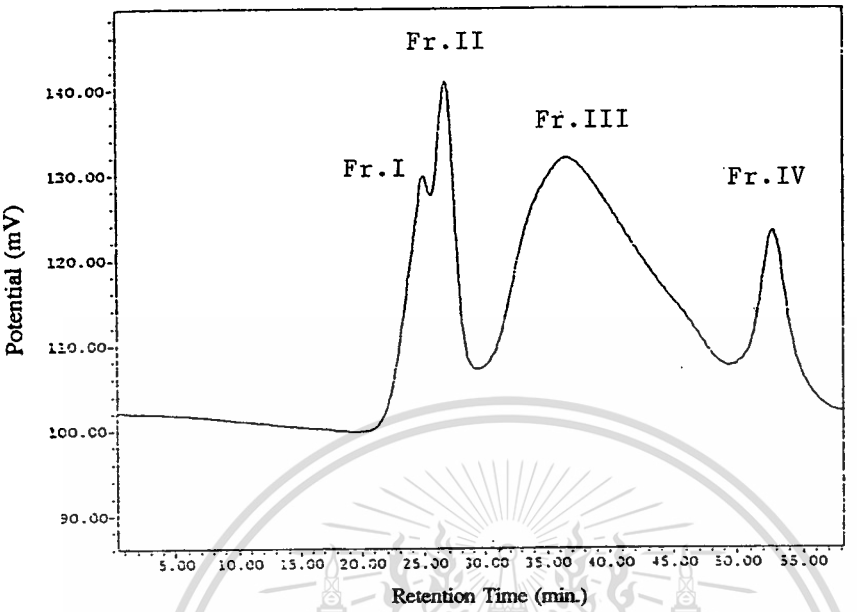


Fig. 4.53 Gel Permeation Chromatogram of Debranched Starch of ST Starch

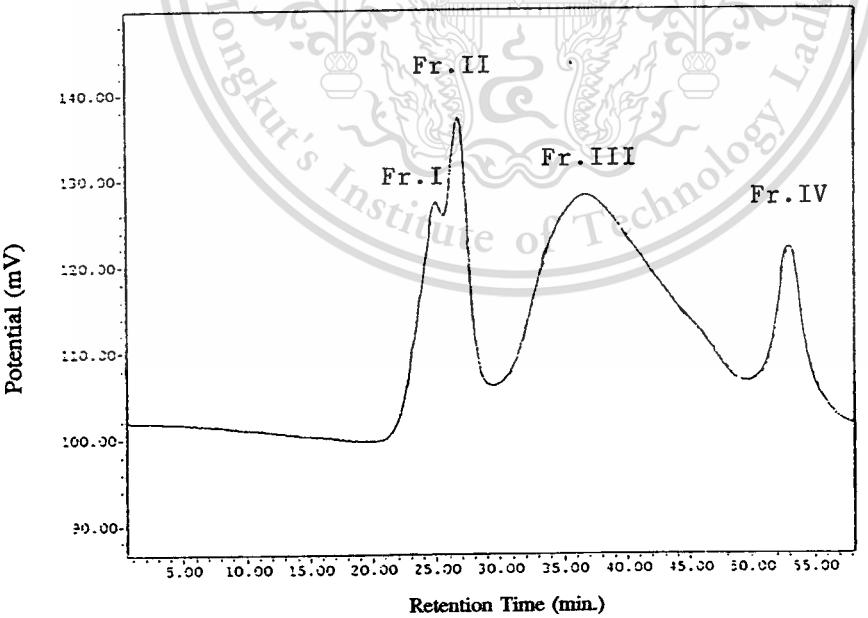


Fig. 4.54 Gel Permeation Chromatogram of Debranched Starch of RO Starch

4.2 PART II : STARCH DERIVATIVES CHARACTERIZATION

To this study, starch derivatives were starch graft copolymers, which were synthesis with emulsion polymerization. There were four monomers (MMA, EA, BA, and 2-EHA) using to graft onto tapioca starch. Effect of monomers and monomer ratios on graft copolymerization were studied. Furthermore, effect of initiator ($K_2S_2O_8$) concentration on graft copolymerization was also studied. These starch derivatives were characterized by gel permeation chromatography (GPC), infrared (IR) spectrophotometer, differential scanning calorimetry (DSC).

4.2.1 Identification of Grafting

Infrared (IR) spectra (Fig. 4.55) of the tapioca starch showed many strong bands in the 1160-1100 cm^{-1} (streching of C-O bonds of COH and COC groups). There is strong broad band at 3200-3600 cm^{-1} (streching of O-H bonds). There is medium weak CH strech absorption at 2920 cm^{-1} and multiple medium bands in the 1460-1200 cm^{-1} region including CH_2 deformation, CH and CH_2 wag, and OH in-plane deformation. There are the characteristic absorption bands of starch at 760 cm^{-1} (in-phase ring stretch) and 920 cm^{-1} (COC out-of-phase stretch), and distinguishing band of α anomers at 855 cm^{-1} [14].

All IR spectra of starch graft copolymers showed broad band at 3200-3600 cm^{-1}, all the characteristic absorption bands of starch, and distinct band of carbonyl group at 1730-1740 cm^{-1}. These results proved that starch molecules were grafted with poly(methyl methacrylate) and other acrylate polymer showed the carbonyl group at 1730-1740 cm^{-1}, so that it was possible to determine copolymer composition.

After hydrolysis, grafted polymers were purified and characterized with IR spectrophotometer. IR spectra of grafted polymers showed no characteristic absoprtion bands of starch. Similarly, all IR spectra of free polymers was almost similar to grafted polymers. These indicated grafted polymers and free polymers were completely purified.

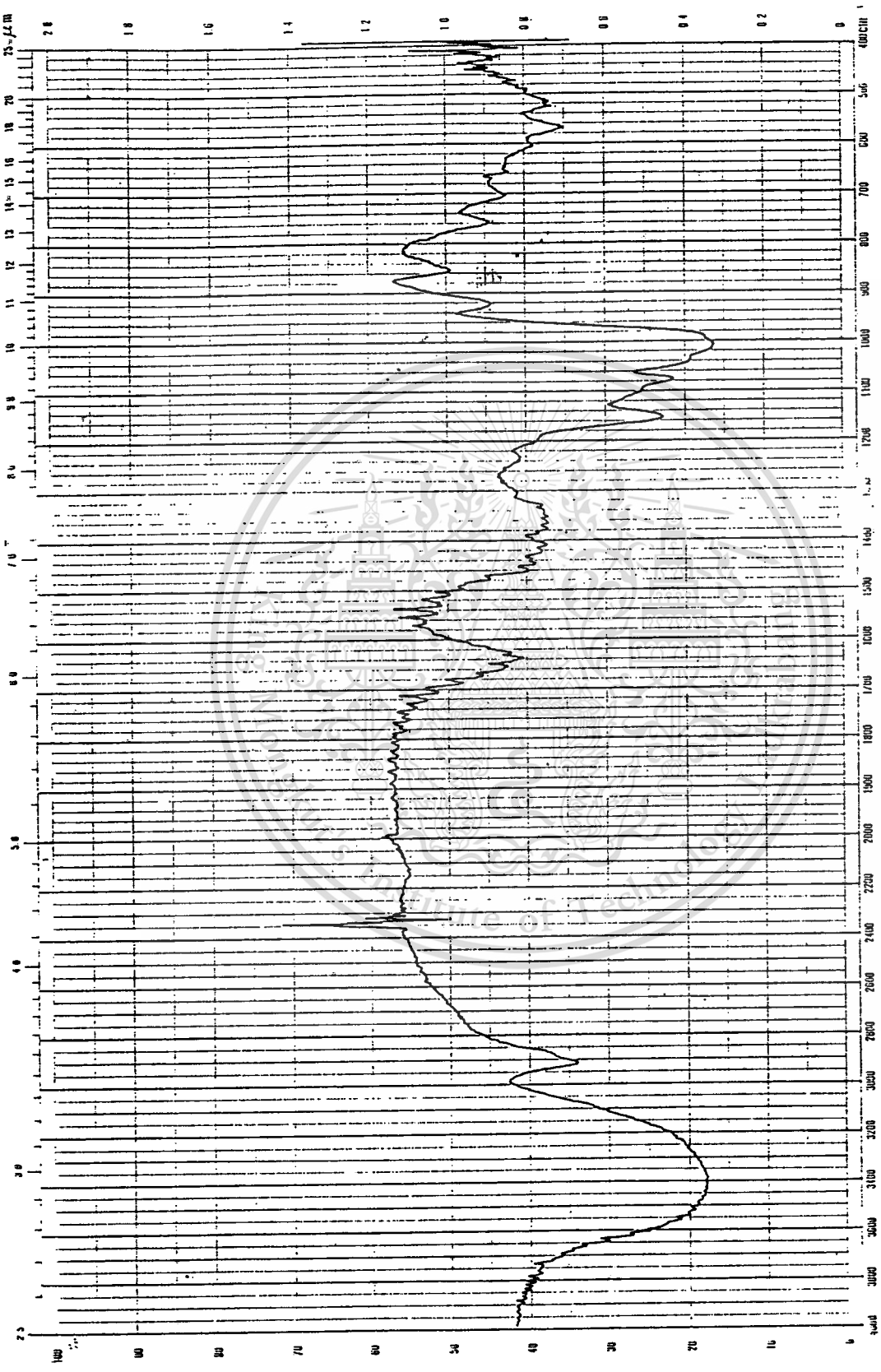


Fig.4.55 IR Spectra of Tapioca Starch

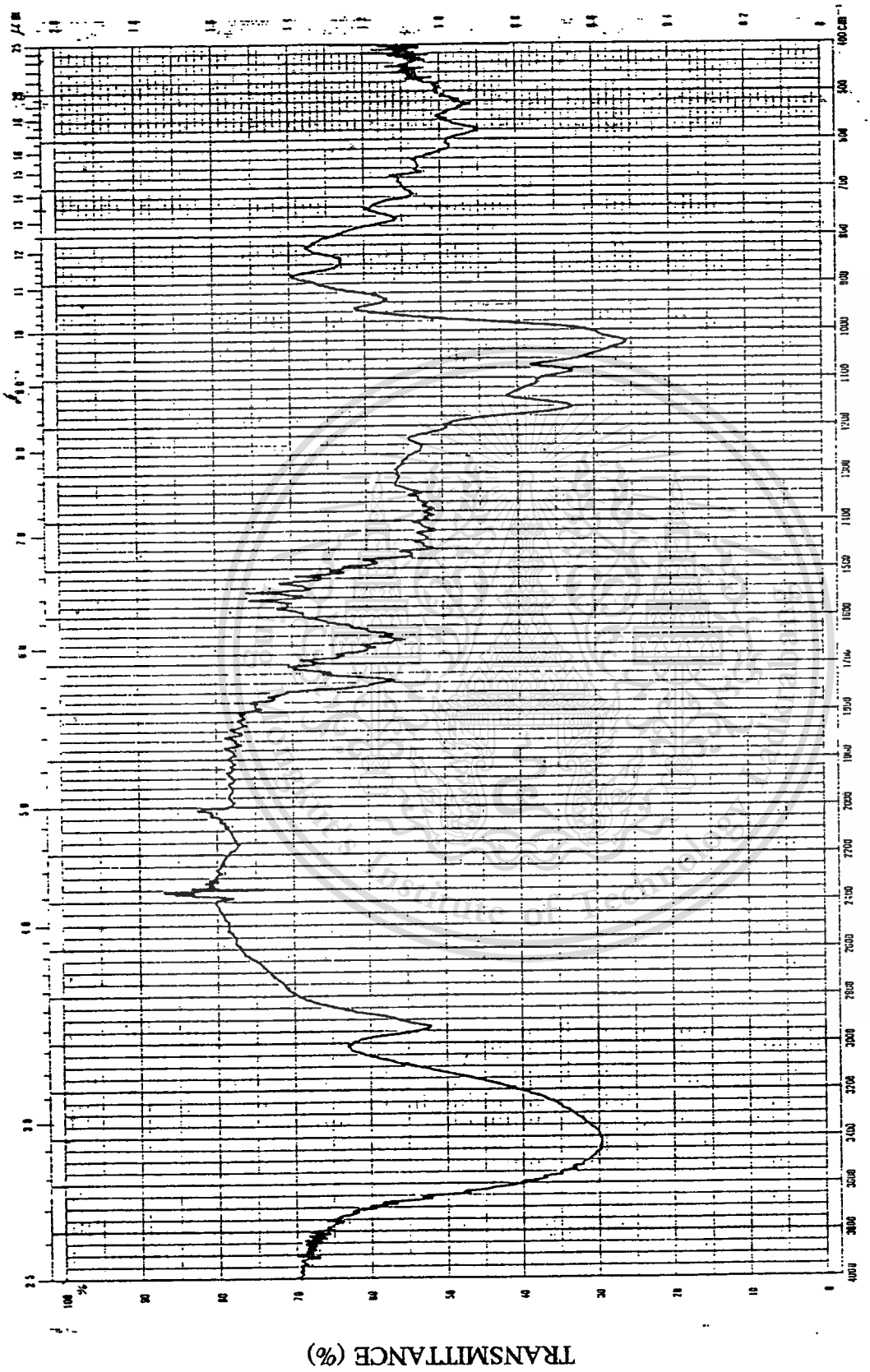


Fig. 4.56 IR Spectra of Starch Graft Poly(MMA)

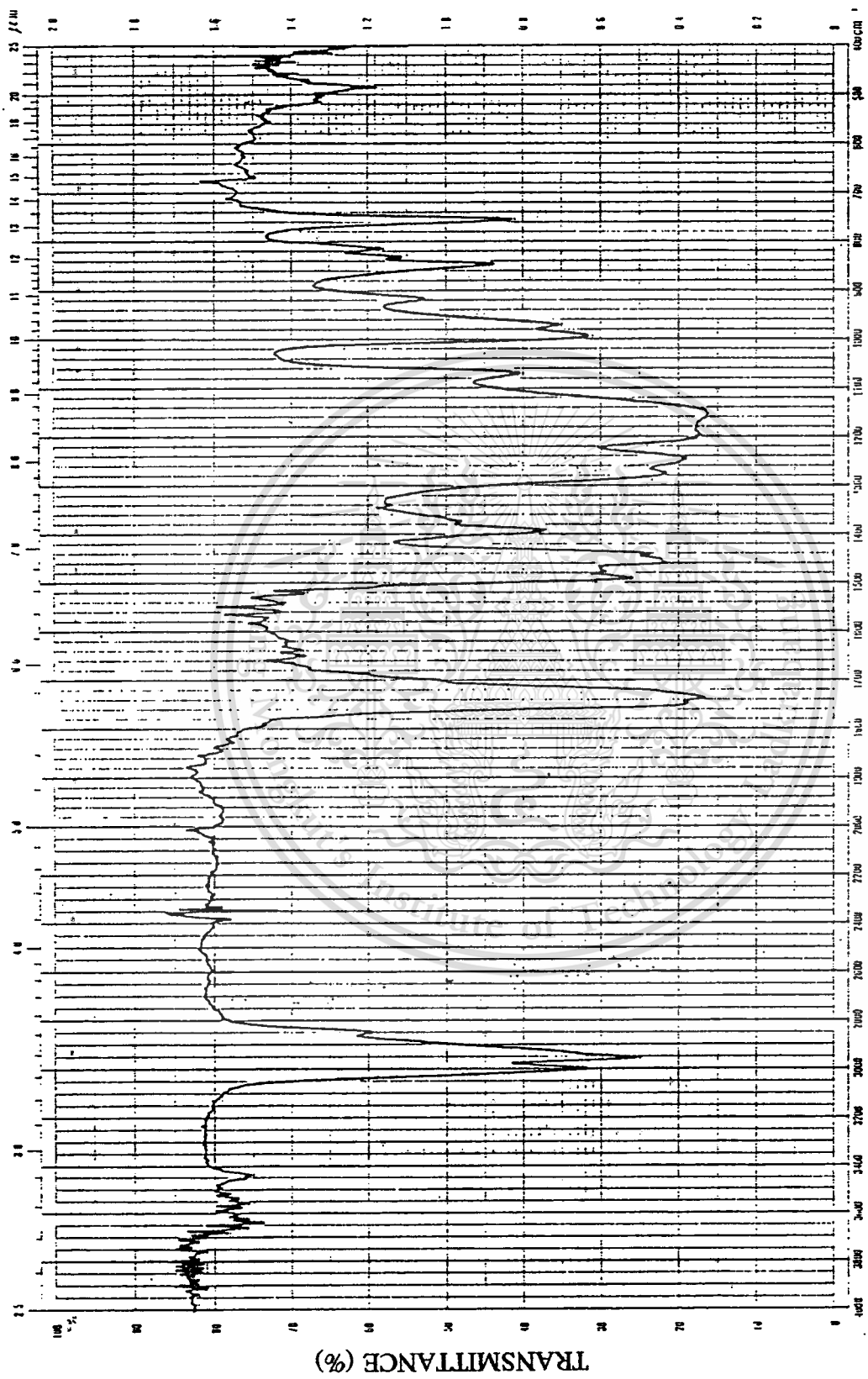


Fig. 4.57 IR Spectra of PMMA Free Polymer

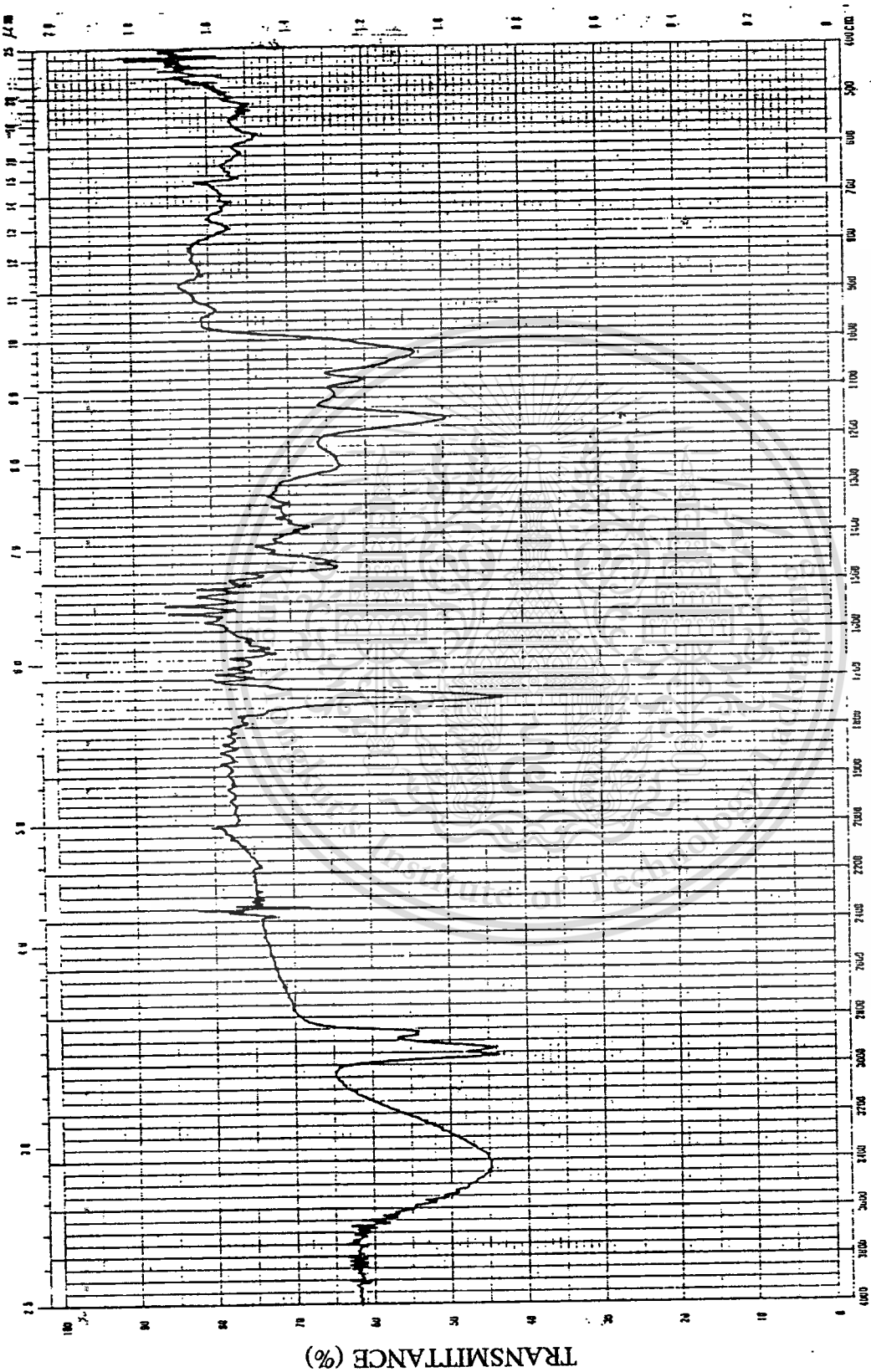


Fig. 4.58 IR Spectra of Starch Graft Poly(2-EHA)

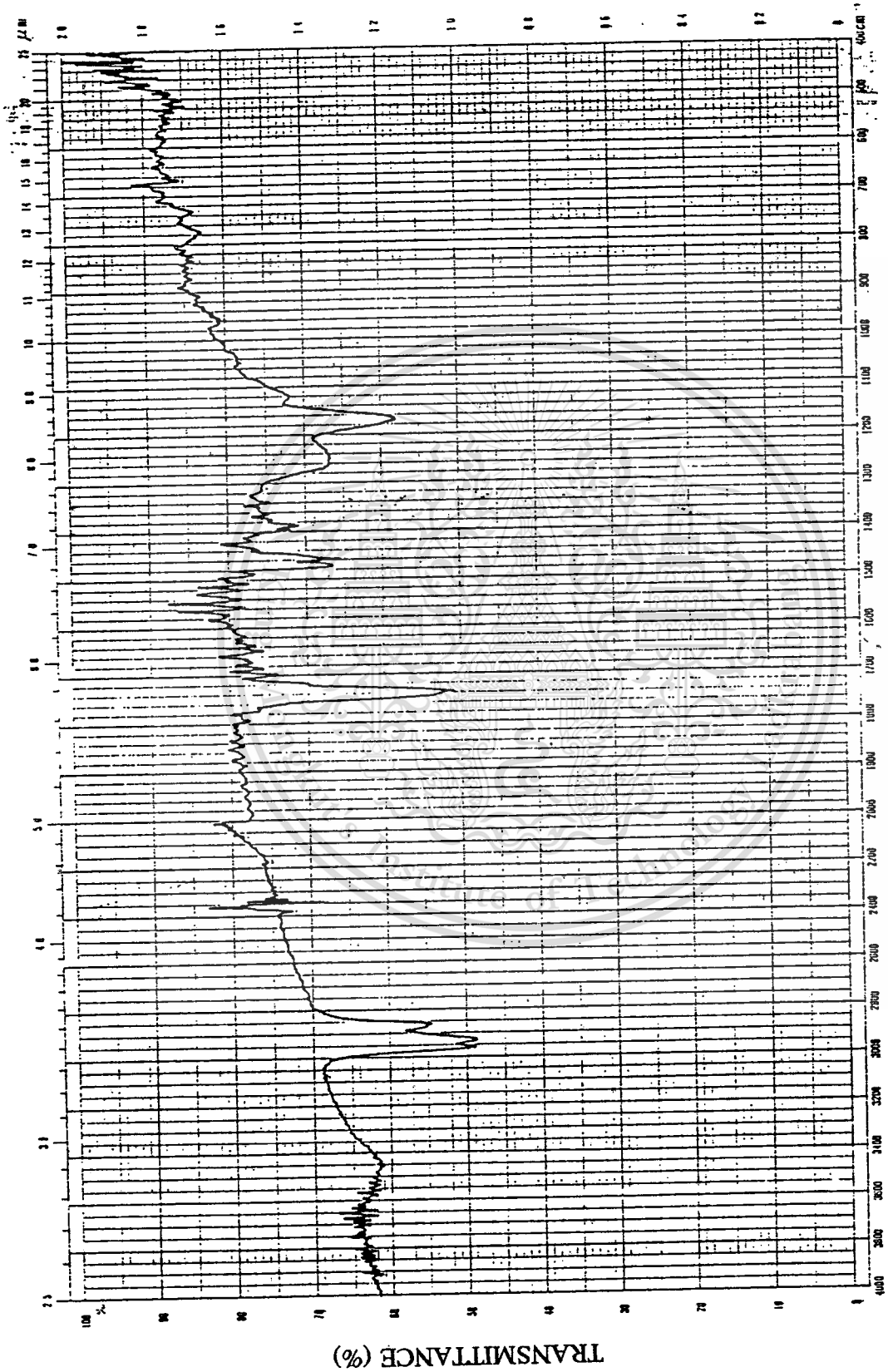


Fig. 4.59 IR Spectra of Poly(2-EHA) Free Polymer

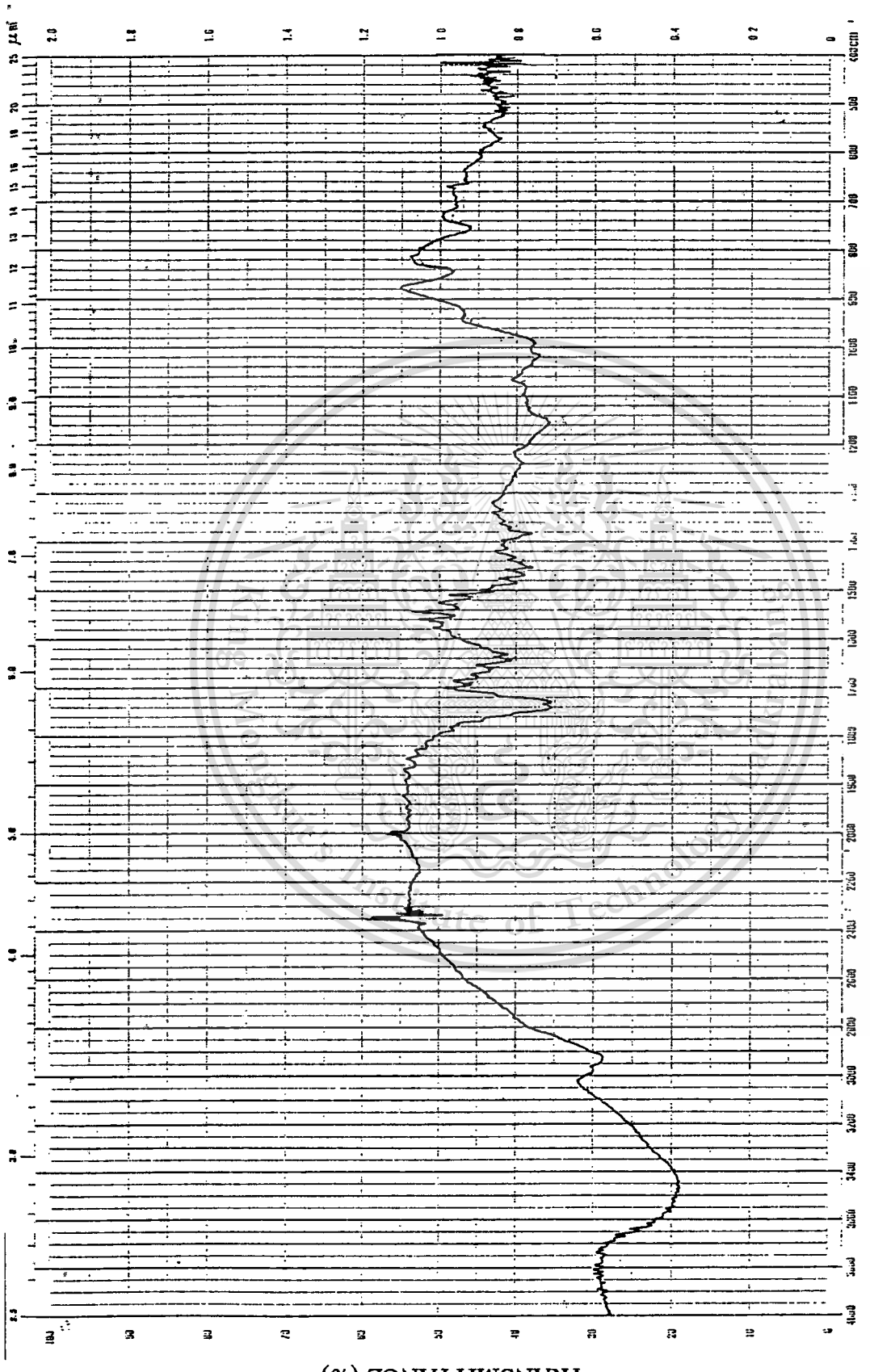


Fig. 4.60 IR Spectra of Starch Graft Poly(MMA/EA)

