CHAPTER II

THEORY and LITERATURE REVIEW

Theoretical Background

1. Starch

Starch, the principle reserve polysaccharide in plants, constitutes a substantial portion of the human diet. It is the component of most seeds, tubers, and roots, cereal grains, green vegetables, and fruits. Starch is a carbohydrate, a very large group of compounds containing carbon, hydrogen, and oxygen in the ratio of 6:10:5 as an empirical formula of $C_6H_{10}O_5$. Besides starch, carbohydrates include such important substances as cellulose, sugars, and gums. Today, starch is used extensively in the paper, textile, explosives, foods, alcoholic beverage, clothing, fuel and adhesive industries.

Carbohydrate is classified as monosaccharides, disaccharides, trisaccharides, and polysaccharides. Monosaccharides are simple sugars with six carbon atoms, which cannot be hydrolyzed to smaller molecules. Glucose is a monosaccharide, while disaccharides, contain twelve carbon atoms and can be hydrolyzed to two single sugars. Disaccharides include sucrose, lactose, and maltose. Trisaccharides contain eighteen carbon atoms and yield three molecules of simple sugars on hydrolysis. Polysaccharides, which are very numerous, are insoluble, and are of high molecular complexity. On hydrolysis, a polysaccharide molecule yields many molecules of monosaccharide sugars. Starch, cellulose and glycogen are polysaccharides (4).

Starch is abundantly available, inexpensive, a desirable source of calories, and occurs in the form of granules with a density of approximately 1.5×10^{-3} kg/cm^3. In a granule form, starch is semi-crystalline, insoluble in cold water but imbibes water reversibly and swells slightly. The granules vary in shape and size ranging from about 1 to 1000 μm diameter depending on the source (5). For cassava starch, the sizes of the granules ranges from 5 to 35 μm. The largest size are usually 25 to 35 μm and the smallest ones are 5 to 15 μm (6).

1.1 Molecular Structure

Most common starches are made up from two different types of polysaccharides of different structures, namely, amylose and amylopectin.

Amylose is essentially a linear polymer of α-D-glucopyranosyl unit linked (1→4) (often called anhydroglucose units, or AGU) (Figure 2.1). This polymer may be separated from the starch by complete gelatinization and vigorous dispersion of the hot starch solution with a complexing agent such as 1-butanol in water (7). On cooling, an amylose-butanol complex crystallizes and may be removed by centrifugation. Recrystallization of the amylose-1-butanol complex and removal of the 1-butanol produce highly pure amylose. Its degree of polymerization (D.P.) will vary with plant variety from which the starch is obtained. Depending on the source of starch, the D.P. will range from about 1,000-1,600 (160,000-2,650,000 in molecular weight). Generally, starches contain about 14-25% amylose. For cassava starch, the amylose content is about 16-22% (8). Amylose is largely crystalline and has several conformations. In the solid state, a stable formation of amylose is six-fold helical structure. In aqueous solution, it seems to be expanded helix that behaves as a random coil. Amylose is completely hydrolyzed by enzyme amylase.