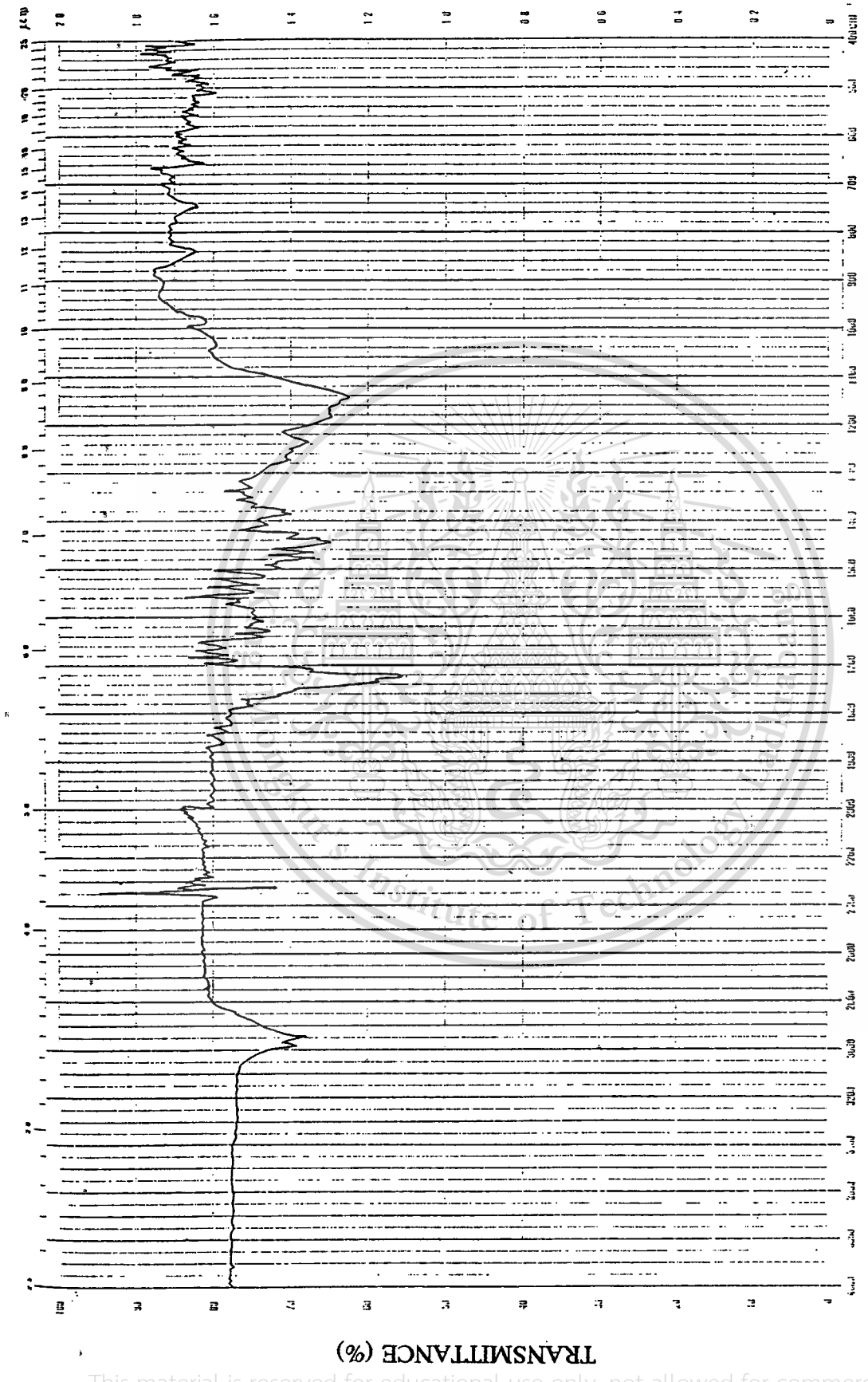


Fig. 4.61 IR Spectra of Poly(MMA/EA) Grafted Polymer

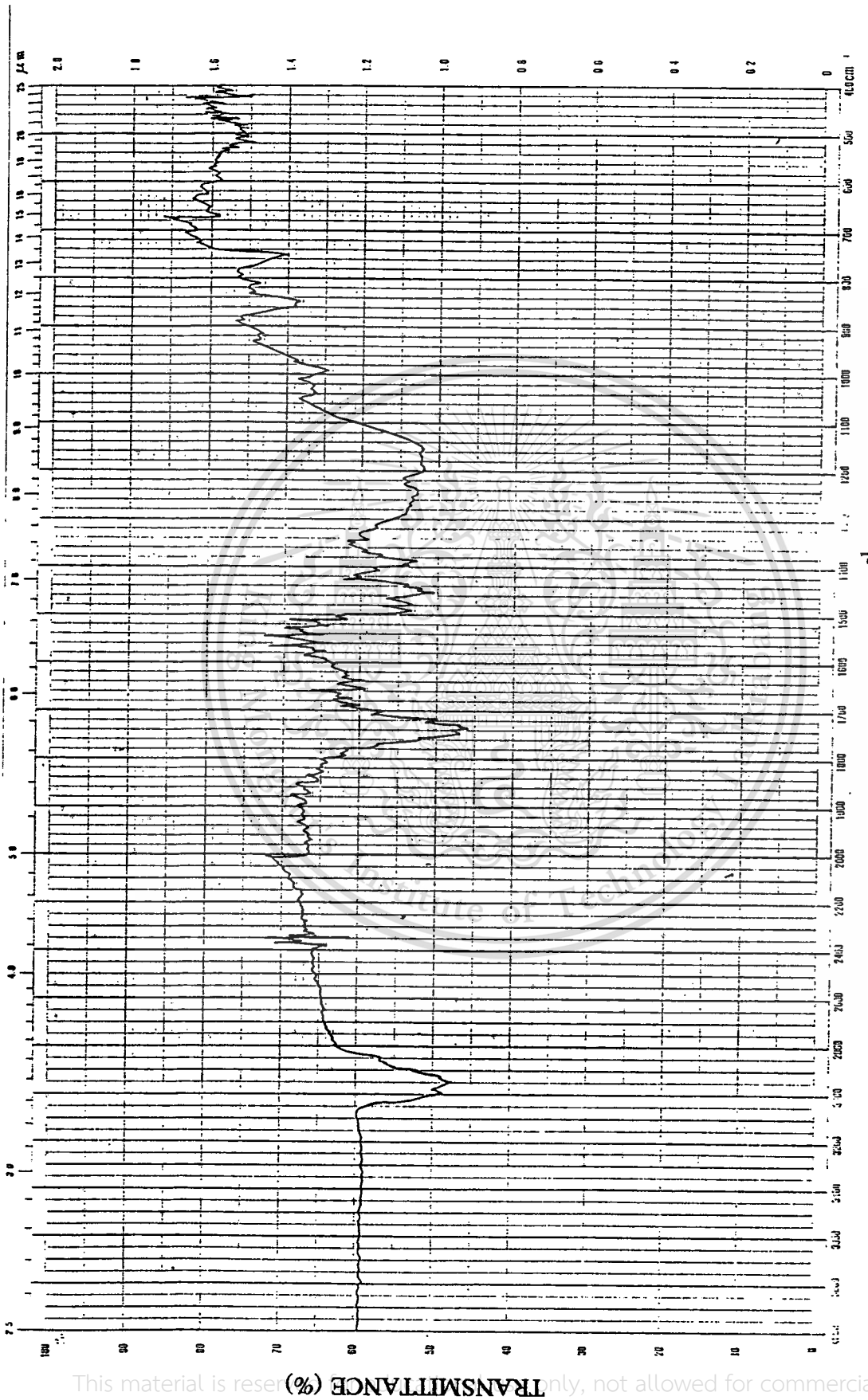


Fig. 4.62 IR Spectra of Poly(MMA/EA) Free Polymer

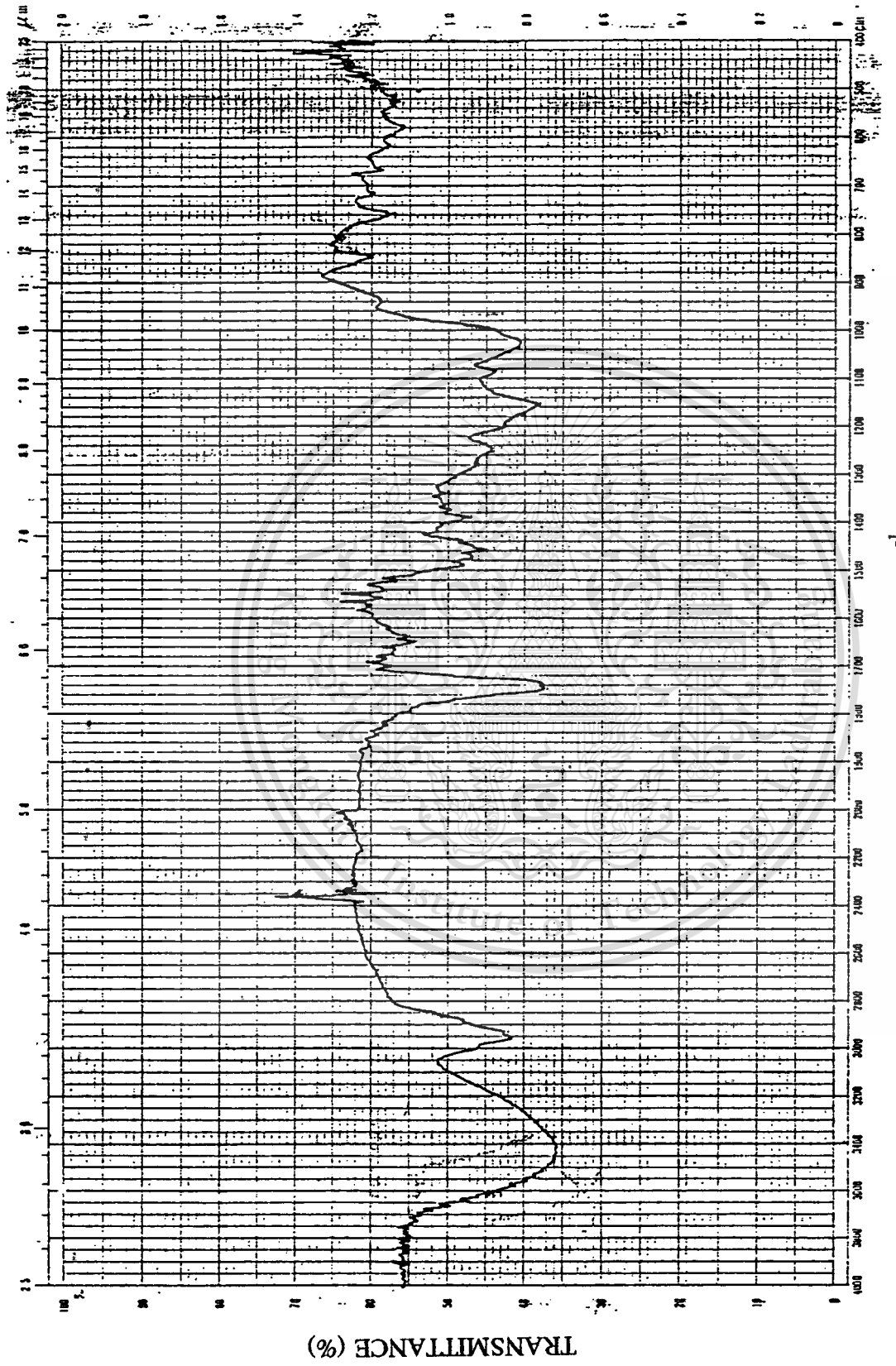


Fig. 4.63 IR Spectra of Starch Graft Poly(MMA/BA)

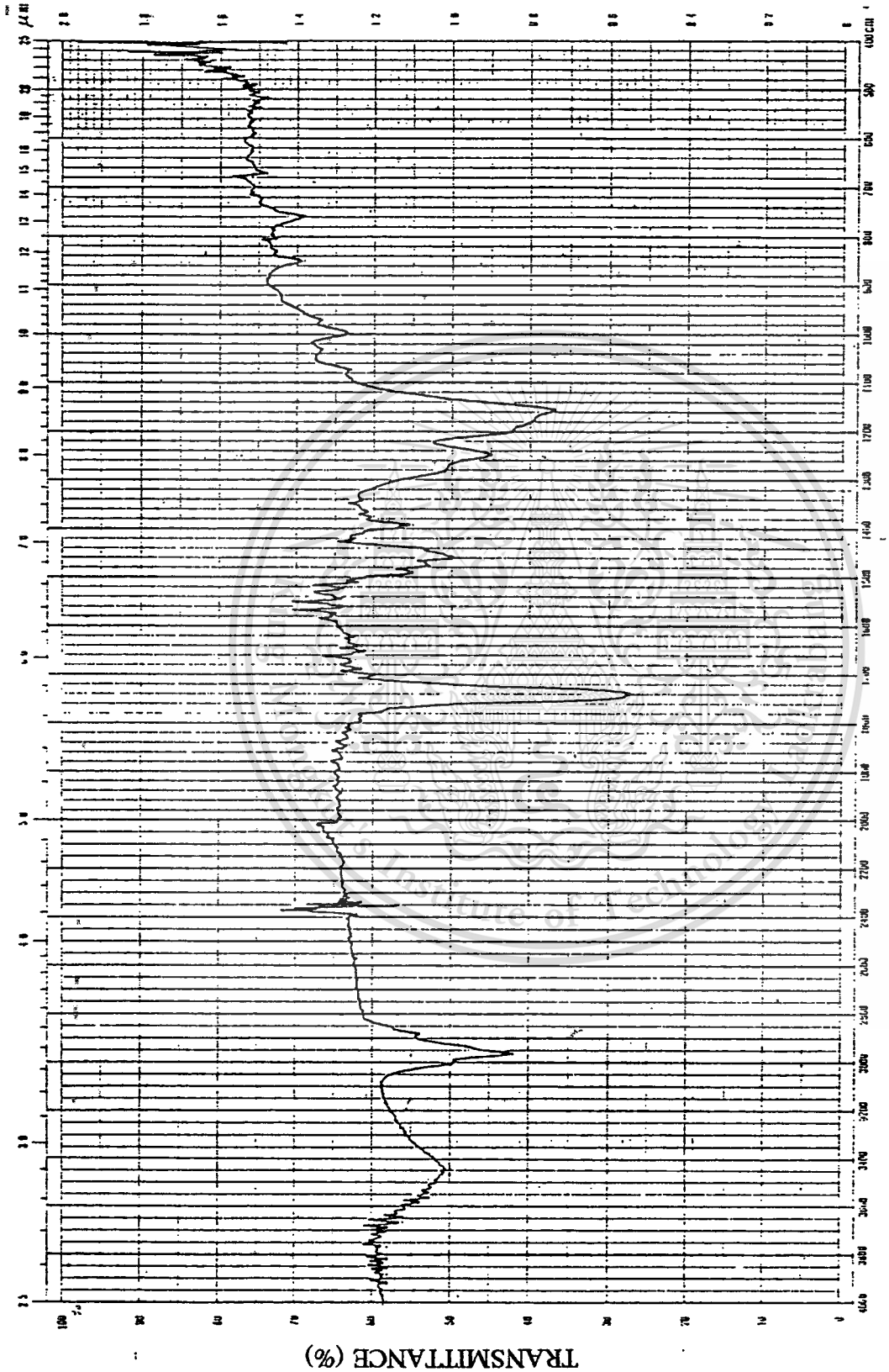


Fig. 4.64 IR Spectra of Poly(MMA/BA) Grafted Polymer

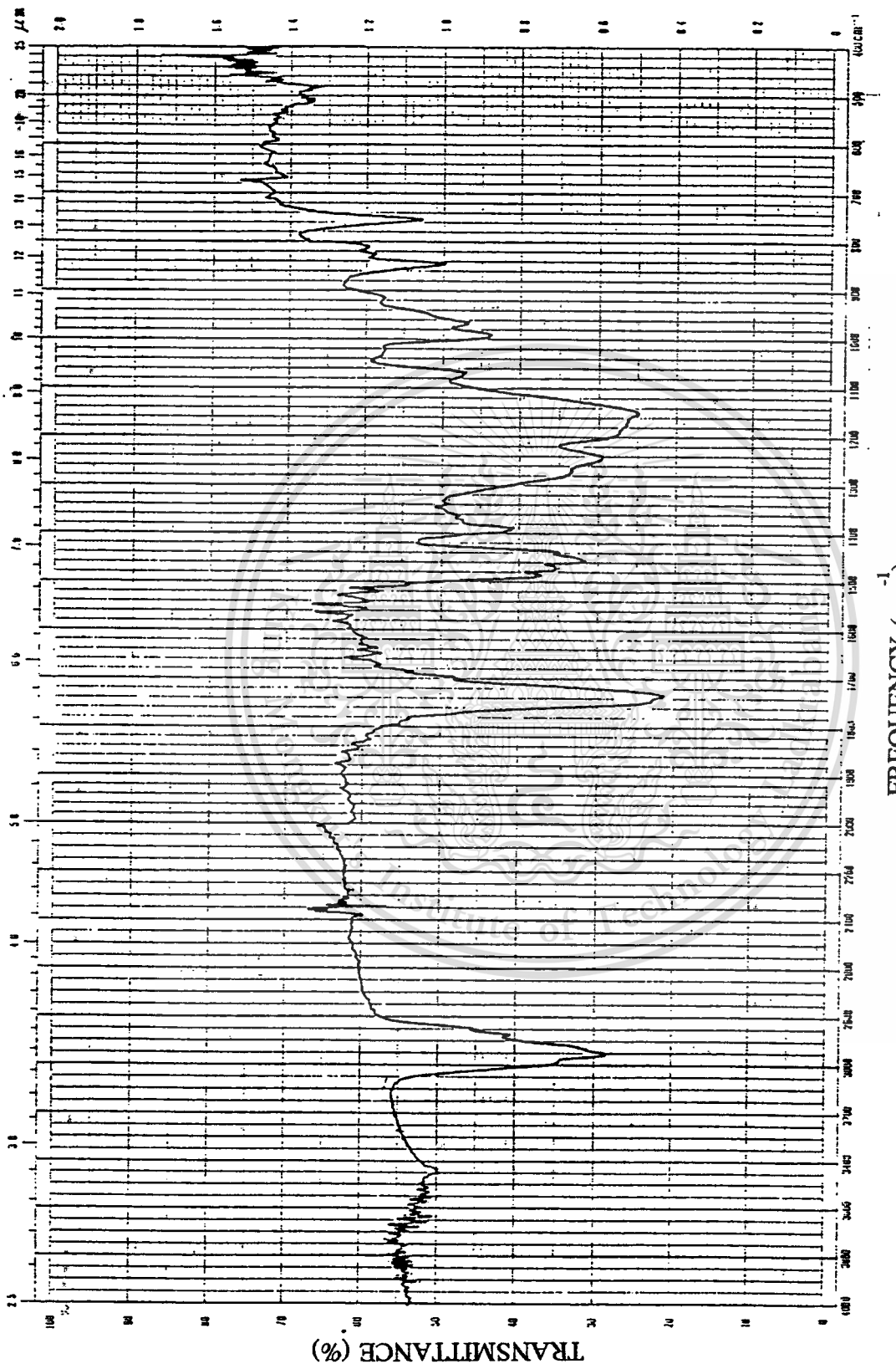


Fig. 4.65 IR Spectra of Poly(MMA/BA) Free Polymer

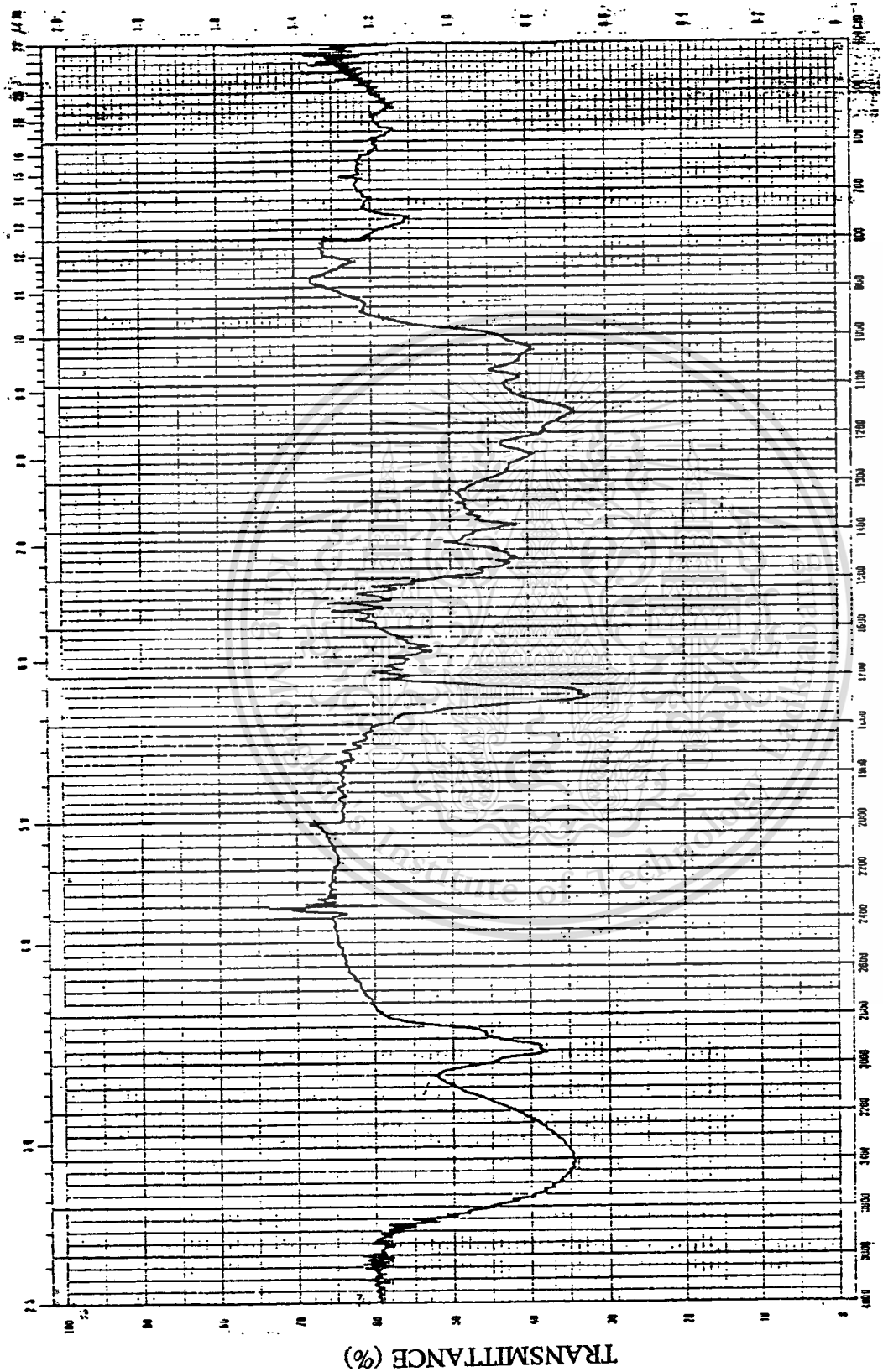


Fig. 4.66 IR Spectra of Starch Graft Poly(MMA/2-EHA)

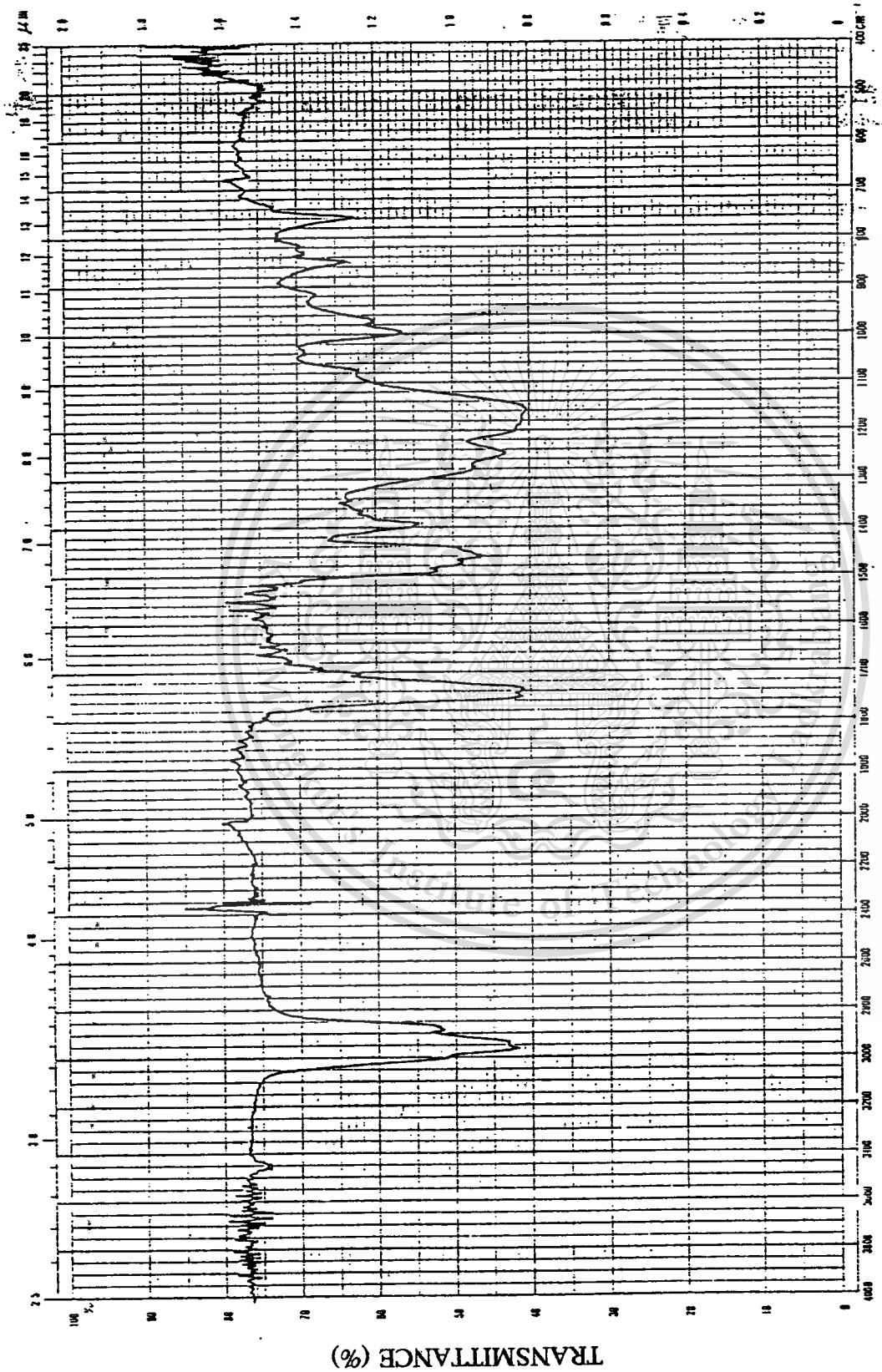


Fig. 4.67 IR Spectra of Poly(MMA/2-EHA) Free Polymer

4.2.2 Effect of Monomers

There were four monomers (MMA, EA, BA, and 2-EHA) which were used to graft onto tapioca starch and using potassium persulfate ($K_2S_2O_8$) as initiator. Each vinyl monomers was independently graft-polymerized onto tapioca starch. The results obtained are set out in Table 4.7.

Table 4.7 Graft Yields for the Graft Copolymerization of Pure Monomers onto Tapioca Starch

Monomer	TSC[c] (%)	Conversion (%)	Free Polymer (%)	G[d] (%)	GE[e] (%)	GF[f] (AGU/Chain)	MW of Grafted Chain
MMA[a]	12.20	78.69	33.38	23.45	31.88	9,771.09	484,968
EA[a]	9.92	63.97	39.99	19.19	19.19	29,406.09	895,110
MMA[b]	11.77	76.47	45.21	25.35	35.93	6,938.30	381,695
BA[b]	17.40	95.74	26.59	49.65	65.12	7,868.62	1,256,995
2-EHA[b]	19.23	90.96	17.46	51.84	74.80	4,585.89	799,681

[a]Reaction Condition : tapioca starch 150 g., propylene glycol (PG) 1.5 g., NP-40 = 10% wt. monomer, and 0.0150 mole of $K_2S_2O_8$, in water 1800 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

[b]Reaction Condition : tapioca starch 100 g., propylene glycol (PG) 1.0 g., NP-40 = 10% wt. monomer, Na_2EDTA 25 ml., and 0.0100 mole of $K_2S_2O_8$, in water 1200 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

[c]TSC = Total solid content

[d]G = Pecentage of grafting

[e]GE = Grafting efficiency

[f]GF = Grafting frequency

Khalil et al. (1993) concluded that differences in grafting with different monomers could be interpreted in terms of differences among different monomers (acrylamide,

acrylonitrile, methacrylic acid, and acrylic acid) with respect to (1) polarizability of the vinyl double bond, (2) solubility, (3) affinity of the monomer to starch and its ability to diffuse into the starch molecules and (4) ability of monomer to homopolymerization [37].

Graft copolymerization with butyl acrylate (BA) and 2-ethyl hexyl acylate (2-EHA) monomers onto tapioca starch backbones had higher conversion, > 90%, and total solid content (TSC) than grafting with methyl methacrylate (MMA) monomer. Percentage of grafting (G) and grafting efficiency (GE) of starch graft copolymerization with 2-EHA and BA monomers were also higher than grafting with MMA monomer. These results indicated that affinity of BA and 2-EHA monomer to tapioca starch and their ability to diffuse into starch molecules were higher than MMA monomer. In addition, homopolymerization of grafting with 2-EHA and BA monomers were less than grafting with MMA monomer, so that monomers were conversed into grafted polymers more than homopolymers.

Starch graft copolymers were hydrolyzed to separate grafted polymer and were characterized with GPC. Molecular weight of grafted poly(2-EHA) and poly(BA) were higher than grafted poly(MMA) at the same condition.

Grafting frequency (GF) were ordered with monomer as following: 2-EHA<MMA <BA. Grafting frequency indicates average distance of AGU per one grafted chain, so higher grafting frequency is more distance between each grafted chain and good graft polymerization will be low GF. From the results, 2-EHA monomer was the best among 4 monomers for grafting on tapioca starch which using $K_2S_2O_8$ as initiator.

Starch graft copolymerization with ethyl acrylate (EA) monomer was lower TSC, conversion, G and GE than with methyl methacrylate (MMA) monomer at the same condition. Similarly, free polymer, grafting frequency and molecular weight of grafted polymer of starch graft copolymerization with EA monomer was higher than with MMA monomer. These results pointed EA monomer had less efficiency in starch graft copolymerization than MMA monomer.

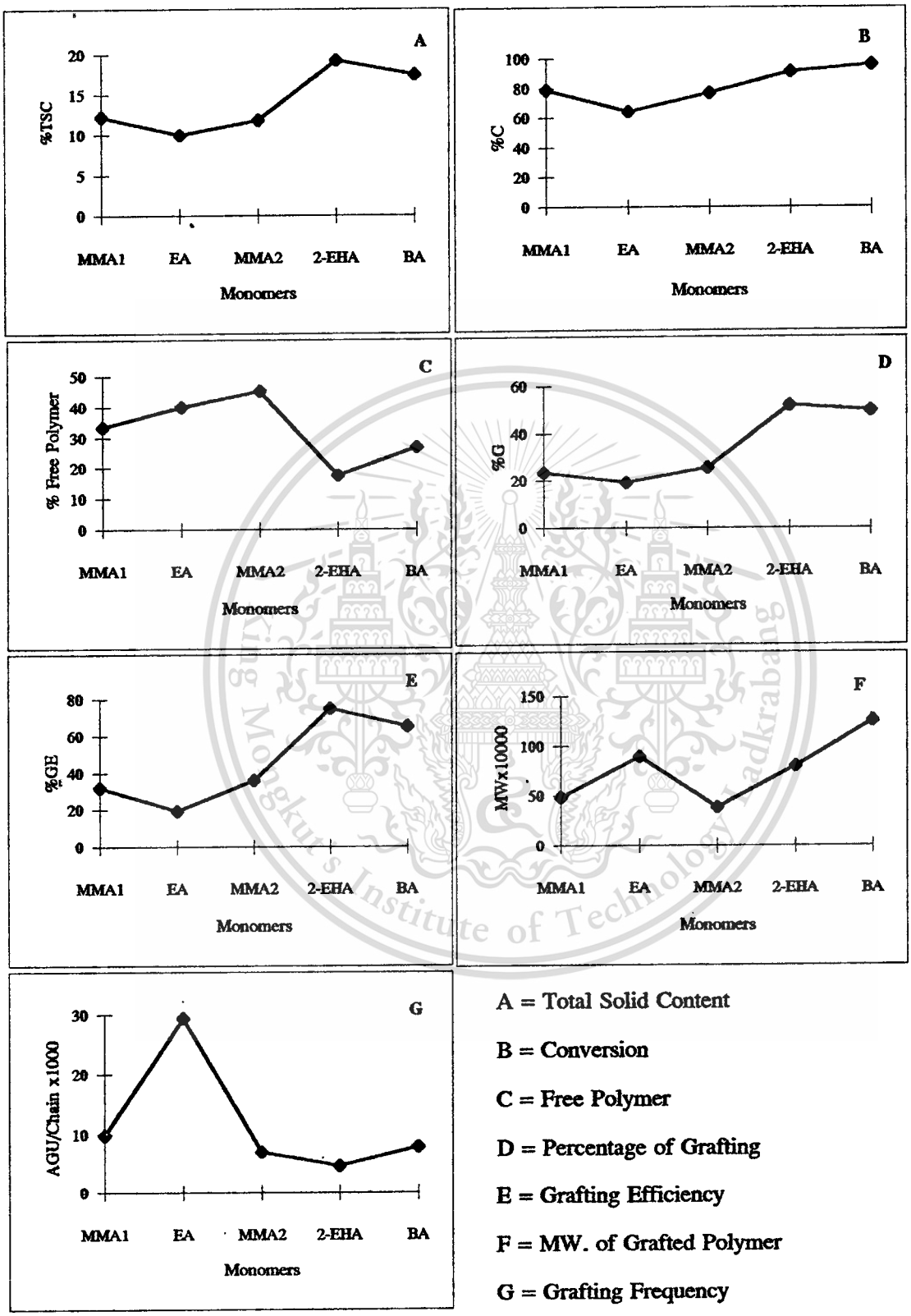


Fig. 4.68 Effect of Monomers on Tapioca Starch Graft Copolymerization

In conclusion, 4 monomers were different efficiency in graft copolymerization onto tapioca starch because of their nature. However, from the data it is clear that efficiency of monomer to graft onto tapioca starch were following order : 2-EHA>BA>MMA>EA.

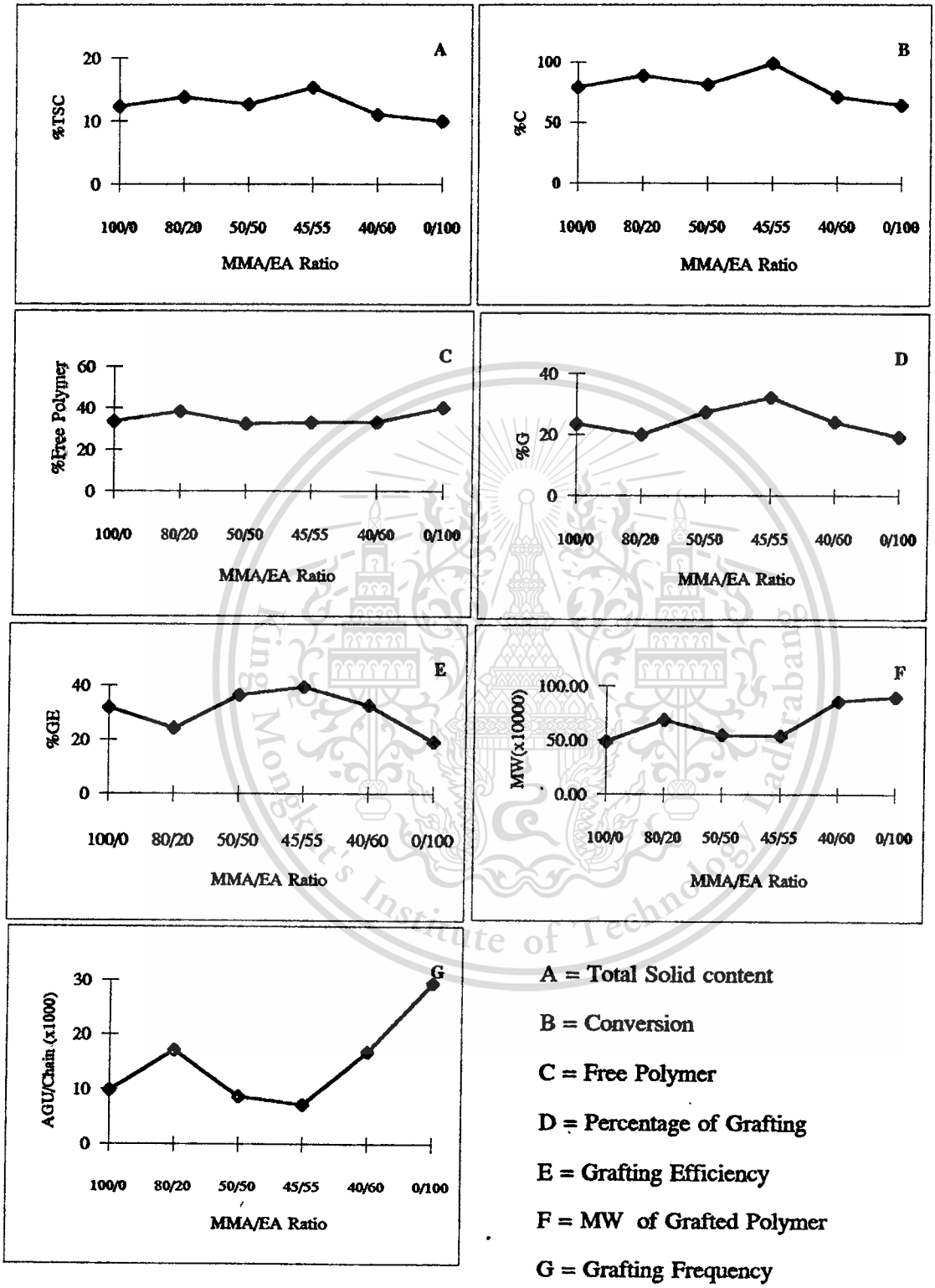
4.2.3 Effect of Monomer Ratios

Tapioca starch were used to graft polymerization with a mixed monomers between methacrylate monomer (MMA) and acrylate monomer (2-EHA, BA, and EA). The mixed monomers were MMA/EA, MMA/BA, and MMA/2-EHA with variable with set up ratio at 200% mole monomer.

Table 4.8 Graft Yields for the Graft Copolymerization of a Mixture of MMA and EA Monomers onto Tapioca Starch[a]

Monomer Ratio MMA/EA	TSC (%)	Conversion (%)	Free Polymer (%)	G (%)	GE (%)	GF (AGU/chain)	MW of Grafted Polymer
100/0	12.20	78.69	33.38	23.45	31.88	9,771.09	484,968
80/20	13.69	88.33	38.29	19.86	24.25	17,024.32	683,522
50/50	12.57	81.10	32.27	27.32	36.45	8,579.39	546,427
45/55	15.30	98.73	32.98	32.02	39.42	7,083.25	540,468
40/60	10.99	70.94	33.08	23.98	32.66	16,745.40	855,348
0/100	9.92	63.97	39.99	19.19	19.19	29,406.09	895,110

[a]Reaction Condition : tapioca starch 150 g., propylene glycol (PG) 1.5 g., NP-40 = 10% wt. monomer, and 0.0150 mole of $K_2S_2O_8$, in water 1800 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.



A = Total Solid content

B = Conversion

C = Free Polymer

D = Percentage of Grafting

E = Grafting Efficiency

F = MW of Grafted Polymer

G = Grafting Frequency

Fig. 4.69 Effect of a Mixture of MMA and EA Monomer on Tapioca Starch Graft Copolymerization

The mixture of MMA and EA monomer feed composition (MMA/EA) were 100/0, 80/20, 50/50, 45/55, 40/60 and 0/100. Table 4.8 showed the values of grafting parameters obtained with each feed compositions. Comparison of the values of grafting parameters obtained showed that TSC, conversion, G and GE from reaction in which mixtures of monomers used had higher values than those obtained from pure monomers, with the maximum value for MMA/EA = 45/55. In addition, free polymer, molecular weight of grafted chain and grafting frequency (GF) of starch graft polymerization with a mixture of MMA/EA were lower than graft polymerization with pure monomers (MMA or EA).

These results agree with results of Goni et al. for the study of study of starch graft copolymerization with a mixture of MMA with EA on maize amylose by C^{13} n.m.r. that grafting with a mixture monomers had grafting parameter values better than obtained grafting with pure monomers. However, the ratio of monomers of Goni's studying [17] which maximum graft yields were 20/80 (MMA/EA). This would be suggested that different starches, grafting methods and initiator made different ratio of MMA/EA to maximum graft yields.

Consequently, a mixture of methyl methacrylate (MMA) and butyl acrylate (BA) used to graft onto tapioca starch was studied. The mixture of monomer (MMA/BA) feed composition were 100/0, 95/5, 80/20, 70/30, 60/40, and 0/100. The grafting characteristics were shown in Table 4.9 and Fig. 4.70.

TSC, conversion, G, and GE values of grafting with a mixture of MMA/BA monomers were higher than grafting with pure MMA monomer. Higher BA monomer composition in feed monomer tended to increase these values (TSC, conversion, G, and GE), while free polymer, molecular weight of grafted polymer and grafting frequency tended to decrease. These results indicated that BA monomer increased efficiency of reaction.

Table 4.9 Graft Yields for the Graft Copolymerization of a Mixture of MMA and BA Monomers onto Tapioca Starch[a]

Monomer Ratio MMA/BA	TSC (%)	Conversion (%)	Free Polymer (%)	G (%)	GE (%)	GF (AGU/chain)	MW of Grafted Polymer
100/0	11.77	76.47	45.21	25.35	35.93	6,938.30	381,695
95/5	15.51	92.34	51.64	29.70	36.52	7,893.24	540,221
80/20	15.80	96.88	52.86	32.49	38.09	9,218.05	718,680
70/30	16.01	93.88	49.69	33.73	40.43	7,320.15	603,579
60/40	16.22	96.06	42.50	38.58	47.58	6,004.09	610,963
0/100	17.40	95.74	26.59	49.65	65.12	7,868.62	1,256,995

[a]Reaction Condition : tapioca starch 100 g., propylene glycol (PG) 1.0 g., NP-40 = 10% wt. monomer, Na_2EDTA 25 ml., and 0.0100 mole of $K_2S_2O_8$, in water 1200 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

Contrustly, increasing MMA monomer composition in feed monomer of graft polymerization with a mixture of MMA/BA decreased TSC, conversion, G and GE when comparison with starch graft copolymerization with pure BA monomer. The tendency of free polymer, MW of grafted chain and GF increased following MMA monomer feed composition ratio, increased. These probably suggested that MMA monomer had lower affinity and ability to diffuse into starch molecule, but reactivity of MMA monomer was higher than BA monomer, so that MMA monomer would interrupt the starch graft polymerization system with BA monomer.

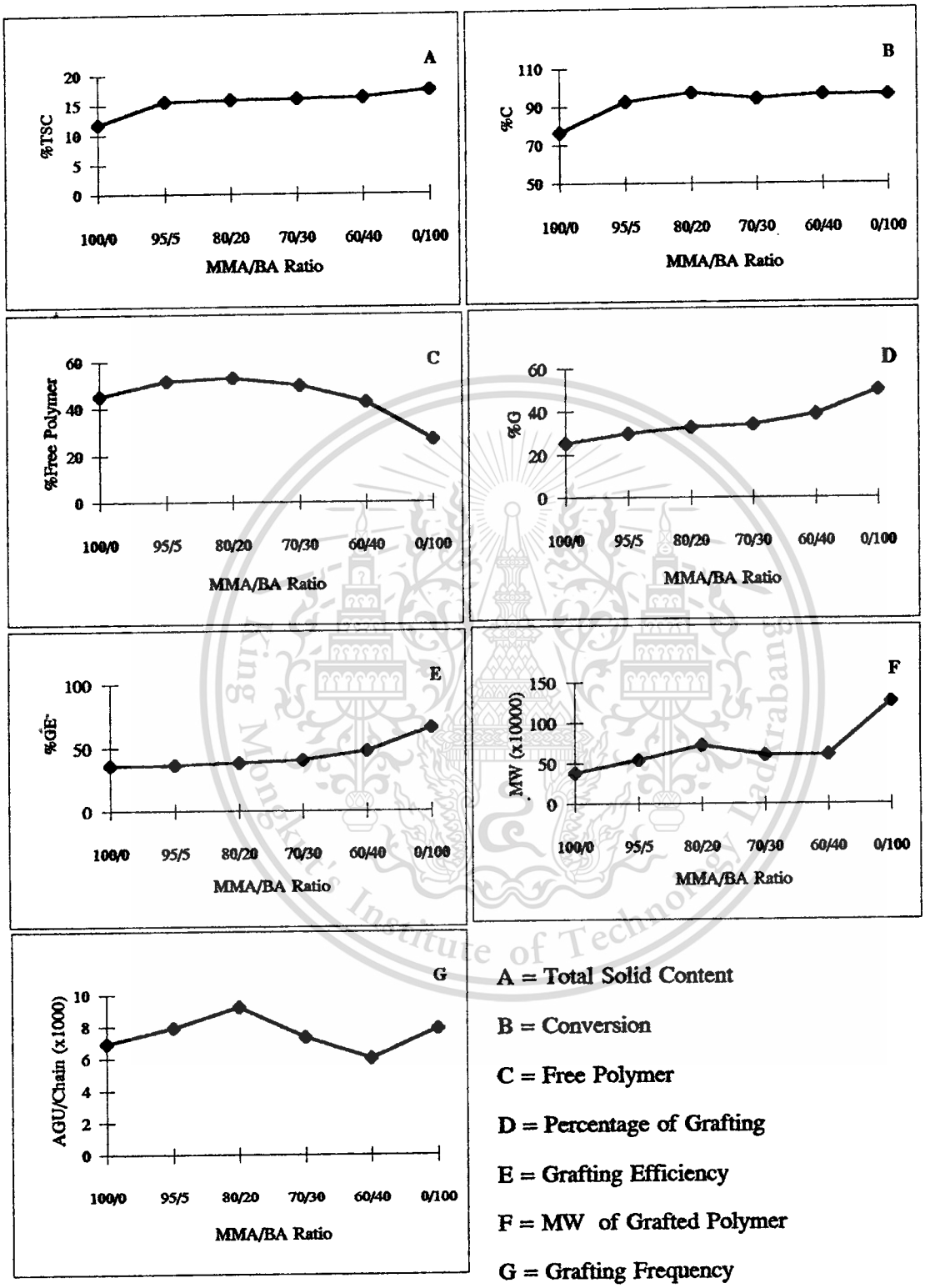


Fig. 4.70 Effect of a Mixture of MMA and BA Monomers on Tapioca Starch Graft Copolymerization

Finally, the mixture of MMA with 2-EHA used to graft onto tapioca starch were studied. The feed monomers composition of MMA/2-EHA monomers were 100/0, 95/5, 80/20, 70/30, 60/40, and 0/100. The grafting characteristics were shown in Table 4.10 and Fig. 4.71.

Table 4.10 Graft Yields for the Graft Copolymerization of a Mixture of MMA and 2-EHA Monomers onto Tapioca Starch[a]

Monomer Ratio MMA/2-EHA	TSC (%)	Conversion (%)	Free Polymer (%)	G (%)	GE (%)	GF (AGU/Chain)	MW of Grafted Polymer
100/0	11.77	76.47	45.21	25.35	35.93	6938.30	381,695
95/5	15.71	95.28	53.63	31.02	36.64	8317.12	605,908
80/20	16.62	97.71	52.37	36.78	41.26	7590.47	715,387
70/30	17.21	95.33	46.90	40.64	46.42	7671.11	850,811
60/40	17.81	96.97	39.43	46.38	54.05	4613.25	646,736
0/100	19.23	90.96	17.46	51.84	74.80	4585.89	799,681

[a]Reaction Condition : tapioca starch 100 g., propylene glycol (PG) 1.0 g., NP-40 = 10% wt. monomer, Na_2EDTA 25 ml., and 0.0100 mole of $K_2S_2O_8$, in water 1200 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

The tendency of increasing 2-EHA monomer in feed monomers composition of grafting system with a mixture of MMA/2-EHA monomer to graft polymerization was similar to increasing BA monomer in feed monomer of grafting system with a mixture of MMA/BA monomers. From the data obtained it is clear that 2-EHA monomer increased efficiency of graft polymerization with a mixture of MMA/2-EHA monomers when comparison with graft polymerization with pure MMA monomer. However, if grafting parameter values (TSC, conversion, G, and GE) of graft polymerization with a mixture of MMA/2-EHA monomers were compared with graft polymerization with pure 2-EHA monomer, these values would be decrease when increasing of MMA monomer in feed composition of graft polymerization with a mixture of MMA/2-EHA monomers. As the suggestion of graft polymerization

with a mixture of MMA/BA, MMA monomer added in grafting system with a mixture of MMA/2-EHA monomer might interrupt grafting polymerization system which reducing efficiency of reaction.

In conclusion, starch graft polymerizations of a mixture of methacrylate monomer (MMA) and acrylate monomer (EA, BA, 2-EHA) were different results depending on nature of monomers. The starch graft polymerization with a mixture of MMA/EA monomers had higher graft yields than grafting with pure monomer, which maximum grafting yield at MMA/EA ratio = 45/55. The graft yields of starch graft polymerization with a mixture of MMA/BA monomers were increased following ratio of BA monomer in feed monomer composition of MMA/BA increased. Similarly, the graft yields of starch graft polymerizations with MMA/2-EHA monomer also tended to increase following ratio of 2-EHA monomer in feed composition increased. However, both graft yields of starch graft polymerization with a mixture of MMA/BA and MMA/2-EHA monomers were less than grafting with pure BA and 2-EHA monomers, respectively.

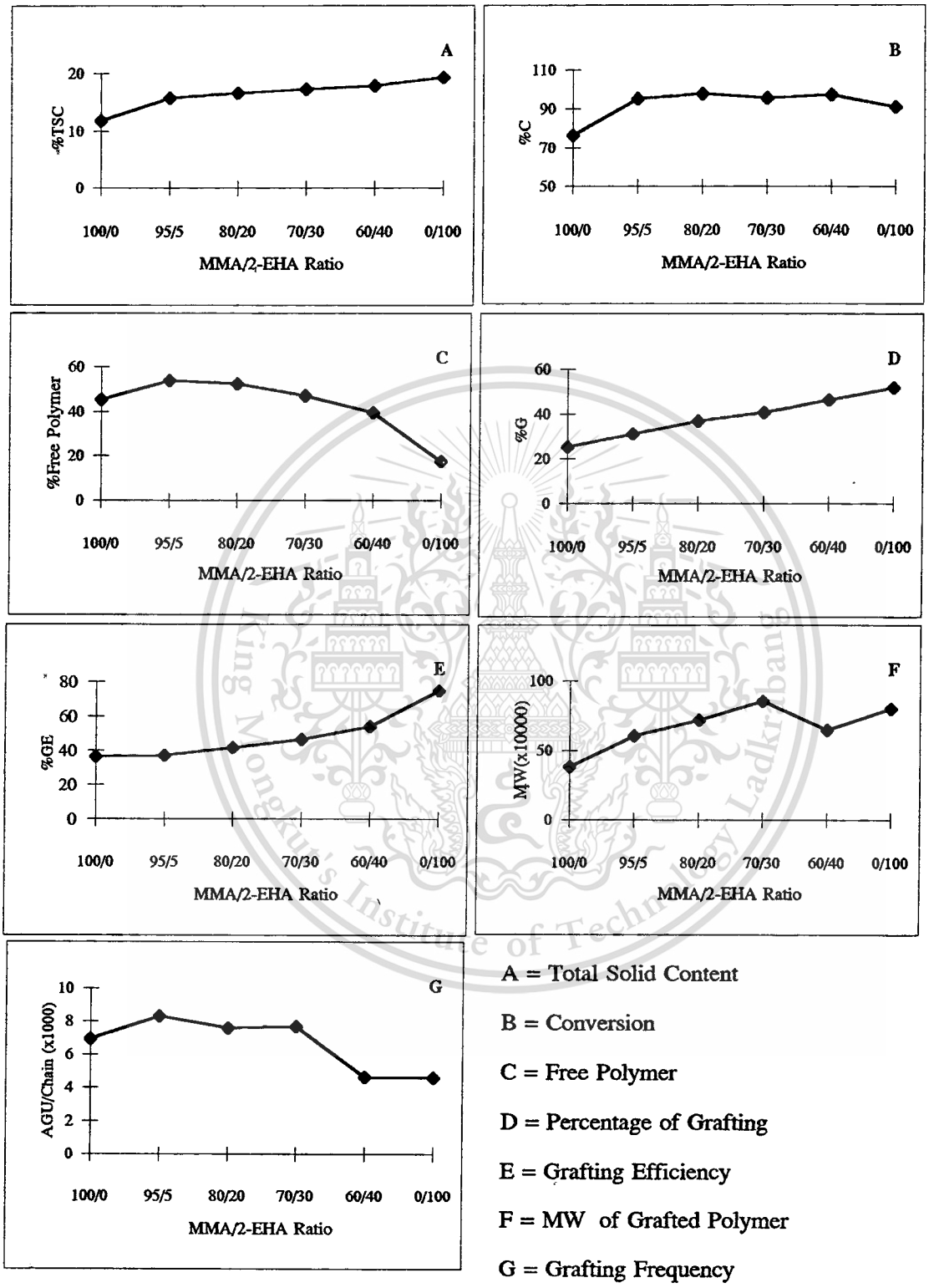


Fig. 4.71 Effect of a Mixture of MMA and 2-EHA Monomers on Tapioca Starch Graft Copolymerization

4.2.4 Effect of Initiator Concentration

Table 4.11 shows the effect of potassium persulfate ($K_2S_2O_8$) concentration on the magnitude of grafting of a mixture of MMA/EA monomers onto tapioca starch. The $K_2S_2O_8$ concentration were 0.0100-0.0200 mole. Total solid content (TSC) (Fig. 4.72 A) was increased with the increasing of $K_2S_2O_8$ at the beginning, and then slighly increased with further increasing of $K_2S_2O_8$ over 0.0150 mole. Similarly, conversion of reaction were also same results.

The tendency of increasing $K_2S_2O_8$ concentration on percentage of grafting (G) are shown in Fig. 4.72, which G was rapidly increased with increasing of $K_2S_2O_8$ concentration from 0.0100 mole to 0.0150 mole, and then rapidly decreased with further increasing of $K_2S_2O_8$ concentration. The results of increasing $K_2S_2O_8$ concentration on grafting efficiency (GF) were samilar to percentage of grafting.

Table 4.11 Effect of Initiator Concentration on Graft Yields of Tapioca Starch Graft Copolymerization with a Mixture of MMA/EA Monomers.

Amount of $K_2S_2O_8$ (mole)	TSC (%)	Conversion (%)	Free Polymer (%)	G (%)	GE (%)	GF (AGU/chain)	MW of Grafted Polymer
0.0100	12.83	83.46	8.89	8.77	47.15	48,417.86	754,234
0.0125	14.54	95.47	29.64	6.95	14.26	-	-
0.0150	15.30	98.73	32.98	32.02	39.42	7,083.25	540,468
0.0175	15.56	98.61	32.44	4.90	10.34	154,718.50	929,929
0.0200	15.62	99.85	29.83	3.57	6.90	109,697.70	915,725

[a]Reaction Condition : tapioca starch 150 g., propylene glycol (PG) 1.5 g., NP-40 = 10% wt. monomer, and 200% mole monomer with MMA/EA monomer ratio = 45/55, in water 1800 ml., temp.= 60°C 3 hr. and 90°C 1 hr.

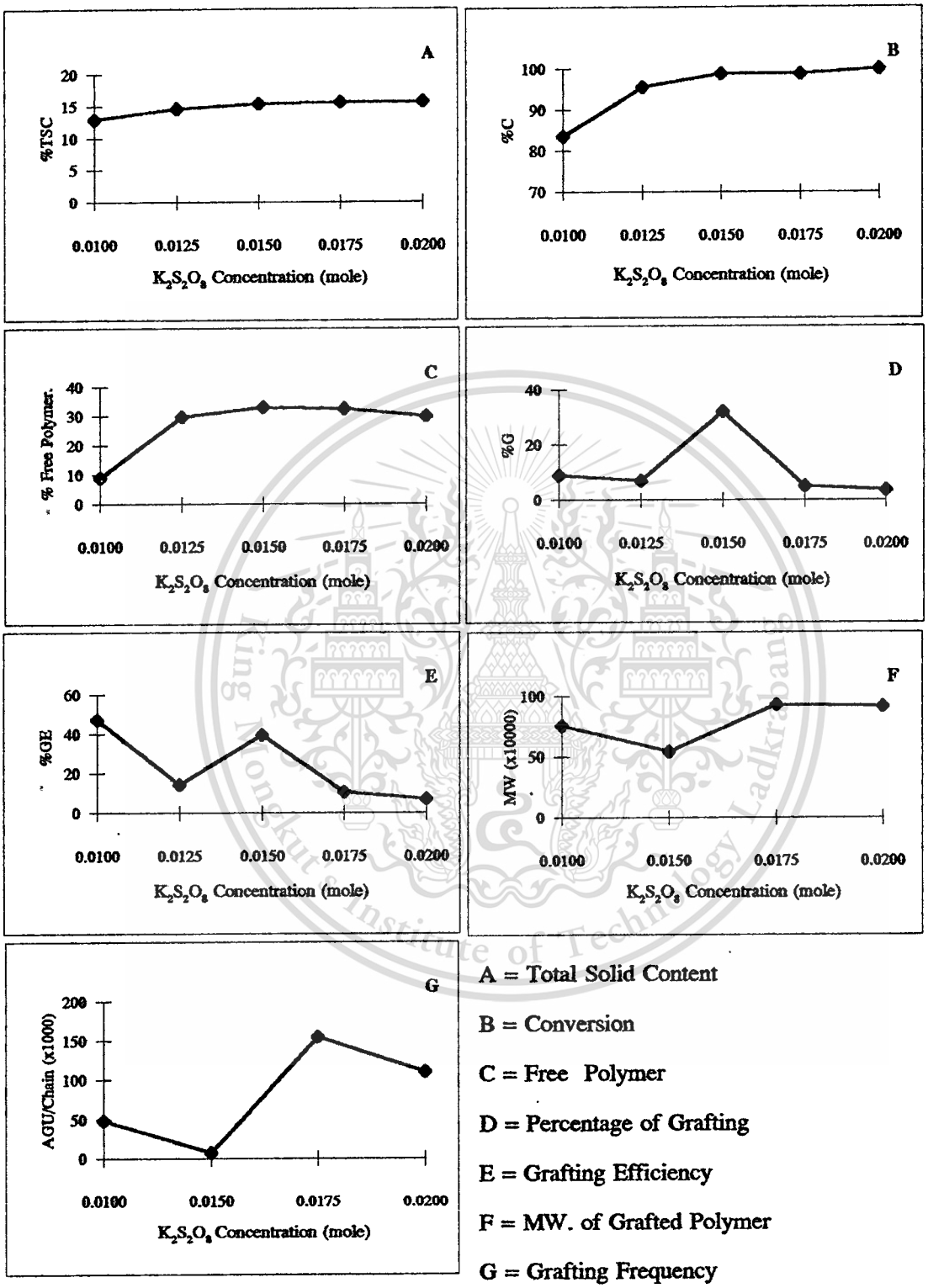


Fig. 4.72 Effect of Potassium Persulfate Concentration on Tapioca Starch Graft Copolymerization

Molecular weights (MW) of grafted polymers were analyzed and grafting frequency (GF) were calculated. Both MW and GF were decreased with increasing of $K_2S_2O_8$ concentration at beginning and then increasing with further increasing of $K_2S_2O_8$ concentration over 0.0150 mole.

Previous works [22] have shown that the procedure of sulfate ion-radicals, which were gennerated from decomposition of persulfate ion heated in aqueous solution :

$$S_2O_8^{2-} \longrightarrow 2\ SO_4^{-\bullet} \qquad (4.1)$$

Then, the sulfate radicals may react with water to procuce hydroxy radicals :

$$SO_4^{-\bullet} + H_2O \longrightarrow HSO_4^{-} + HO^{\bullet} \qquad (4.2)$$

Next, the sulfate and the hydroxy free radicals attrack the starch macromolecules via hydrogen abstraction, thereby starch macroradicals were generated and initiating vinyl grafting :

$$St\text{-}H + SO_4^{-\bullet} \longrightarrow St^{\bullet} + HSO_4^{-} \qquad (4.3)$$

$$St\text{-}H + HO^{\bullet} \longrightarrow St^{\bullet} + H_2O \qquad (4.4)$$

Thus, starch macroradicals will initiate grafting when vinyl monomers were in system.

$$St^{\bullet} + CH_2{=}CH(X) \longrightarrow St\text{-}CH_2\text{-}CH(X)^{\bullet} \qquad (4.5)$$

Generally, the amount of radicals will be higher with increasing of persulfate concentration. From the data, it was suggested that at beginning, presence of $K_2S_2O_8$ in polymerization system generated free radical, which participate mainly used in graft initiation, so that the magnitude of grafting was increased.

$$2\,HO^{\bullet} \longrightarrow H_2O_2 \qquad (4.6)$$

$$H_2O_2 + HO^{\bullet} \longrightarrow HO_2^{\bullet} + H_2O \qquad (4.7)$$

$$HO_2^{\bullet} + S_2O_8^{2-} \longrightarrow O_2 + HSO_4^{-} + SO_4^{-\bullet} \qquad (4.8)$$

However, presence of higher $K_2S_2O_8$ concentrations leads to plenty of free radicals. Eq.(4.1) and (4.2) are one of suggestions of free radicals generated. The other forms are shown in Eq. (4.6) - (4.8), which may change manitude of grafting. Besides free radicals generate starch macroradicals and initiating of grafting, they can be generated self-termination of starch macroradicals and growing chain radicals. The plenty of free radicals may increase opportunity to macroradicals self-termination and growing chain radicals. These will decrease the magnitude of grafting and as seeing in results.

4.2.5 Glass-Transition Temperature

Besides using for determination of starch gelatinization, differential scanning calorimetry (DSC) were used to characterized glass-transition temperature (T_g) of starch and starch graft copolymers. Tapioca starch, Rose brand, was characterized with DSC before grafting with vinyl monomers. T_g of pure polymers tabulated in Table 4.12 were reported from other researcher [10]. T_g of starch graft copolymers were analyzed with DSC and reported in Table 4.12. As expected, T_g of starch graft poly(MMA) and starch graft poly(EA) lied between the T_g of corresponding homopolymers. The T_g of starch graft poly(BA) and starch graft poly(2-EHA) were higher than the T_g of corresponding homopolymers. This rising temperature may be due to some specific chain stiffening effect or the possibility of hydrogen bonding between the comonomer residues. Besides molecular structure, moisture content may correspond to T_g of starch graft copolymers.

Table 4.12 Glass-Transition Temperature (T_g) of Tapioca Starch , Pure Polymers, and Tapioca Starch Graft Copolymers

Polymer	T_g (°C)
PMMA[a]	105
PBA[a]	-22
PEA[a]	-54
PEHA[a]	-85
Tapioca Starch[b]	120.70
Tapioca starch graft poly(MMA)[c]	101.50
Tapioca Starch graft poly(BA)[c]	112.10
Tapioca starch graft poly(2-EHA)[c]	135.20
Tapioca starch graft poly(MMA)[d]	100.30
Tapioca starch graft poly(EA)[d]	99.90

[a] Reference from : [10]

[b] at moisture content 10.93 %

[c] Reaction Condition : tapioca starch 150 g., propylene glycol (PG) 1.5 g., NP-40 = 10% wt. monomer, and 0.0150 mole of $K_2S_2O_8$, in water 1800 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

[d] Reaction Condition : tapioca starch 100 g., propylene glycol (PG) 1.0 g., NP-40 = 10% wt. monomer, Na_2EDTA 25 ml., and 0.0100 mole of $K_2S_2O_8$, in water 1200 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

Table 4.13 was showed T_g of tapioca starch graft poly(MMA/EA). T_g of starch graft poly(MMA/EA) at various ratios were a little different values, 94.2-105.4 °C. This may be due to T_g of starch graft poly(MMA) and starch graft poly(EA) were close. However, T_g of these starch graft poly(MMA/EA) were difficult to compare, because grafted materials had different values of grafting, grafting frequency, and molecular weight of grafted polymers. In addition, composition of MMA and EA in grafted polymers were not clear. Although T_g of these

grafted materials could not compare, T_g indicated molecular structure of starch was changed or grafting with these vinyl monomer onto tapioca starch effect to thermal properties of starch.

Table 4.13 Glass-Transition Temperature (T_g) of Starch Graft Poly(MMA/EA) Copolymers

Monomer ratio MMA/EA[a]	T_g (°C)
100/0	100.3
80/20	105.4
50/50	94.2
45/55	100.5
40/60	95.7
0/100	99.9

[a]Reaction Condition : tapioca starch 150 g., propylene glycol (PG) 1.5 g., NP-40 = 10% wt. monomer, and 0.0150 mole of $K_2S_2O_8$, in water 1800 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

CHAPTER 5

CONCLUSIONS

Cassava in an economic plant in Thailand. Annually, tapioca or cassava starch and cassava products are exported and make many thousand million baht. However, almost of cassava products are exported, the problems of cassava products usually occur because of unstability of cassava products price in world market.

To investigate new products from tapioca starch is one of interesting procedures. This does not only reduce problems of cassava products in exporting, but also increase values of cassava products. In addition, the products from tapioca starch will save environment because the susceptibility of tapioca starch to be degraded by bacteria.

Between 1975-1992, Department of Agriculture, Ministry of Agriculture and Cooperatives has recommended 7 varieties of cassava to farmers. These recommended-variety cassava have been developed into more starch containing and resistance to diseases. The physico-chemical properties and molecular structure of tapioca starch from these recommended varieties were not interesting to analyze.

To this study, the physico-chemical properties of these recommended-variety tapioca starches were analyzed. The results were summarized in Table 5.1. These values showed slight difference in group and were fell within range with the other researchers.

In addition, two brands of commercial tapioca starches were analyzed in order to compare with the recommended-variety tapioca starches. From the data, moisture and phosphorus contents were found that there were different values from recommended-variety tapioca starch group. These would be the effect of starch preparations. Besides, the physical properties were also effected from preparation.

Table 5.1 The Physico-Chemical Properties of Tapioca Starches

Chemical & Physical Property	Recommended-Variety Tapioca Starch	Commercial Tapioca Starch
Moisture (%)	7.02 - 9.12	10.93 - 12.24
Ash (%)	0.220 - 0.227	0.220 - 0.226
Phosphorus (%)	0.014 - 0.017	0.007
Crude Fat (%)	0.019 - 0.104	0.036 - 0.041
Crude Protein (%)	0.097 - 0.190	0.048 - 0.152
Amylose (%)	12.66 - 16.74	13.42 - 13.97
Average Granular Size (μm)	12.64 - 15.67	18.54 - 22.79
Gelatinization Temp. (^{o}C)	60.45 - 69.75	64.50 - 71.25
Maximum Viscosity (B.U.)	315 - 495	355 -585
% Transmittance (%)	80.06 - 83.50	79.44 - 79.72

The molecular structure of both tapioca starch groups were analyzed by GPC. All starch chromatograms showed same pattern. There were three fractions, which one amylopectin fraction, and two amylose fractions. These results were confirmed by starch fractionation. The average molecular weights of amylopectins were 2.70-3.40 x 10^{7}, large amyloses were 3.50-4.20 x 10^{6}, and small amyloses were 0.62-1.45 x 10^{6}. However, after fractionation average molecular weights of all fractions were lower. These probably were the effect of fractionation.

All starches were debranched with isoamylase and were analyzed by GPC. Debranched starch chromatograms showed 4 fractions, which could be separated into 3 groups following their patterns and compositions. The debranched starches of commercial tapioca starches had same patterns with first group but different ratios.

Tapioca starch, Rose brand, was modified to derivatives, starch graft copolymers, with emulsion polymerization using potassium persulfate as initiator. The factors effected to graft polymerization were monomers, monomer ratios, and initiator concentration.

1. Effect of monomers : At the same graft polymerization condition, the effect of methacrylate monomer, methyl methacrylate (MMA), on starch graft polymerization were compared with acrylate monomers, ethyl acrylate (EA) ; butyl acrylate (BA) ; and 2-ethyl hexylacrylate (2-EHA). Graft yields of starch graft polymerization with EA monomer were less than grafting with MMA monomer, while graft yields of starch graft polymerization with BA and 2-EHA monomers were higher than grafting with MMA monomer. The effect of monomers to graft polymerization ordered following graft yields were EA<MMA<BA <2-EHA, respectively.

2. Effect of monomer ratios : A mixture of bimonomers were used to graft onto tapioca starch. MMA monomer was used as base monomer and each acrylate monomers was used as co-monomer. To investigate starch graft poly(MMA/EA) showed that graft yields were higher than starch grafted with pure monomers, maximum graft yields at MMA/EA ratio = 45/55. To investigate starch graft poly(MMA/BA), graft yields tended to decrease following MMA monomer composition increased in feed monomers. Similarly, the tendency of graft yields of starch graft poly(MMA/2-EHA) were same as starch graft poly(MMA/BA).

3. Effect of initiator concentration : At beginning, to increase potassium persulfate ($K_2S_2O_8$) tended to increase graft yield. However, when $K_2S_2O_8$ was added higher than 0.0150 mole, TSC and conversion were nearly stable, G and GE were decreased and GF and MW of grafted polymer were increased. This may be due to the plenty of persulfate ions formed termination faster than formed radical sites. The optimum of initiator concentration was 0.0150 mole.

In addition, thermal properties of starch graft copolymers were studied by differential scanning calorimetry (DSC). T_g of starch graft poly(MMA) and starch graft poly(EA) were in

range between tapioca starch and pure monomer as expected. However, T_g of starch graft poly (BA) and starch graft poly(2-EHA) were higher than expectation. This may be due to some specific chain stiffening or possibility of hydrogen bonding between the comonomer residues. Besides above reasons, moisture content, molecular structure (G, GF, MW of grafted polymers) may be the reasons.

T_g of starch graft poly(MMA/EA) at various ratios, excepted MMA/EA ratio = 80/20, were between T_g of starch graft poly(MMA) and starch graft poly(EA). However, the accuracy composition of MMA and EA monomer in starch graft poly(MMA/EA) were not clear, so that T_g raised were only indicated that starch graft poly(MMA/EA) were different compositions.

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APPENDICES

APPENDIX A

Table A-1 Moisture Content of Tapioca Starches (%)

Sample No.	R1	R3	R5	R60	R90	KU50	SR	ST	RO
1	7.19	7.72	8.71	8.16	7.48	8.47	9.21	12.86	11.35
2	7.16	8.03	8.71	9.21	7.06	9.30	9.12	12.91	11.58
3	6.97	7.84	8.52	8.80	7.40	6.95	9.07	13.06	11.62
4	7.22	7.79	8.75	7.98	7.55	8.52	8.96	12.78	11.67
5	7.35	7.74	8.24	8.44	7.53	8.54	8.94	12.96	11.64
6	6.76	7.76	9.16	8.43	7.31	8.72	9.11	11.50	10.47
7	6.60	7.73	9.23	8.67	7.12	8.00	9.12	11.58	10.24
8	6.76	7.86	9.10	8.77	7.17	7.90	9.48	11.55	10.23
9	7.16	7.74	8.72	8.83	7.52	8.33	9.31	11.67	10.38
10	7.03	-	8.99	8.73	-	8.31	8.89	11.57	10.47
Mean	7.02	7.82	8.81	8.50	7.35	8.30	9.12	12.24	10.93
S.D.	0.23	0.11	0.29	0.29	0.18	0.58	0.17	0.67	0.66

Table A-2 Ash Content of Tapioca Starches (%)

Sample No.	R1	R3	R5	R60	R90	KU50	SR	ST	RO
1	0.260	0.270	0.260	0.280	0.250	0.220	0.210	0.250	0.230
2	0.230	0.280	0.260	0.280	0.260	0.220	0.220	0.270	0.210
3	0.230	0.270	0.290	0.270	0.250	0.220	0.240	0.270	0.220
Mean	0.240	0.273	0.270	0.277	0.253	0.220	0.223	0.263	0.220
S.D.	0.014	0.005	0.014	0.005	0.005	0.000	0.012	0.009	0.008

Table A-3 Phosphorus Content of Tapioca Starches (%)

Sample No.	R1	R3	R5	R60	R90	KU50	SR	ST	RO
1	0.016	0.016	0.015	0.015	0.014	0.017	0.016	0.007	0.007
2	0.016	0.016	0.016	0.016	0.014	0.017	0.016	0.008	0.007
3	0.016	0.017	0.016	0.015	0.015	0.017	0.016	0.007	0.006
Mean	0.016	0.016	0.016	0.015	0.014	0.017	0.016	0.007	0.007
S.D.	0.0003	0.0003	0.0001	0.0004	0.0002	0.0002	0.0002	0.0002	0.0004

Table A-4 Crude Fat Content of Tapioca Starches (%)

Sample No.	R1	R3	R5	R60	R90	KU50	SR	ST	RO
1	0.057	0.049	0.096	0.015	0.049	0.096	0.051	0.040	0.049
2	0.055	0.038	0.093	0.022	0.038	0.096	0.054	0.038	0.045
3	0.056	0.046	0.123	0.019	0.045	0.098	0.045	0.032	0.028
Mean	0.057	0.044	0.104	0.019	0.044	0.097	0.050	0.036	0.041
S.D.	0.002	0.005	0.013	0.003	0.005	0.001	0.004	0.003	0.009

Table A-5 Crude Protein Content of Tapioca Starches (%)

Sample No.	R1	R3	R5	R60	R90	KU50	SR	ST	RO
1	0.105	0.183	0.156	0.133	0.123	0.152	0.124	0.049	0.166
2	0.097	0.202	0.153	0.128	0.128	0.129	0.129	0.049	0.156
3	0.099	0.193	0.141	0.128	0.112	0.119	0.115	0.044	0.145
4	0.091	0.183	0.135	0.130	0.113	0.130	0.129	0.059	0.141
5	0.098	-	-	-	0.125	-	0.124	0.049	-
Mean	0.097	0.190	0.146	0.130	0.120	0.133	0.124	0.048	0.152
S.D.	0.003	0.008	0.009	0.002	0.006	0.012	0.005	0.002	0.010

Table A-6 Amylose Content of Tapioca Starches (%)

Sample No.	R1	R3	R5	R60	R90	KU50	SR	ST	RO
1	15.65	14.66	13.55	14.29	13.59	12.74	13.19	13.29	13.49
2	14.64	15.06	14.41	18.00	13.75	12.47	13.49	14.14	14.17
3	14.97	14.83	13.96	17.93	12.02	12.78	13.64	12.84	14.24
Mean	15.09	14.85	13.97	16.74	13.12	12.66	13.44	13.42	13.97
S.D.	0.42	0.17	0.35	1.73	0.78	0.14	0.19	0.54	0.34

Table A-7 Percent Transmittance of Tapioca Starches (%)

Sample No.	R1	R3	R5	R60	R90	KU50	SR	ST	RO
1	80.20	83.70	84.00	82.50	81.00	81.30	81.30	79.00	81.00
2	80.00	84.30	83.50	81.00	82.80	80.00	79.50	80.80	79.30
3	80.70	82.50	81.50	82.00	80.00	80.20	79.30	79.30	78.00
Mean	80.28	83.50	83.00	81.83	81.28	80.50	80.06	79.72	79.44
S.D.	0.28	0.76	1.08	0.62	1.17	0.59	0.91	0.80	1.23

Table A-8 Thermal Properties of Tapioca Starches

Sample	No.	T_o (°C)	T_p (°C)	T_c (°C)	ΔH(mJ)
R1	1	67.30	73.50	82.10	-29.918
	2	67.30	74.20	82.60	-25.387
	3	67.10	74.00	83.30	-28.613
	Mean	67.23	73.90	82.67	-27.973
	S.D.	0.09	0.29	0.49	1.904

Table A-8 Thermal Properties of Tapioca Starches (continued)

Sample	No.	T_o (°C)	T_p (°C)	T_c (°C)	ΔH(mJ)
R8	**1**	65.50	70.70	82.20	-27.521
	2	65.40	70.70	80.20	-24.372
	Mean	65.45	70.70	81.20	-25.947
	S.D.	0.05	0.00	1.00	1.575
R5	**1**	64.80	70.20	84.10	-24.540
	2	65.10	70.80	81.00	-22.922
	3	64.60	70.80	80.60	-20.182
	Mean	64.83	70.60	81.90	-22.548
	S.D.	0.21	0.28	1.56	1.799
R60	**1**	65.00	70.70	82.70	-24.996
	2	65.00	70.70	82.60	-26.919
	3	65.60	70.50	81.30	-29.892
	Mean	65.20	70.63	82.20	-27.269
	S.D.	0.28	0.09	0.64	2.014
R90	**1**	66.30	73.30	83.50	-27.433
	2	67.00	73.30	82.80	-27.778
	Mean	66.65	73.30	83.15	-27.606
	S.D.	0.35	0.00	0.35	0.172
KU50	**1**	67.10	73.30	83.90	-28.778
	2	69.00	72.00	82.50	-39.438
	Mean	68.05	72.65	83.20	-34.108
	S.D.	0.95	0.65	0.70	5.330

Table A-8 Thermal Properties of Tapioca Starches (continued)

Sample	No.	T_o (°C)	T_p (°C)	T_c (°C)	ΔH(mJ)
SR	1	65.60	71.50	88.40	-37.716
	2	65.40	70.90	80.40	-23.298
	Mean	65.50	71.20	84.40	-30.507
	S.D.	0.10	0.30	4.00	7.209
ST	1	63.60	74.10	82.40	-26.490
	2	62.40	73.70	83.80	-30.158
	3	65.90	73.80	83.30	-38.926
	Mean	63.97	73.87	83.17	-31.858
	S.D.	1.45	0.17	0.58	5.217
RO	1	65.10	72.70	83.50	-24.605
	2	63.50	72.20	82.80	-29.767
	3	63.80	72.00	82.90	-21.718
	Mean	64.13	72.30	83.07	-25.363
	S.D.	0.69	0.29	0.31	3.329

APPENDIX B

Table B-1 Retention Time of Pollulan Standard

Molecular Weight (M_n)	Retention Time (min.)
1,660,000	28.017
380,000	31.183
186,000	33.533
100,000	36.283
48,000	39.450
23,700	42.633
12,200	45.150
5,800	47.600

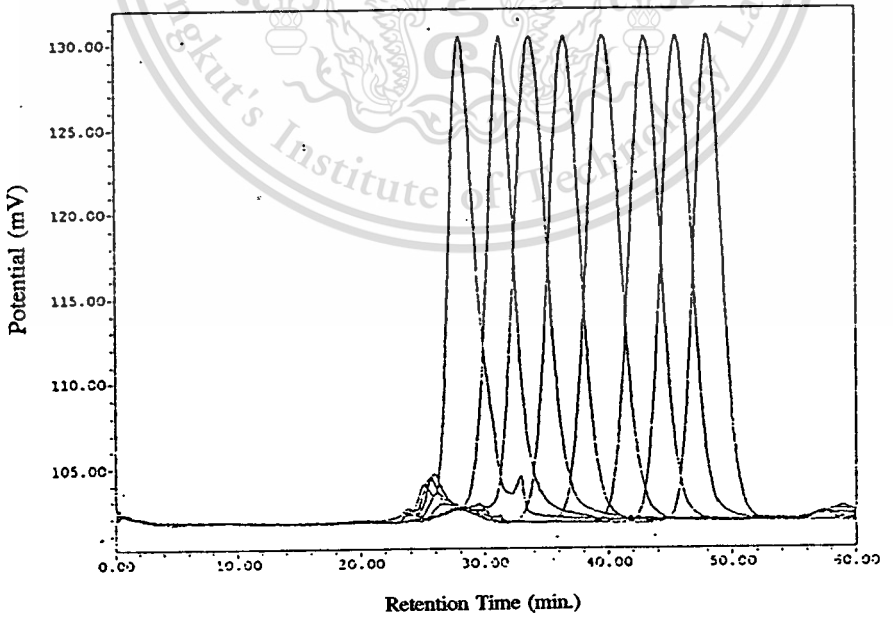


Fig. B-1 Chromatogram of Pollulan Standard

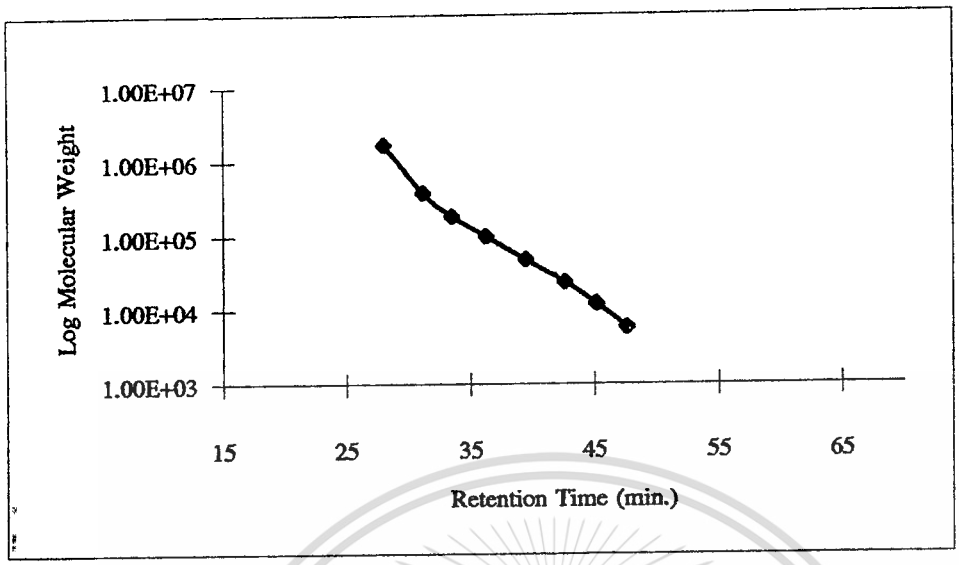


Fig. B-2 Calibration Curve of Pollulan Standard

Table B-2 Retention Time of Tapioca Starch Chromatograms

Sample	Retention Time (min.)		
	Fr.I	Fr.II	Fr.III
R1	21.000	26.183	28.767
R3	20.817	26.100	28.867
R5	20.667	25.950	30.183
R60	20.717	25.950	28.467
R90	20.600	25.717	28.583
KU50	20.683	25.833	30.467
SR	20.450	25.800	28.433
ST	20.817	26.000	31.133
RO	20.683	25.800	31.017

Table B-3 Retention Time of Amylopectin Fraction Chromatograms

Sample	Retention Time (min.)
R1	22.150
R3	21.733
R5	21.583
R60	21.750
R90	21.517
KU50	21.367
SR	21.683
ST	21.450
RO	21.350

Table B-4 Retention Time of Amylose Fraction Chromatograms

Sample	Retention Time (min.)	
	Fr.II	Fr.III
R1	28.117	31.150
R3	27.067	30.067
R5	28.650	32.050
R60	28.467	32.933
R90	27.200	31.450
KU50	26.133	34.367
SR	27.383	32.200
ST	26.800	32.500
RO	26.983	32.283

Table B-5 Retention Time of Debranched Starches of Tapioca Starch Chromatograms

Sample	Retention Time (min.)			
	Fr.I	Fr.II	Fr.III	Fr.IV
R1	25.883	27.700	36.550	52.517
R3	25.533	27.550	36.500	52.633
R5	25.617	27.567	37.100	52.683
R60	26.067	27.600	42.317	52.550
R90	25.400	27.333	40.650	52.567
KU50	25.450	27.150	42.250	52.583
SR	25.483	27.100	44.333	52.683
ST	24.950	26.700	36.367	52.733
RO	25.050	26.717	36.717	52.933

Table B-6 Retention Time of Polystyrene Standard

detector 1 Calibration Report

Printed: 15-FEB-1996 15:21:31

METHOD NAME :
Calibration Type : Narrow Standards

Curve Type : 4th Order
Equation of Curve : log MW = + 3.35E+00 + 3.74E+00*R - 1.11E+00*R^2 + 1.21E-01*R^3 - 4.71E-03*R^4

Correlation Coef : r^2 = 1.00000000
Std Err of Estimate: 0.00000000

Calibration Points :

Ret Time (min)	Specified Molecular Wt	Calculated Molecular Wt	Valid
5.87	1090000	1090000	Yes
6.23	706000	706000	Yes
6.88	355000	355000	Yes
7.57	190000	190000	Yes
8.38	96400	96400	Yes

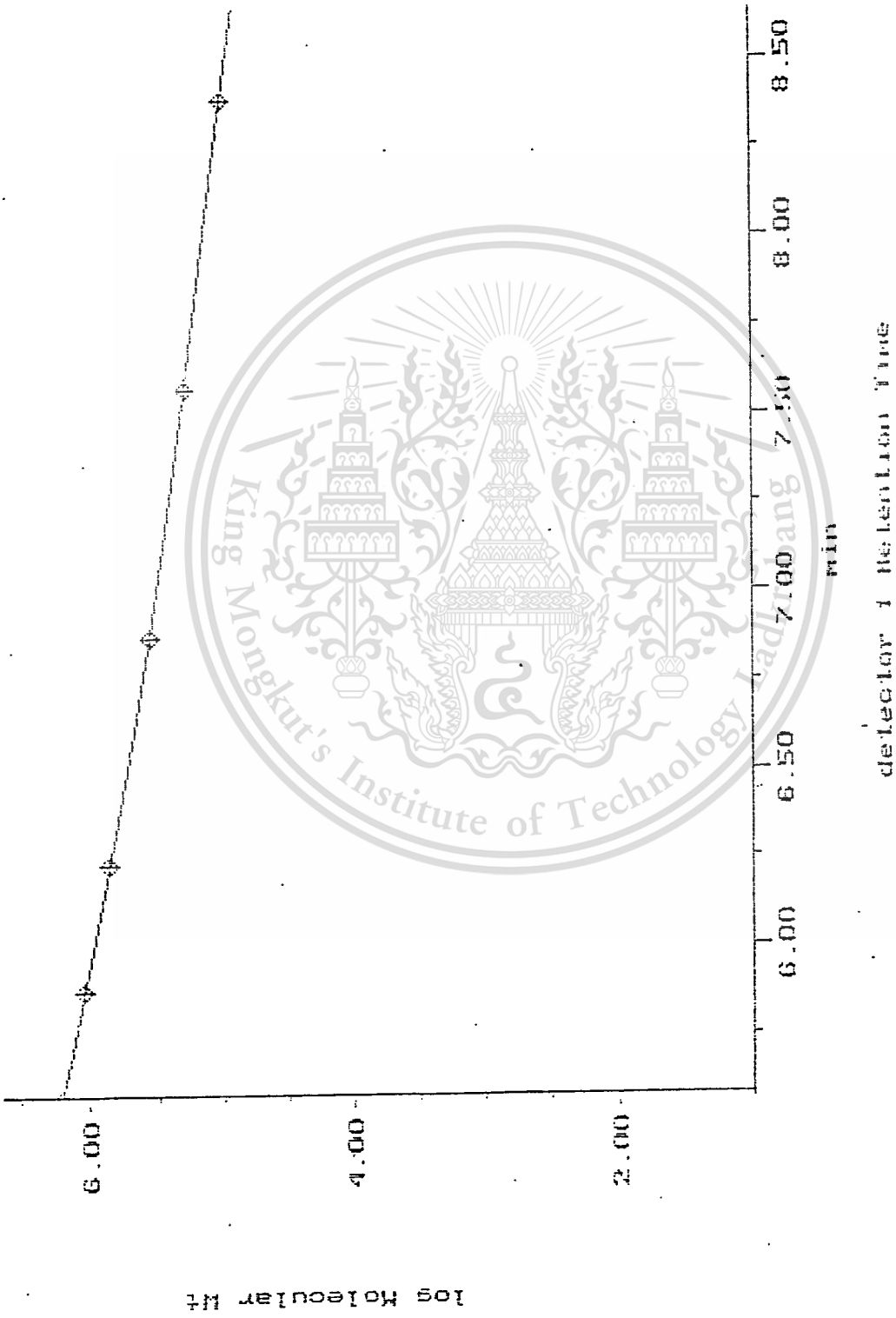


Fig. B-3 Calibration Curve of Polystyrene Standard

Table B-7 Molecular Weight of Grafted Poly(MMA/EA)

Monomer ratio MMA/EA	Mn	Mw	Mv	Mz	Mz+1	polydispersity
100/0	165,194	484,968	484,968	1,232,321	2,020,290	2.938
80/20	268,241	683,522	683,522	1,426,712	2,179,371	2.548
50/50	171,010	546,427	546,427	1,335,150	2,148,390	3.195
45/55	174,247	540,468	540,468	1,340,706	2,188,223	3.102
40/60	303,914	855,348	855,348	1,734,770	2,452,681	2.814
0/100	253,029	895,110	895,110	1,763,204	2,142,485	3.537

Reaction Condition : tapioca starch 150 g., propylene glycol (PG) 1.5 g., NP-40 = 10% wt. monomer, and 0.0150 mole of $K_2S_2O_8$, in water 1800 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

Table B-8 Molecular Weight of Grafted Poly(MMA/BA)

Monomer ratio MMA/BA	Mn	Mw	Mv	Mz	Mz+1	polydispersity
100/0	32,155	381,695	381,695	1,733,985	3,611,886	11.87
95/5	46,466	540,221	540,221	1,987,337	3,845,874	11.63
80/20	37,089	718,680	718,680	2,766,907	4,882,315	19.38
70/30	119,328	603,579	603,579	2,305,963	4,318,051	5.06
60/40	57,842	610,963	610,963	2,296,922	4,285,276	10.56
0/100	92,628	1,256,995	1,256,995	3,468,483	5,173,510	13.57

Reaction Condition : tapioca starch 100 g., propylene glycol (PG) 1.0 g., NP-40 = 10% wt. monomer, Na_2EDTA 25 ml., and 0.0100 mole of $K_2S_2O_8$, in water 1200 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

Table B-9 Molecular Weight of Grafted Poly(MMA/2-EHA)

Monomer ratio MMA/2-EHA	Mn	Mw	Mv	Mz	Mz+1	polydispersity
100/0	32,155	381,695	381,695	1,733,985	3,611,886	11.87
95/5	35,023	605,908	605,908	2,322,564	4,234,641	17.30
80/20	57,594	715,387	715,387	2,468,116	4,297,430	12.42
70/30	66,126	850,811	850,811	3,174,254	5,330,125	12.87
60/40	44,047	646,736	646,736	2,616,554	4,792,870	14.68
0/100	61,148	799,681	799,681	2,750,367	4,728,406	13.08

Reaction Condition : tapioca starch 100 g., propylene glycol (PG) 1.0 g., NP-40 = 10% wt. monomer, Na_2EDTA 25 ml., and 0.0100 mole of $K_2S_2O_8$, in water 1200 ml., 200% mole monomer, temp. 60°C 3 hr. and 90°C 1 hr.

Table B-10 Molecular Weight of Grafted Poly(MMA/EA) at Different Initiator Concentration

Amount of $K_2S_2O_8$ (mole)	Mn	Mw	Mv	Mz	Mz+1	polydispersity
0.0100	349,871	927,929	927,929	1,341,867	2,330,153	2.65
0.0150	174,274	540,468	540,468	1,340,706	2,188,223	3.10
0.0175	254,803	754,234	754,234	1,471,844	2,187,829	2.96
0.0200	310,532	915,725	915,725	1,648,764	2,250,442	2.95

Reaction Condition : tapioca starch 150 g., propylene glycol (PG) 1.5 g., NP-40 = 10% wt. monomer, and 0.0150 mole of $K_2S_2O_8$, in water 1800 ml., 200% mole monomer at ratio 45/55, temp. 60°C 3 hr. and 90°C 1 hr.

APPENDIX C

X-Ray Diffraction Patterns of Tapioca Starches

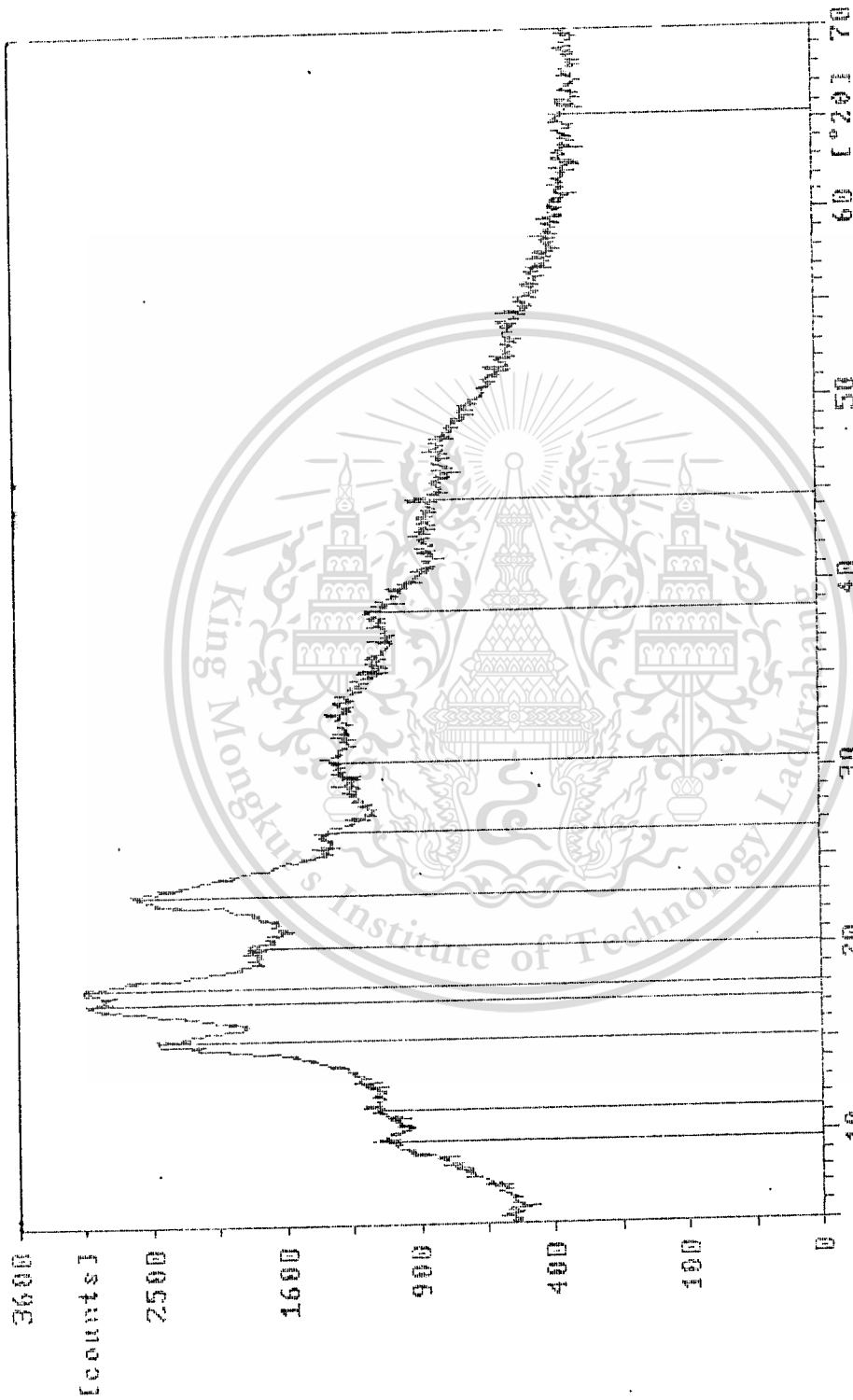


Fig. C-1 X-Ray Diffraction Pattern of R3 Starch

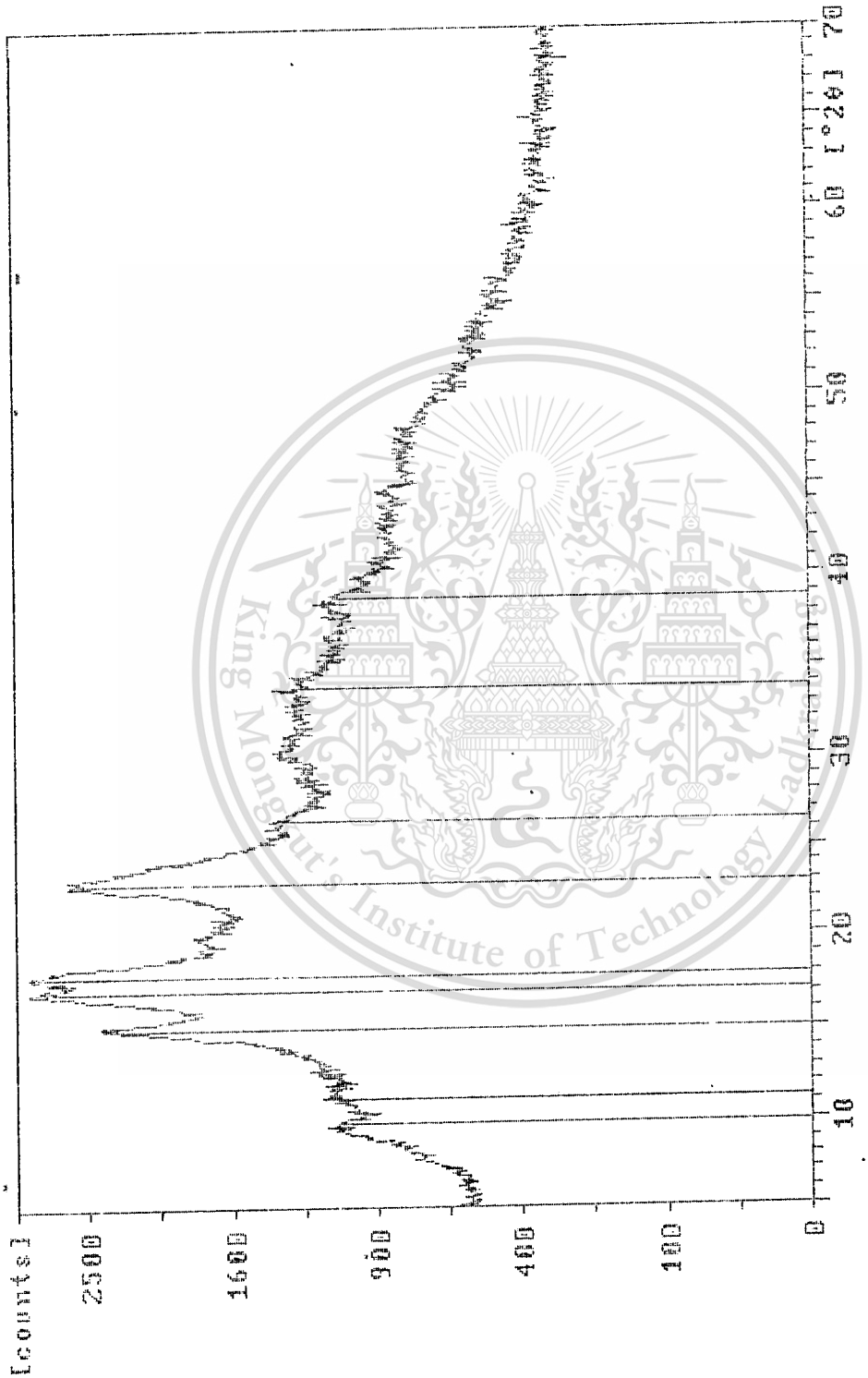


Fig. C-2 X-Ray Diffraction Pattern of R5 Starch

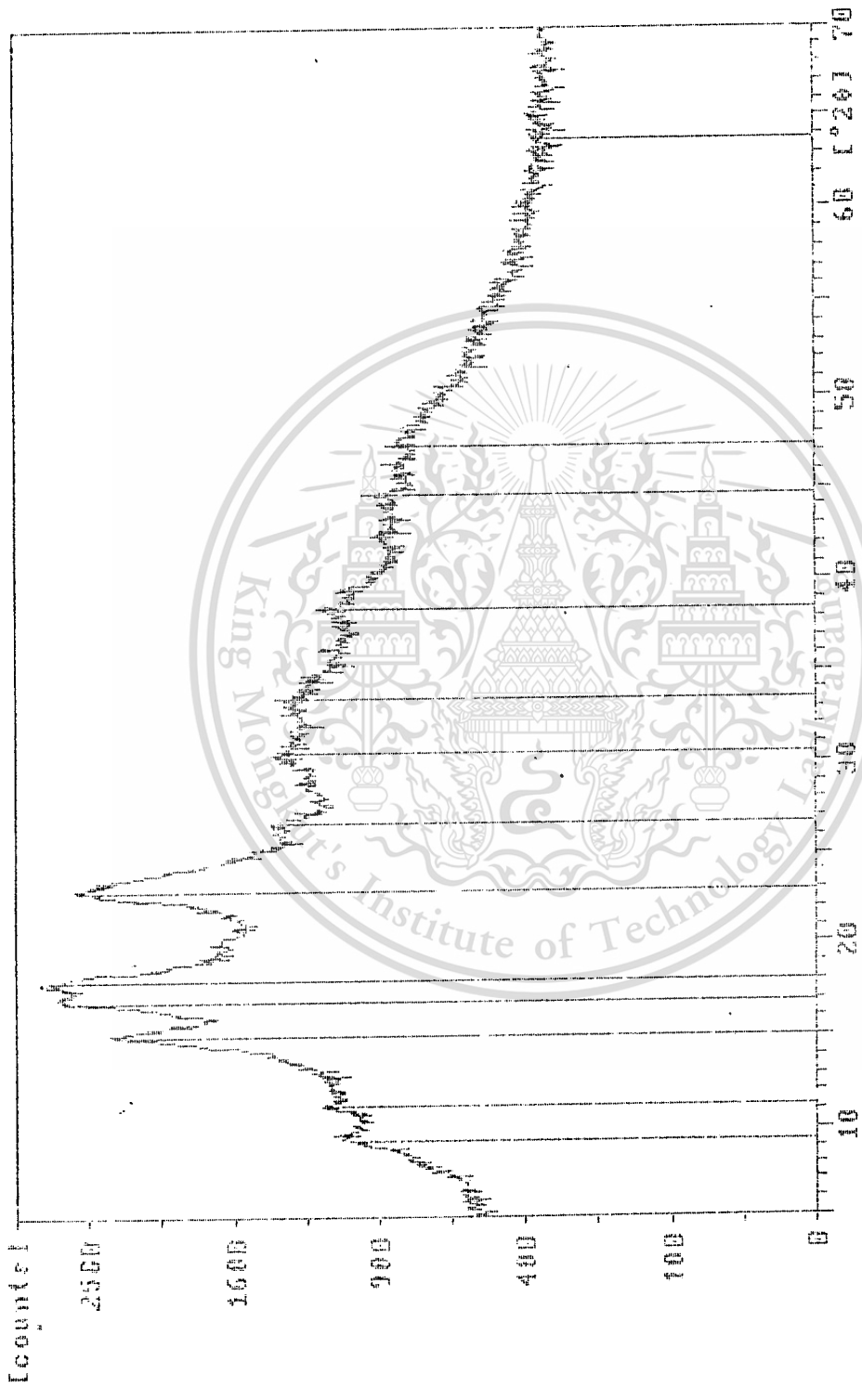


Fig. C-3 X-Ray Diffraction Pattern of R60 Starch

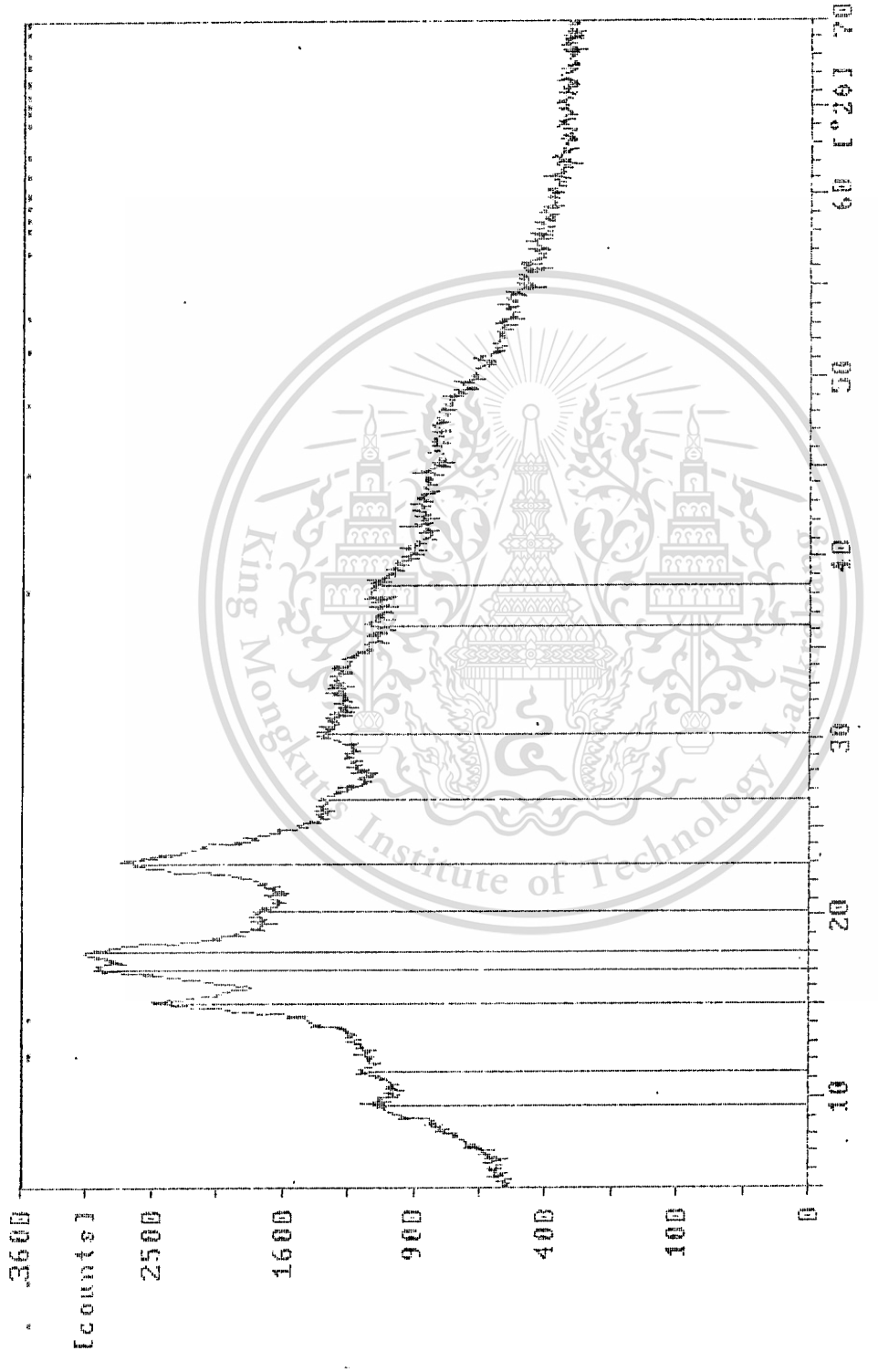


Fig. C-4 X-Ray Diffraction Pattern of R90 Starch

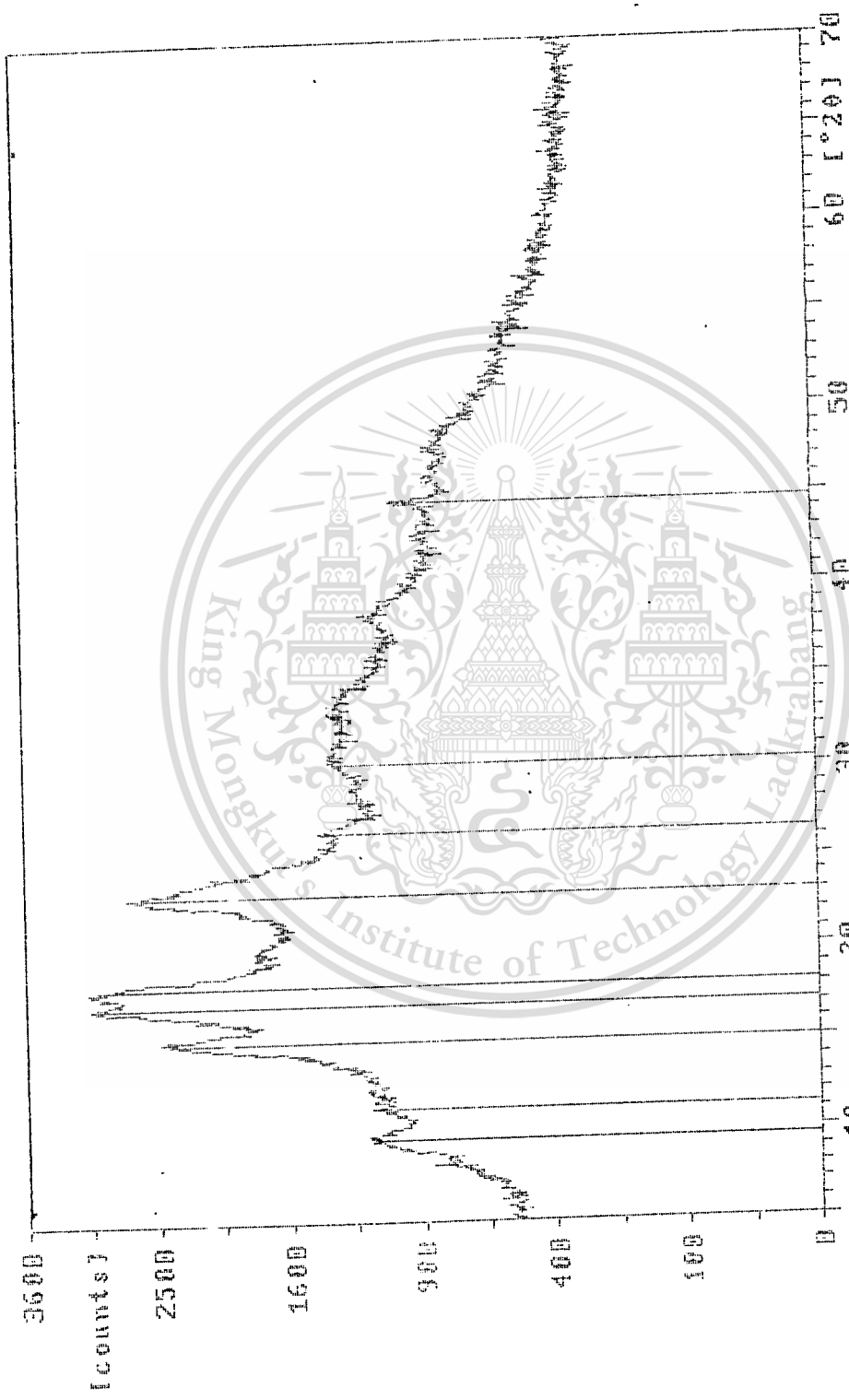


Fig. C-5 X-Ray Diffraction Pattern of KU50 Starch

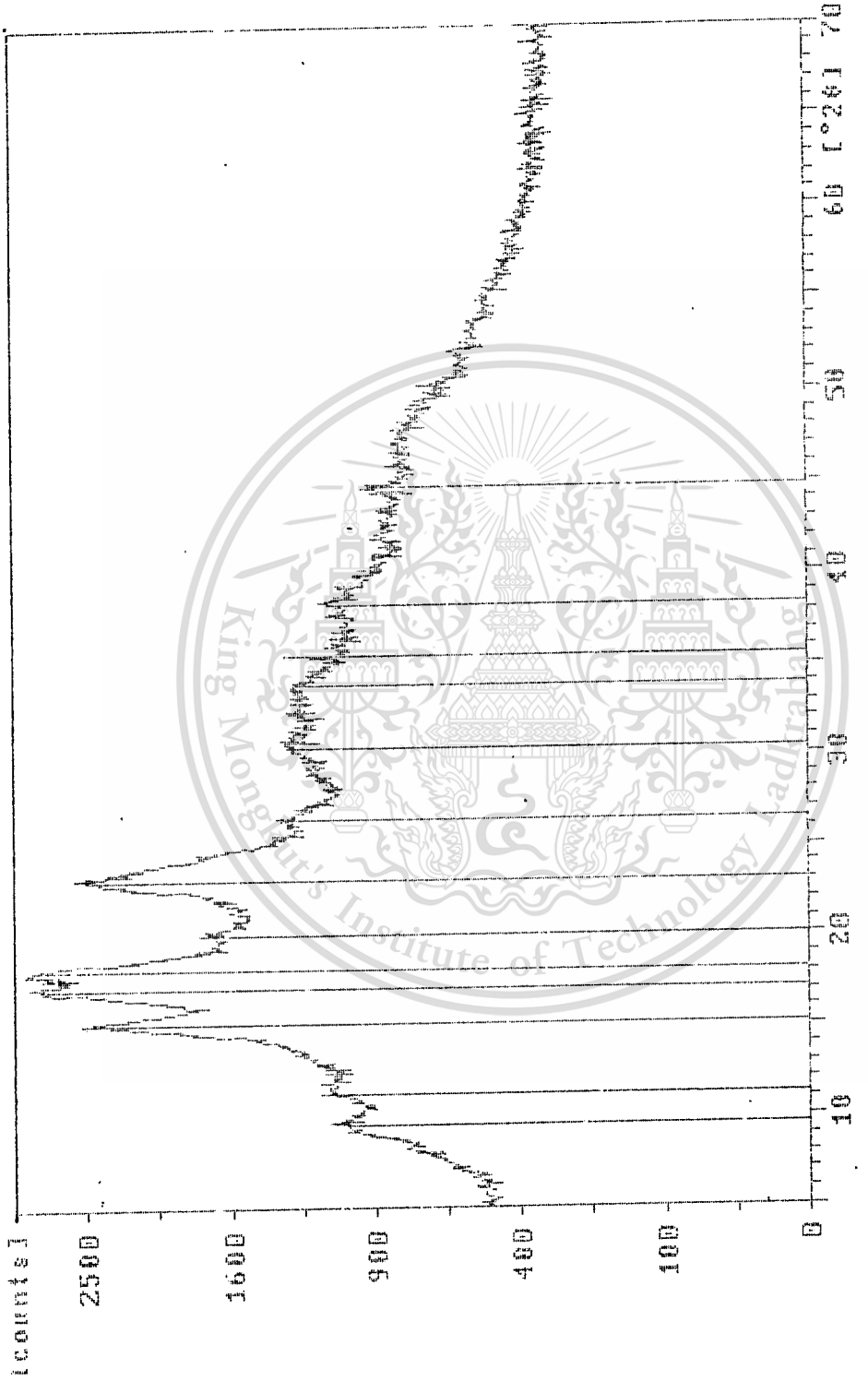


Fig. C-6 X-Ray Diffraction Pattern of SR Starch

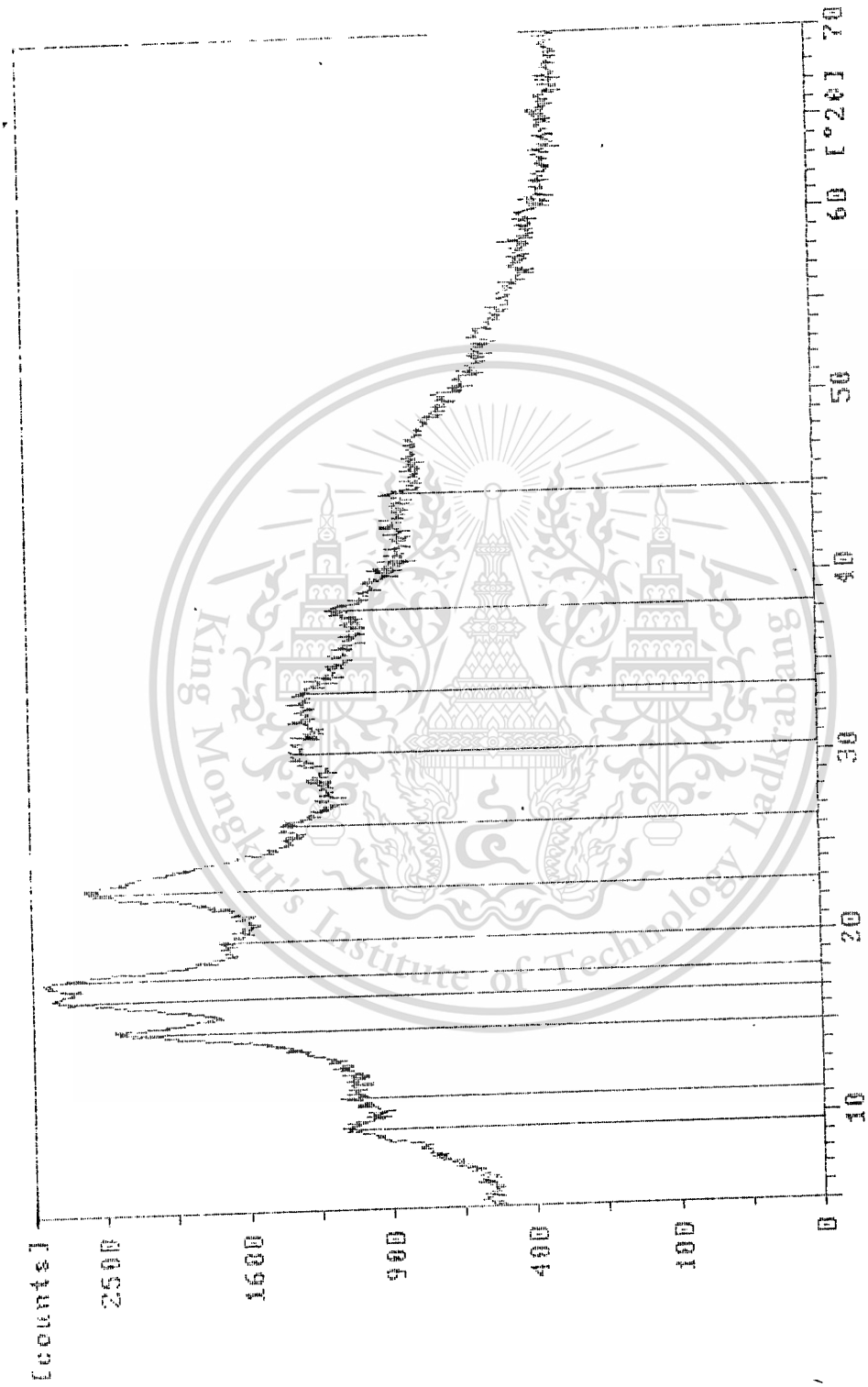


Fig. C-7 X-Ray Diffraction Pattern of ST Starch

APPENDIX D

Master Sizer-X Diagrams of Tapioca Starches

MALVERN MASTERSIZER X

Version 1.1a

Mon, Jun 2, 1997 1:59PM

R1 STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: R1 , Record: 2
Measured on: Mon, Jul 1, 1996 4:29PM Last saved on: Tue, Jul 2, 1996 10:41AM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + I 0.10000
Polydisperse model

Volume Result

Focus = 300 mm.

Residual = 0.211 %
d (0.5) = 13.66 µm
D [4, 3] = 17.37 µm
Sauter Mean (D[3,2]) = 8.21 µm
Specific Surface Area = 0.7311 sq. m. / gm

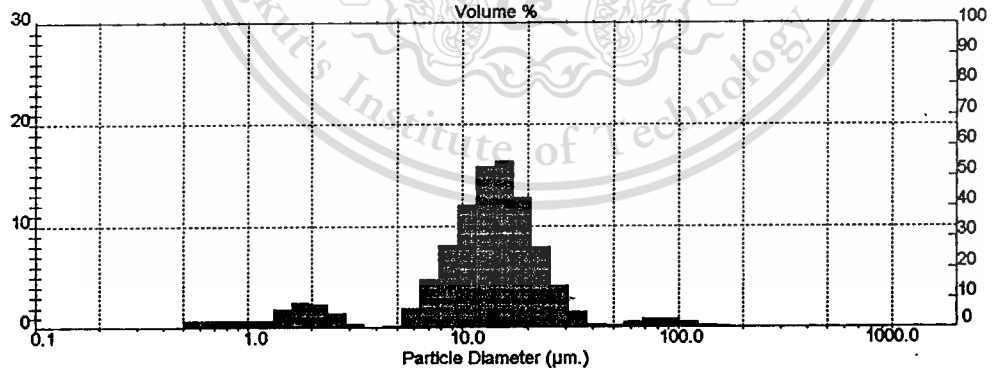
Concentration = 0.142 %
d (0.1) = 5.49 µm
Span = 1.48

Obscuration = 74.21 %
d (0.9) = 25.71 µm

Mode = 14.49 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	0.75	1.32	0.75
1.32	1.90	1.60	2.65
1.60	2.48	1.95	5.13
1.95	2.30	2.38	7.43
2.38	1.50	2.90	8.93
2.90	0.52	3.53	9.45
3.53	0.00	4.30	9.45
4.30	0.30	5.24	9.75
5.24	1.96	6.39	11.71
6.39	4.79	7.78	16.50
7.78	8.13	9.48	24.63
9.48	12.06	11.55	36.69
11.55	15.88	14.08	52.57
14.08	16.36	17.15	68.92
17.15	12.79	20.90	81.71
20.90	7.99	25.46	89.70

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	4.18	31.01	93.88
31.01	1.63	37.79	95.52
37.79	0.40	46.03	95.92
46.03	0.24	56.09	96.16
56.09	0.61	68.33	96.77
68.33	0.93	83.26	97.70
83.26	0.92	101.44	98.63
101.44	0.65	123.59	99.28
123.59	0.34	150.57	99.63
150.57	0.16	183.44	99.78
183.44	0.10	223.51	99.88
223.51	0.08	272.31	99.96
272.31	0.04	331.77	100.00
331.77	0.00	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer X Ver. 1.1a
Serial No.

02 Jun 97 13:59

Fig. D-1 Master Sizer-X Diagram Showing Granular Size Distribution of R1 Starch

MALVERN MASTERSIZER X

Version 1.1a

Mon, Jun 2, 1997 2:13PM

R3 STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: R3 , Record: 1
Measured on: Mon, Jul 1, 1996 4:45PM Last saved on: Mon, Jul 1, 1996 4:46PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + I 0.10000
Polydisperse model

Volume Result

Residual = 0.190 %
d (0 .5) = 13.31 µm
D [4, 3] = 14.38 µm
Sauter Mean (D[3,2]) = 7.63 µm
Specific Surface Area = 0.7864 sq. m. / gm

Concentration = 0.164 %
d (0.1) = 2.82 µm
Span = 1.50

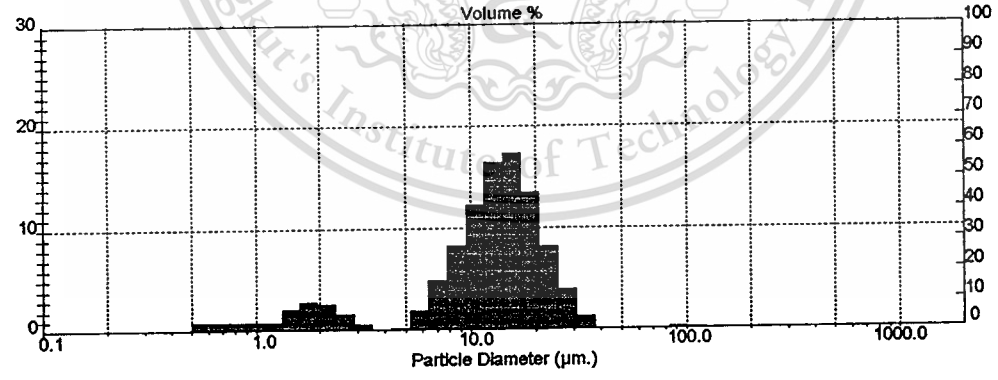
Focus = 300 mm.

Obscuration = 81.46 %
d (0.9) = 22.75 µm

Mode = 14.74 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	0.87	1.32	0.87
1.32	2.18	1.60	3.06
1.60	2.83	1.95	5.89
1.95	2.60	2.38	8.49
2.38	1.69	2.90	10.18
2.90	0.61	3.53	10.79
3.53	0.00	4.30	10.79
4.30	0.26	5.24	11.05
5.24	1.95	6.39	13.00
6.39	4.90	7.78	17.89
7.78	8.29	9.48	26.18
9.48	12.29	11.55	38.47
11.55	16.47	14.08	54.94
14.08	17.29	17.15	72.24
17.15	13.52	20.90	85.76
20.90	8.19	25.46	93.95

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	4.02	31.01	97.97
31.01	1.28	37.79	99.25
37.79	0.01	46.03	99.25
46.03	0.00	56.09	99.25
56.09	0.00	68.33	99.26
68.33	0.13	83.26	99.39
83.26	0.23	101.44	99.62
101.44	0.15	123.59	99.77
123.59	0.05	150.57	99.82
150.57	0.03	183.44	99.85
183.44	0.14	223.51	100.00
223.51	0.00	272.31	100.00
272.31	0.00	331.77	100.00
331.77	0.00	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer X Ver. 1.1a
Serial No.

02 Jun 97 14:13

Fig. D-2 Master Sizer-X Diagram Showing Granular Size Distribution of R3 Starch

MALVERN INSTRUMENTS

MASTERSIZER X

Version 1.1a

Mon, Jun 2, 1997 2:32PM

R5 STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: R5 , Record: 1
Measured on: Mon, Jul 1, 1996 5:03PM Last saved on: Mon, Jul 1, 1996 5:05PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + I 0.10000
Polydisperse model

Volume Result

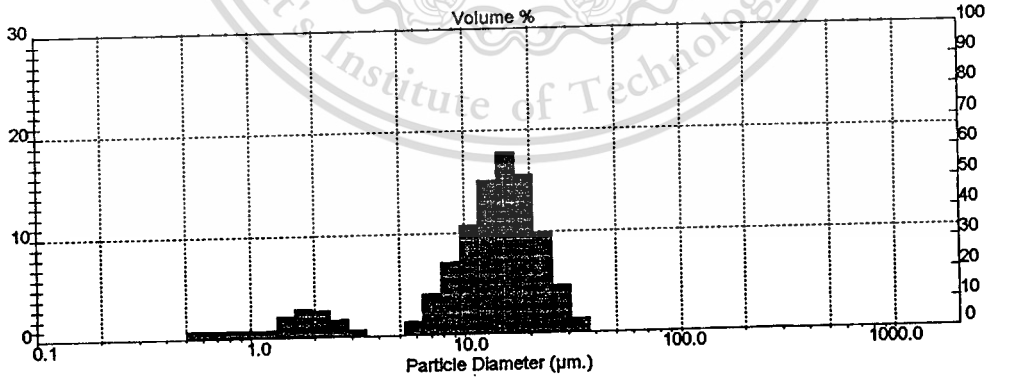
Residual = 0.206 %
d (0 .5) = 14.07 µm
D [4, 3] = 15.32 µm
Sauter Mean (D[3,2]) = 7.72 µm
Specific Surface Area = 0.7768 sq. m. / gm

Concentration = 0.197 %
d (0.1) = 2.72 µm
Span = 1.47

Focus = 300 mm.
Obscuration = 86.47 %
d (0.9) = 23.39 µm
Mode = 15.67 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	0.88	1.32	0.88
1.32	2.22	1.60	3.10
1.60	2.89	1.95	5.99
1.95	2.68	2.38	8.67
2.38	1.81	2.90	10.48
2.90	0.76	3.53	11.24
3.53	0.03	4.30	11.27
4.30	0.11	5.24	11.38
5.24	1.47	6.39	12.85
6.39	4.13	7.78	16.98
7.78	7.18	9.48	24.16
9.48	10.75	11.55	34.91
11.55	15.16	14.08	50.07
14.08	17.80	17.15	67.87
17.15	15.62	20.90	83.49
20.90	9.86	25.46	93.35

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	4.74	31.01	98.08
31.01	1.46	37.79	99.54
37.79	0.01	46.03	99.55
46.03	0.00	56.09	99.55
56.09	0.00	68.33	99.55
68.33	0.00	83.26	99.55
83.26	0.00	101.44	99.55
101.44	0.00	123.59	99.55
123.59	0.00	150.57	99.55
150.57	0.00	183.44	99.55
183.44	0.08	223.51	99.63
223.51	0.16	272.31	99.79
272.31	0.17	331.77	99.96
331.77	0.04	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer X Ver. 1.1a
Serial No.

02 Jun 97 14:32

Fig. D-3 Master Sizer-X Diagram Showing Granular Size Distribution of R5 Starch

MALVERN MASTERSIZER X

Version 1.1a

Mon, Jun 2, 1997 3:16PM

R60 STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: R60 , Record: 1
Measured on: Mon, Jul 1, 1996 5:13PM Last saved on: Mon, Jul 1, 1996 5:14PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + I 0.10000
Polydisperse model

Volume Result

Residual = 0.186 %
d (0 .5) = 13.00 µm
D [4, 3] = 14.57 µm
Sauter Mean (D[3,2]) = 7.09 µm
Specific Surface Area = 0.8467 sq. m. / gm

Concentration = 0.189 %
d (0.1) = 2.41 µm
Span = 1.56

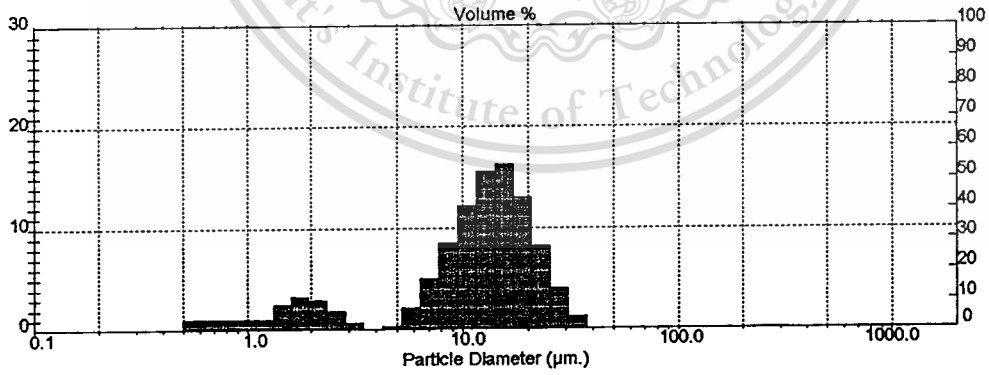
Focus = 300 mm.

Obscuration = 87.66 %
d (0.9) = 22.70 µm

Mode = 14.68 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	1.08	1.32	1.08
1.32	2.58	1.60	3.65
1.60	3.27	1.95	6.92
1.95	2.93	2.38	9.85
2.38	1.87	2.90	11.72
2.90	0.72	3.53	12.44
3.53	0.07	4.30	12.51
4.30	0.43	5.24	12.94
5.24	2.15	6.39	15.09
6.39	5.13	7.78	20.22
7.78	8.51	9.48	28.73
9.48	12.21	11.55	40.94
11.55	15.64	14.08	56.59
14.08	16.26	17.15	72.84
17.15	13.06	20.90	85.90
20.90	8.18	25.46	94.08

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	4.09	31.01	98.17
31.01	1.33	37.79	99.50
37.79	0.01	46.03	99.51
46.03	0.00	56.09	99.51
56.09	0.00	68.33	99.51
68.33	0.00	83.26	99.51
83.26	0.00	101.44	99.51
101.44	0.00	123.59	99.51
123.59	0.00	150.57	99.51
150.57	0.00	183.44	99.51
183.44	0.09	223.51	99.60
223.51	0.18	272.31	99.78
272.31	0.18	331.77	99.96
331.77	0.04	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer X Ver. 1.1a
Serial No.

02 Jun 97 15:16

Fig. D-4 Master Sizer-X Diagram Showing Granular Size Distribution of R60 Starch

MALVERN MASTERSIZER X

Version 1.1a

Wed, Apr 29, 1998 11:46AM

R90 STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: R90 , Record: 1
Measured on: Mon, Jul 1, 1996 5:49PM Last saved on: Mon, Jul 1, 1996 5:50PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + i 0.10000
Polydisperse model

Volume Result

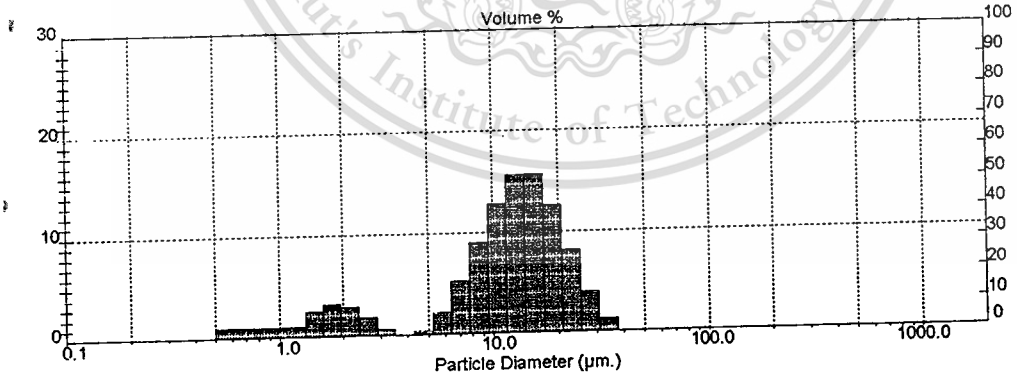
Residual = 0.249 %
d (0 .5) = 12.79 µm
D [4, 3] = 13.61 µm
Sauter Mean (D[3,2]) = 7.09 µm
Specific Surface Area = 0.8463 sq. m. / gm

Concentration = 0.187 %
d (0.1) = 2.43 µm
Span = 1.58

Focus = 300 mm.
Obscuration = 87.40 %
d (0.9) = 22.69 µm
Mode = 14.29 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	1.05	1.32	1.05
1.32	2.53	1.60	3.57
1.60	3.24	1.95	6.81
1.95	2.93	2.38	9.74
2.38	1.88	2.90	11.63
2.90	0.70	3.53	12.32
3.53	0.04	4.30	12.36
4.30	0.45	5.24	12.82
5.24	2.23	6.39	15.05
6.39	5.31	7.78	20.36
7.78	9.01	9.48	29.37
9.48	12.81	11.55	42.18
11.55	15.60	14.08	57.78
14.08	15.62	17.15	73.40
17.15	12.59	20.90	85.99
20.90	8.08	25.46	94.06

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	4.08	31.01	98.14
31.01	1.35	37.79	99.49
37.79	0.05	46.03	99.54
46.03	0.00	56.09	99.54
56.09	0.00	68.33	99.54
68.33	0.13	83.26	99.67
83.26	0.18	101.44	99.85
101.44	0.11	123.59	99.96
123.59	0.04	150.57	100.00
150.57	0.00	183.44	100.00
183.44	0.00	223.51	100.00
223.51	0.00	272.31	100.00
272.31	0.00	331.77	100.00
331.77	0.00	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



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Malvern, U.K.

MasterSizer X Ver. 1.1a
Serial No.

29 Apr 98 11:46

Fig. D-5 Master Sizer-X Diagram Showing Granular Size Distribution of R90 Starch

MALVERN MASTERSIZER X

Version 1.1a

Mon, Jun 2, 1997 1:45PM

KU50 STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: KU50 , Record: 1
Measured on: Mon, Jul 1, 1996 6:44PM Last saved on: Mon, Jul 1, 1996 6:45PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + I 0.10000
Polydisperse model

Volume Result

Residual = 0.208 %
d (0 .5) = 13.36 µm
D [4, 3] =. 13.56 µm
Sauter Mean (D[3,2]) = 7.33 µm
Specific Surface Area = 0.8183 sq. m. / gm

Concentration = 0.181 %
d (0.1) = 2.59 µm
Span = 1.50

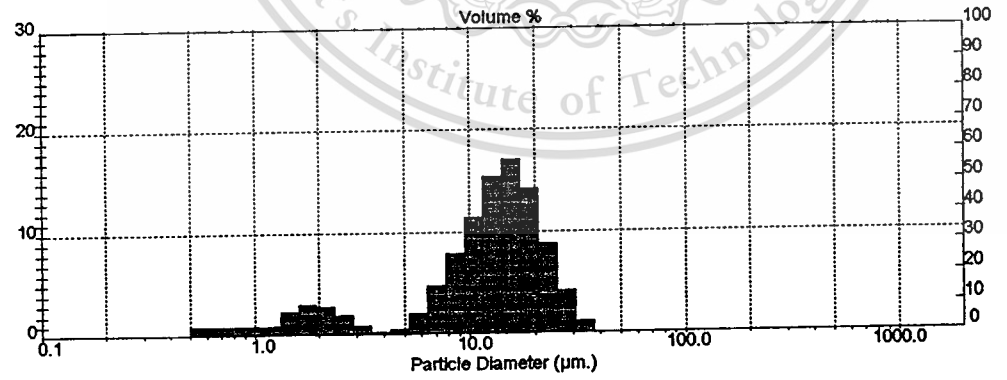
Focus = 300 mm.

Obscuration = 85.53 %
d (0.9) = 22.60 µm

Mode = 15.25 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	0.92	1.32	0.92
1.32	2.32	1.60	3.24
1.60	3.01	1.95	6.25
1.95	2.80	2.38	9.05
2.38	1.93	2.90	10.99
2.90	0.93	3.53	11.92
3.53	0.31	4.30	12.23
4.30	0.57	5.24	12.79
5.24	2.09	6.39	14.89
6.39	4.83	7.78	19.72
7.78	7.88	9.48	27.60
9.48	11.41	11.55	39.00
11.55	15.39	14.08	54.39
14.08	17.08	17.15	71.48
17.15	14.26	20.90	85.74
20.90	8.81	25.46	94.55

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	4.21	31.01	98.76
31.01	1.24	37.79	100.00
37.79	0.00	46.03	100.00
46.03	0.00	56.09	100.00
56.09	0.00	68.33	100.00
68.33	0.00	83.26	100.00
83.26	0.00	101.44	100.00
101.44	0.00	123.59	100.00
123.59	0.00	150.57	100.00
150.57	0.00	183.44	100.00
183.44	0.00	223.51	100.00
223.51	0.00	272.31	100.00
272.31	0.00	331.77	100.00
331.77	0.00	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



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MasterSizer X Ver. 1.1a

Fig.D-6 Master Sizer-X Diagram Showing Granular Size Distribution of KU50 Starch

MALVERN INSTRUMENTS

MASTERSIZER X

Version 1.1a

Mon, Jun 9, 1997 9:52PM

SR STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: SR , Record: 1
Measured on: Mon, Jul 1, 1996 6:00PM Last saved on: Mon, Jul 1, 1996 6:01PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + i 0.10000
Polydisperse model

Volume Result

Focus = 300 mm.

Residual = 0.190 %
d (0.5) = 11.48 µm
D [4, 3] = 12.22 µm
Sauter Mean (D[3,2]) = 6.52 µm
Specific Surface Area = 0.9207 sq. m. / gm

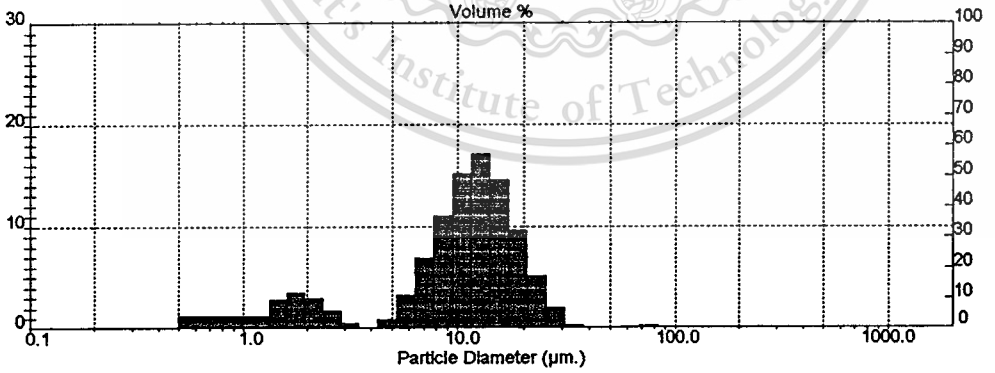
Concentration = 0.157 %
d (0.1) = 2.27 µm
Span = 1.54

Obscuration = 84.85 %
d (0.9) = 19.93 µm

Mode = 12.64 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	1.26	1.32	1.26
1.32	2.84	1.60	4.10
1.60	3.51	1.95	7.60
1.95	3.01	2.38	10.61
2.38	1.76	2.90	12.37
2.90	0.55	3.53	12.92
3.53	0.10	4.30	13.01
4.30	0.95	5.24	13.97
5.24	3.32	6.39	17.28
6.39	6.97	7.78	24.25
7.78	11.07	9.48	35.32
9.48	15.16	11.55	50.48
11.55	17.07	14.08	67.54
14.08	14.56	17.15	82.10
17.15	9.66	20.90	91.76
20.90	5.14	25.46	96.90

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	2.06	31.01	98.97
31.01	0.33	37.79	99.30
37.79	0.00	46.03	99.30
46.03	0.00	56.09	99.30
56.09	0.15	68.33	99.45
68.33	0.28	83.26	99.73
83.26	0.21	101.44	99.95
101.44	0.05	123.59	100.00
123.59	0.00	150.57	100.00
150.57	0.00	183.44	100.00
183.44	0.00	223.51	100.00
223.51	0.00	272.31	100.00
272.31	0.00	331.77	100.00
331.77	0.00	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



Malvern Instruments Ltd.
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MasterSizer X Ver. 1.1a
Serial No.

02 Jun 97 15:52

Fig. D-7 Master Sizer-X Diagram Showing Granular Size Distribution of SR Starch

MALVERN MASTERSIZER X

Version 1.1a

Mon, Jun 2, 1997 1:30PM

4STAR STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: 4STAR , Record: 1
Measured on: Mon, Jul 1, 1996 6:54PM Last saved on: Mon, Jul 1, 1996 6:56PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + I 0.10000
Polydisperse model

Volume Result

Residual = 0.132 %
d (0 .5) = 19.16 µm
D [4, 3] = 22.28 µm
Sauter Mean (D[3,2]) = 9.75 µm
Specific Surface Area = 0.6152 sq. m. / gm

Concentration = 0.265 %
d (0.1) = 6.29 µm
Span = 1.68

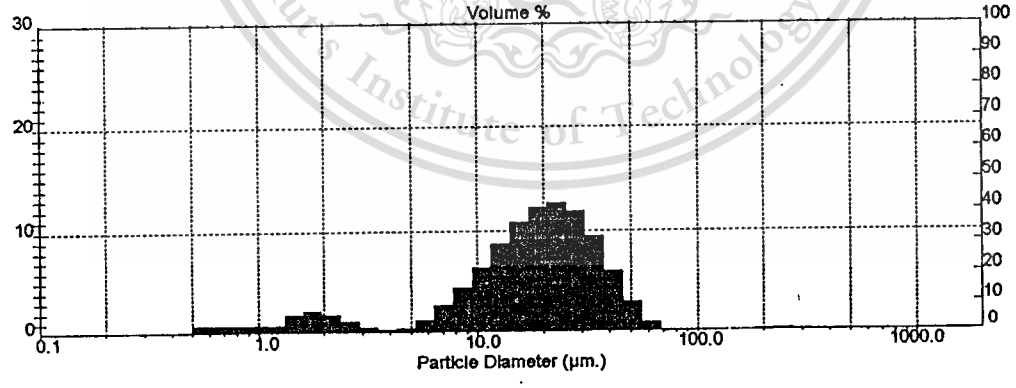
Focus = 300 mm.

Obscuration = 87.97 %
d (0.9) = 36.42 µm

Mode = 22.79 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	0.79	1.32	0.79
1.32	1.79	1.60	2.57
1.60	2.16	1.95	4.73
1.95	1.85	2.38	6.58
2.38	1.18	2.90	7.76
2.90	0.56	3.53	8.32
3.53	0.23	4.30	8.55
4.30	0.40	5.24	8.95
5.24	1.19	6.39	10.14
6.39	2.69	7.78	12.83
7.78	4.43	9.48	17.27
9.48	6.45	11.55	23.71
11.55	8.74	14.08	32.45
14.08	10.81	17.15	43.26
17.15	12.20	20.90	55.46
20.90	12.63	25.46	68.09

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	11.84	31.01	79.93
31.01	9.45	37.79	89.37
37.79	6.08	46.03	95.45
46.03	2.99	56.09	98.44
56.09	0.90	68.33	99.35
68.33	0.01	83.26	99.35
83.26	0.00	101.44	99.35
101.44	0.00	123.59	99.35
123.59	0.00	150.57	99.35
150.57	0.04	183.44	99.39
183.44	0.19	223.51	99.59
223.51	0.23	272.31	99.82
272.31	0.18	331.77	99.99
331.77	0.01	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer X Ver. 1.1a
Serial No.

02 Jun 97 13:30

Fig. D-8 Master Sizer-X Diagram Showing Granular Size Distribution of ST Starch

MALVERN MASTERSIZER X

Version 1.1a

Mon, Jun 2, 1997 3:35PM

ROSE STARCH :Run Number 10

DISPERSE IN METHANOL

Sample File Name: ROSE , Record: 1
Measured on: Mon, Jul 1, 1996 7:05PM Last saved on: Mon, Jul 1, 1996 7:05PM

Source: Analysed

Presentation: (2OHD) 1.330, 1.530 + i 0.10000
Polydisperse model

Volume Result

Residual = 0.120 %
d (0.5) = 17.07 µm
D [4, 3] = 20.18 µm
Sauter Mean (D[3,2]) = 8.81 µm
Specific Surface Area = 0.6811 sq. m. / gm

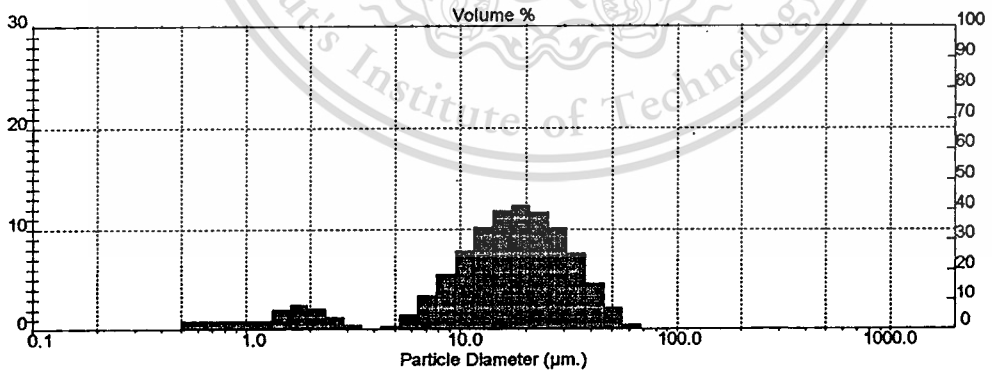
Concentration = 0.252 %
d (0.1) = 5.11 µm
Span = 1.78

Focus = 300 mm.

Obscuration = 89.21 %
d (0.9) = 35.54 µm

Mode = 18.54 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	0.92	1.32	0.92
1.32	2.07	1.60	2.99
1.60	2.49	1.95	5.48
1.95	2.11	2.38	7.59
2.38	1.31	2.90	8.90
2.90	0.57	3.53	9.46
3.53	0.20	4.30	9.66
4.30	0.44	5.24	10.10
5.24	1.49	6.39	11.59
6.39	3.37	7.78	14.96
7.78	5.50	9.48	20.46
9.48	7.83	11.55	28.29
11.55	10.18	14.08	38.48
14.08	11.82	17.15	50.30
17.15	12.33	20.90	62.63
20.90	11.71	25.46	74.34
25.46	10.13	31.01	84.47
31.01	7.58	37.79	92.05
37.79	4.65	46.03	96.70
46.03	2.17	56.09	98.87
56.09	0.58	68.33	99.46
68.33	0.00	83.26	99.46
83.26	0.00	101.44	99.46
101.44	0.00	123.59	99.46
123.59	0.00	150.57	99.46
150.57	0.03	183.44	99.49
183.44	0.15	223.51	99.64
223.51	0.19	272.31	99.83
272.31	0.16	331.77	99.99
331.77	0.01	404.21	100.00
404.21	0.00	492.47	100.00
492.47	0.00	600.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer X Ver. 1.1a
Serial No.

02 Jun 97 15:35

Fig. D-9 Master Sizer-X Diagram Showing Granular Size Distribution of RO Starch

APPENDIX E

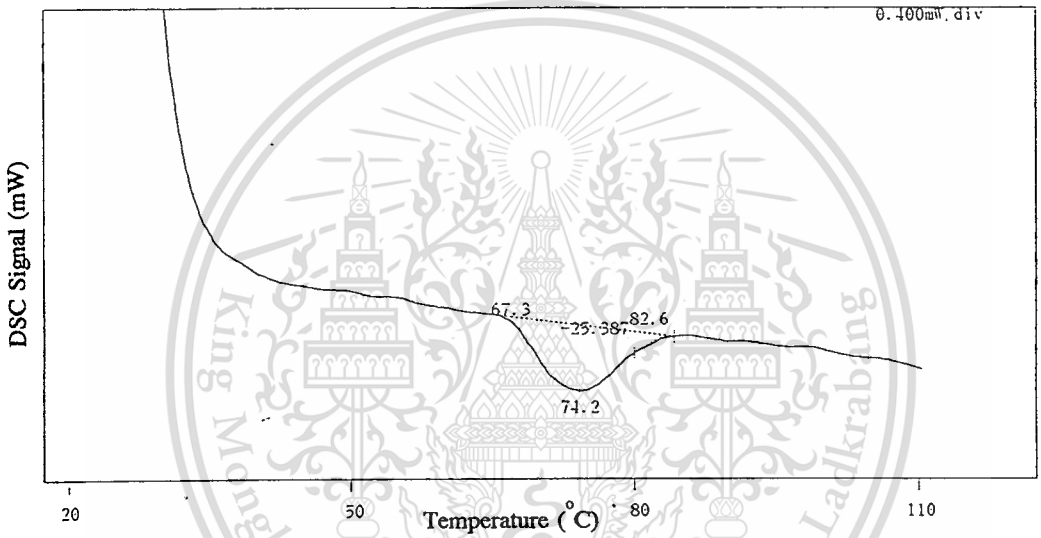
DSC Thermograms of Tapioca Starches

====== THERMAL ANALYSIS REPORT ======

96/08.05

FILE NAME <<R1(2).000>>

			♦ TEMPERATURE PROGRAM ♦				
DATE(y m d)	: 96.08.05		dT.dt	T(hold)	τ(hold)	δT(add)x	n(repeat)
SAMPLE NAME	: R1 STARCH	1:	10	120	0	0	0
COMMENT	: STARCH : WATER (2:8)	2:	0	0	0	0	0
SAMPLE Q'TITY	: 2 mg	3:	0	0	0	0	0
MODULE TYPE	: DSC	4:	0	0	0	0	0
SAMPLING INT	: 1 sec	5:	0	0	0	0	0



[TEMP.]	TEMP('C)	Heat Flow(mW)
1	74.2	-6.2925

[HEAT]	Ti('C)	Tf('C)	Heat(mJ)
1	66	84.2	-25.387

[TANGENT]	Ti('C)	Tf('C)	Intersection('C)
1	66	68.6	67.3
2	80.1	84.2	82.6

E-1 DSC Thermogram of R1 Starch (Starch 2 mg. : water 8 µl.)

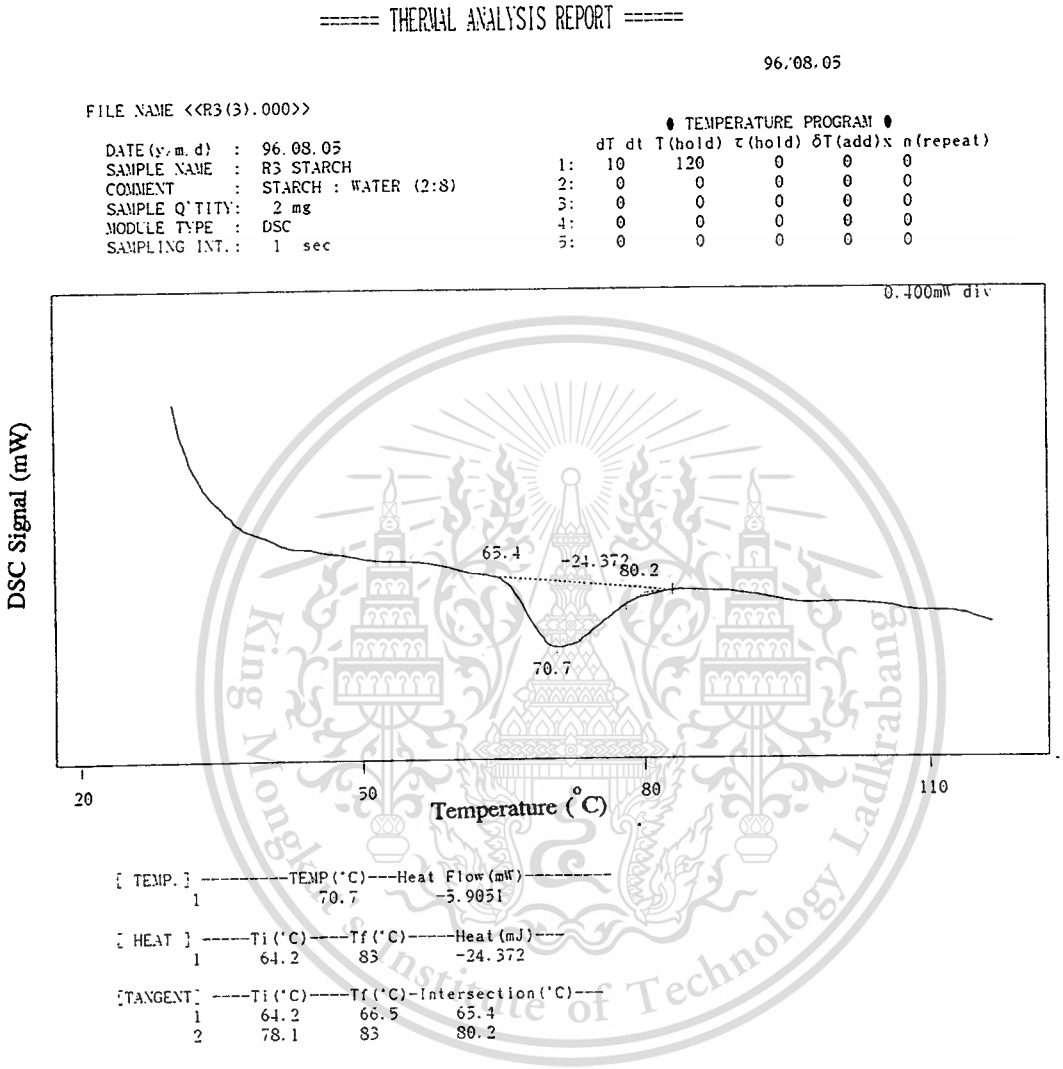


Fig. E-2 DSC Thermogram of R3 Starch (Starch 2 mg. : water 8 µl.)

====== THERMAL ANALYSIS REPORT ======

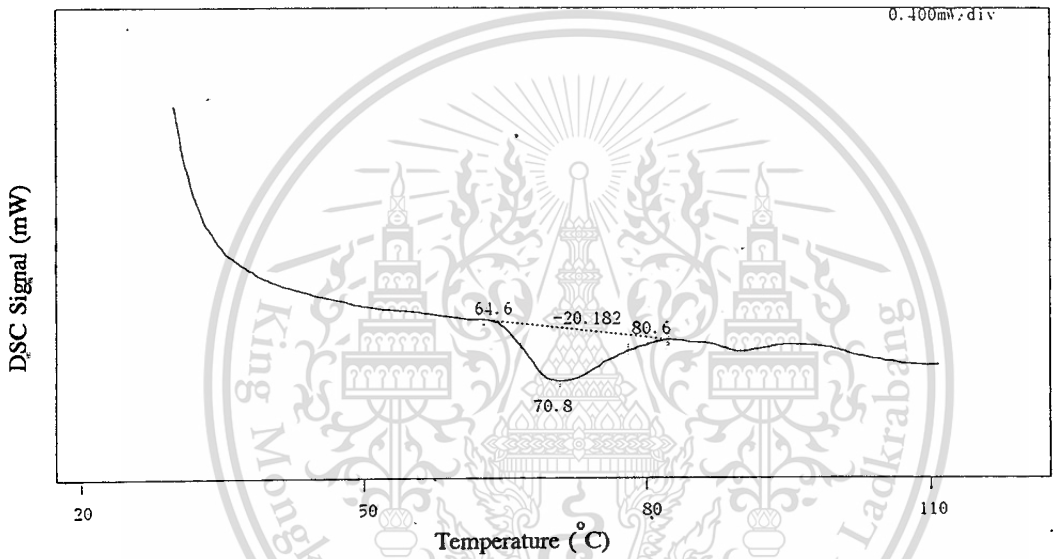
96/08/06

FILE NAME <<R5(3).000>>

DATE(y/m d)	: 96 08 06
SAMPLE NAME	: R5 STARCH
COMMENT	: STARCH : WATER (2:8)
SAMPLE Q'TITY	: 2 mg
MODULE TYPE	: DSC
SAMPLING INT.	: 1 sec

♦ TEMPERATURE PROGRAM ♦

	dT	dt	T(hold)	τ(hold)	δT(add)x	n(repeat)
1:	10		120	0	0	0
2:	0		0	0	0	0
3:	0		0	0	0	0
4:	0		0	0	0	0
5:	0		0	0	0	0



[TEMP.] ---------TEMP(°C)---Heat Flow(mW)---------

1	70.8	-5.8591

[HEAT] -----Ti(°C)-----Tf(°C)-----Heat(mJ)---

1	62.9	82.3	-20.182

[TANGENT] ----Ti(°C)----Tf(°C)-Intersection(°C)---

1	62.7	66.8	64.6
2	78.1	82.1	80.6

Fig. E-3 DSC Thermogram of R5 Starch (Starch 2 mg. : water 8 µl.)

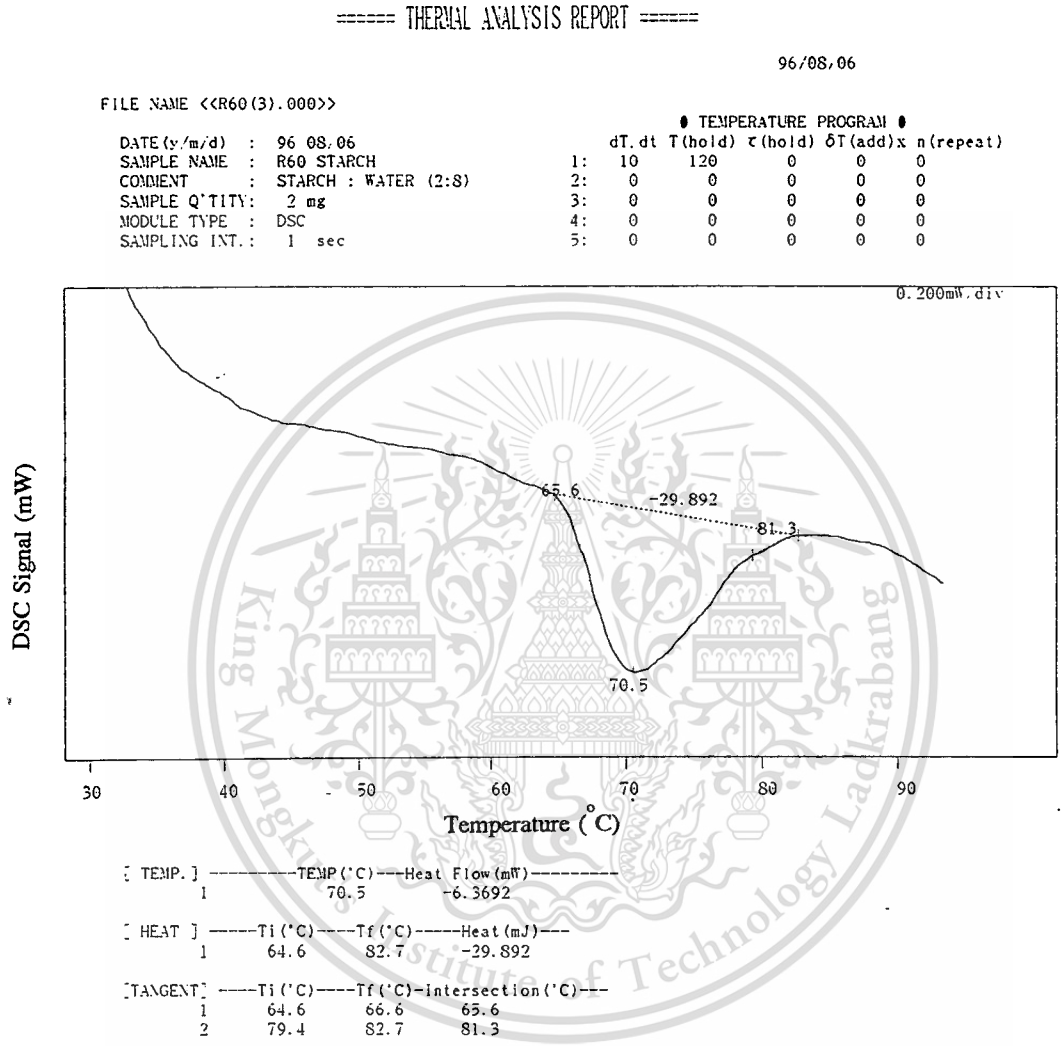


Fig. E-4 DSC Thermogram of R60 Starch (Starch 2 mg. : water 8 µl.)

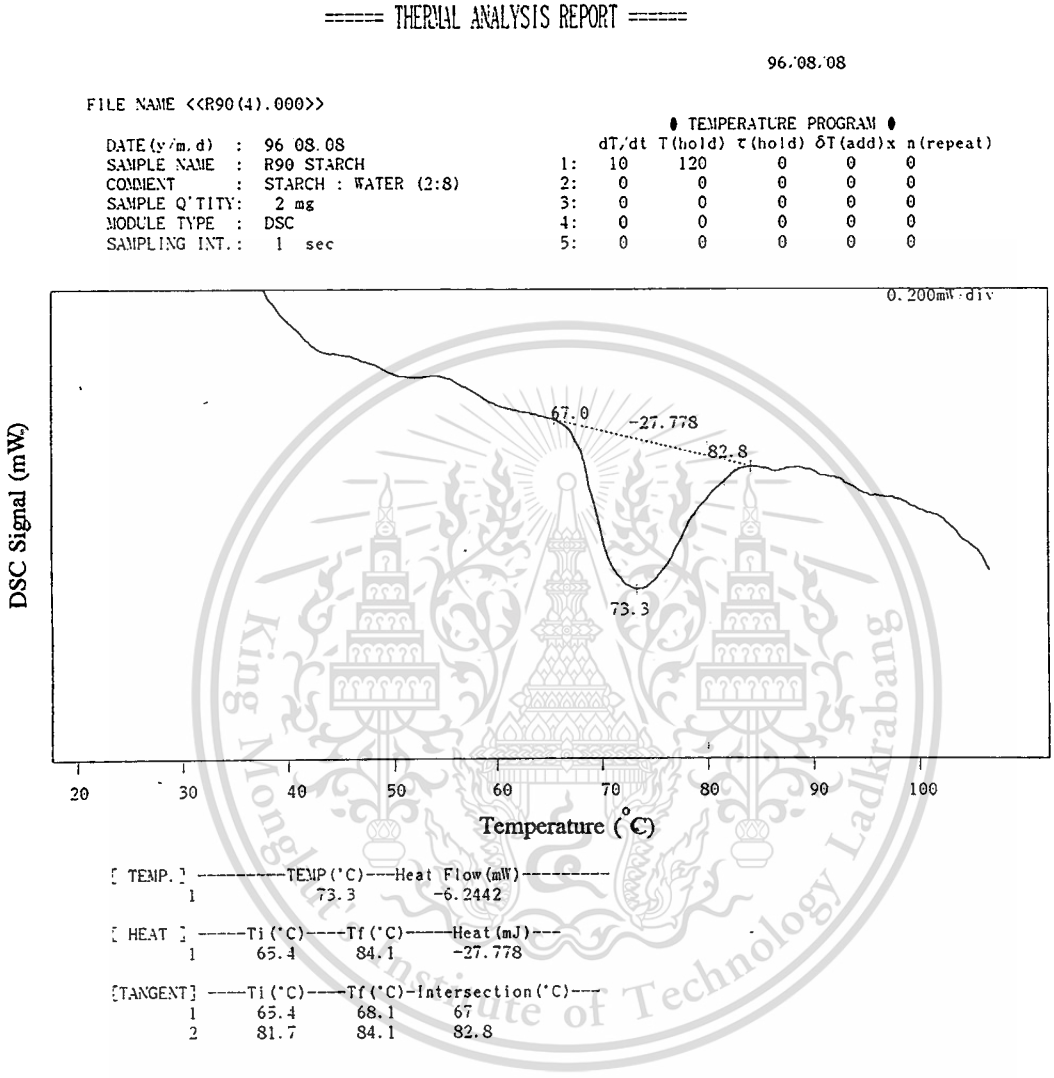


Fig. E-5 DSC Thermogram of R90 Tapioca Starch (Starch 2 mg. : water 8 μl.)

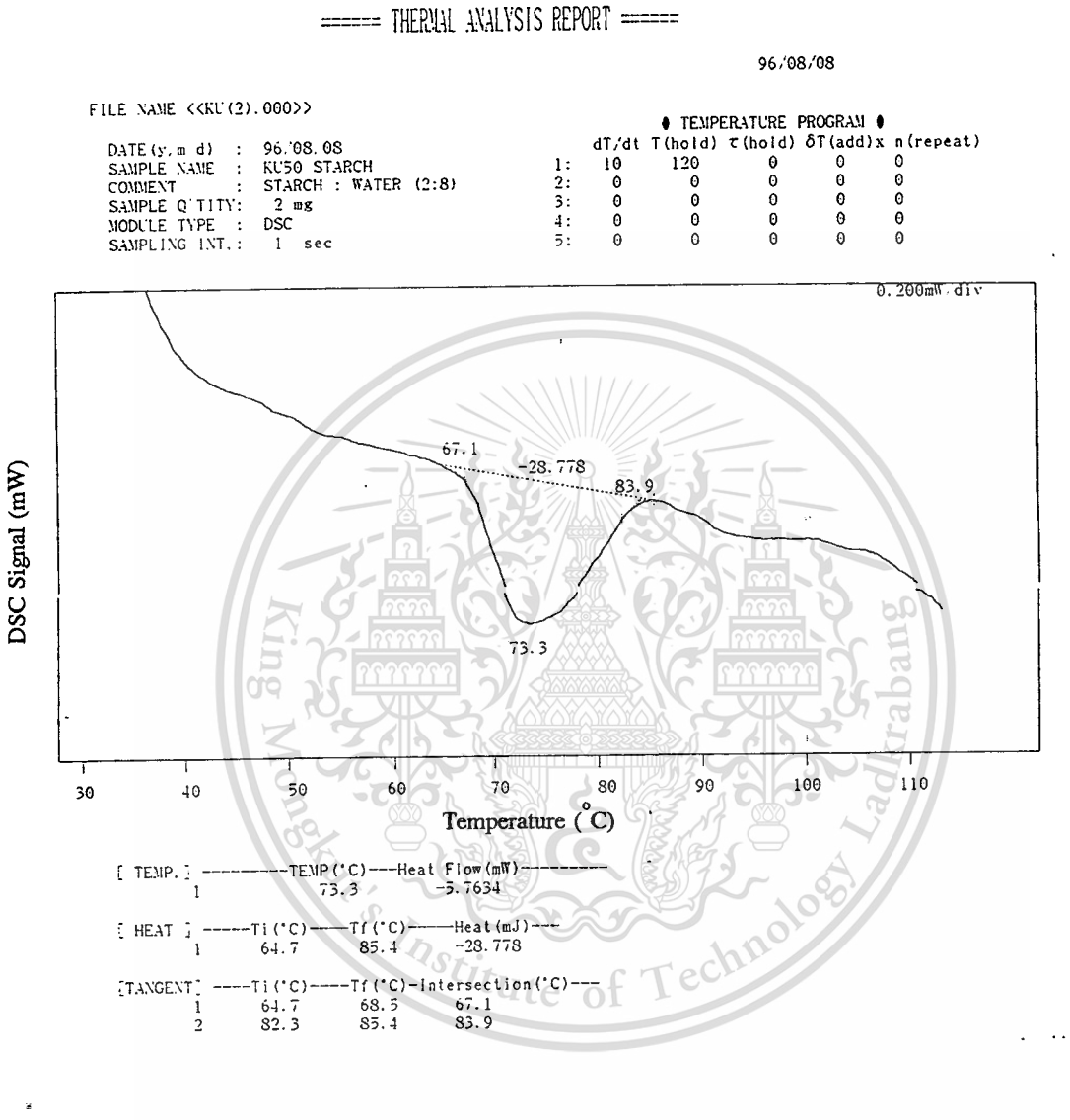


Fig. E-6 DSC Thermogram of KU50 Starch (Starch 2 mg. : water 8 µl.)

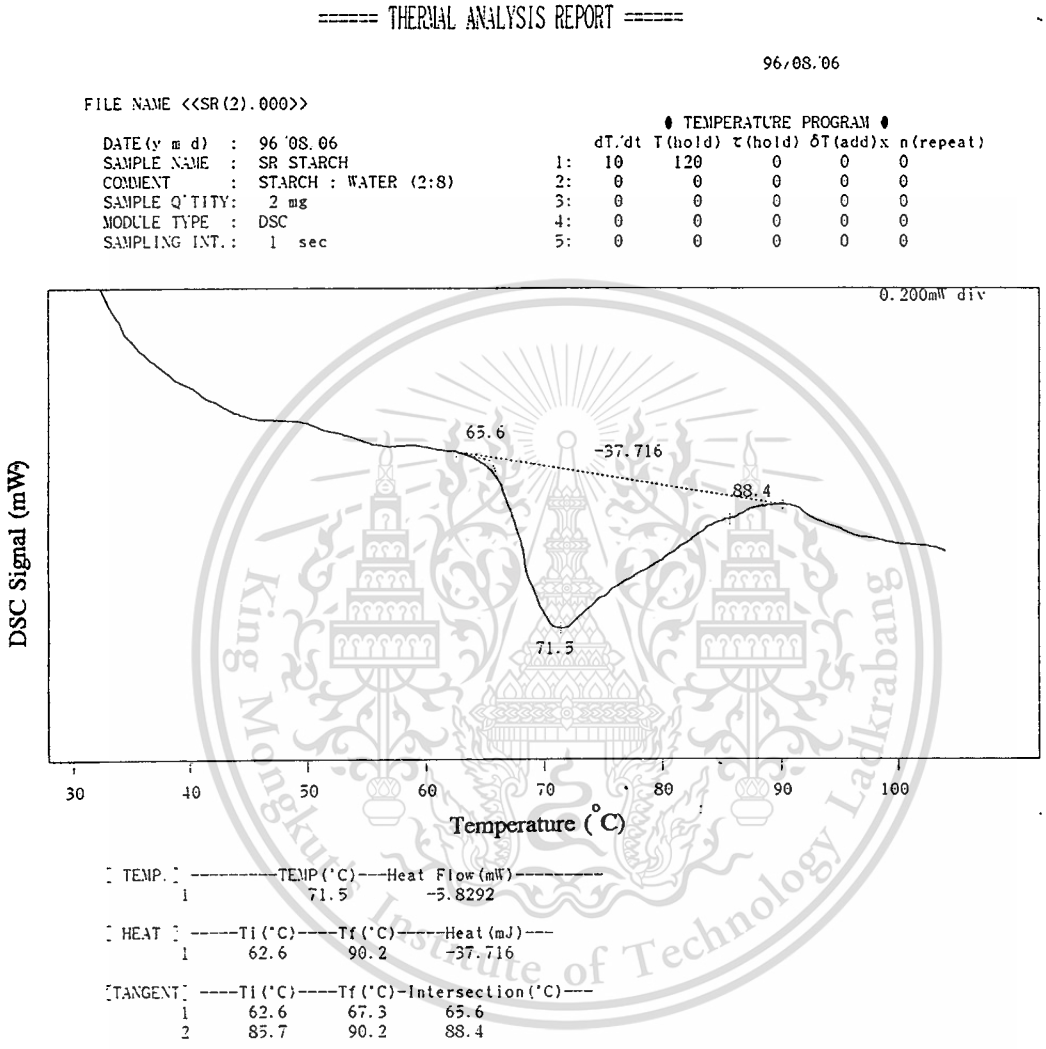


Fig. E-7 DSC Thermogram of SR Starch (Starch 2 mg. : water 8 µl.)

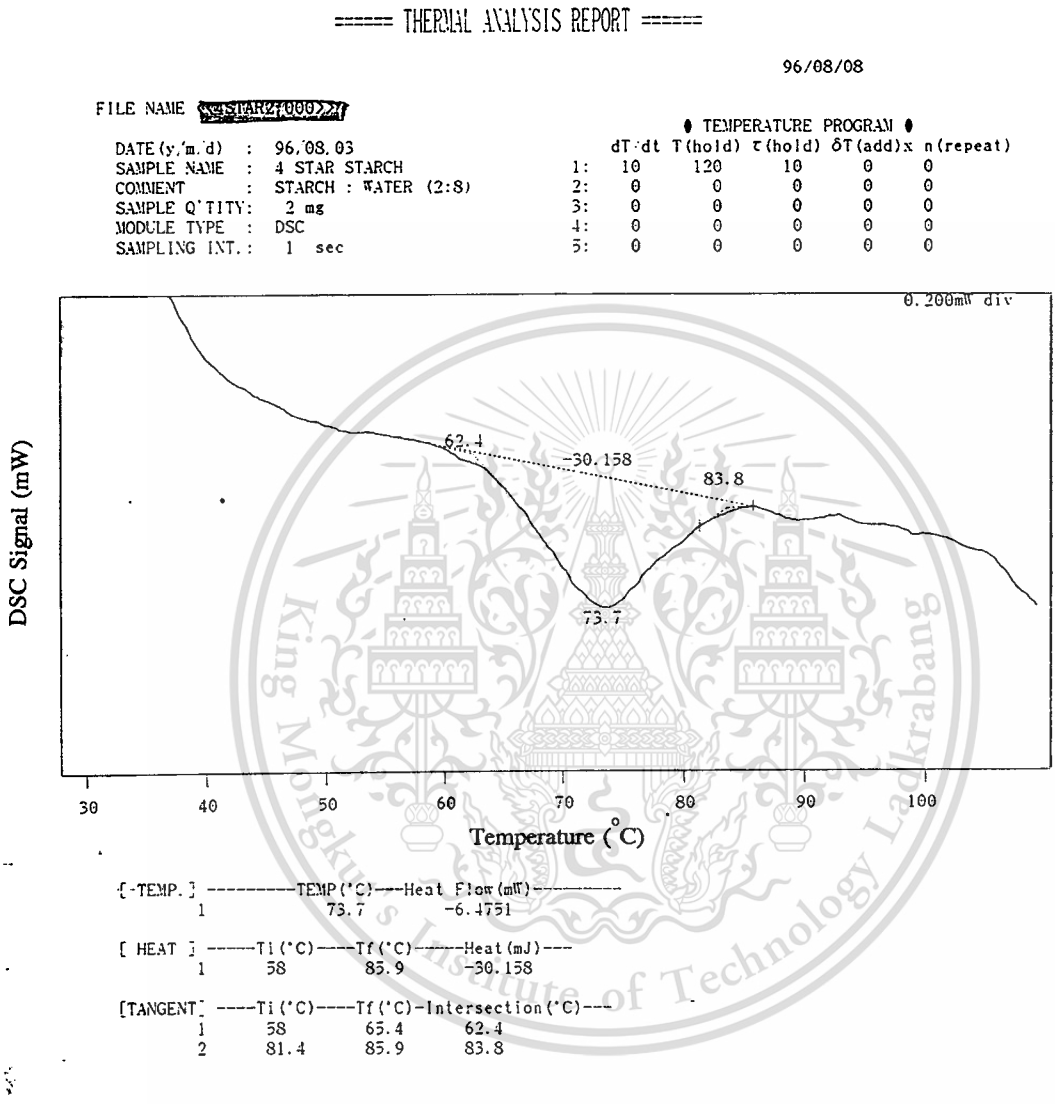


Fig. E-8 DSC Thermogram of ST Starch (Starch 2 mg. : water 8 µl.)

BIOGRAPHY

Miss Sansanee Rungsangporncharoen was born on May 23, 1969. She obtained a Bachelor's Degree of Science in Chemistry at Ramkhamhaeng University in 1991.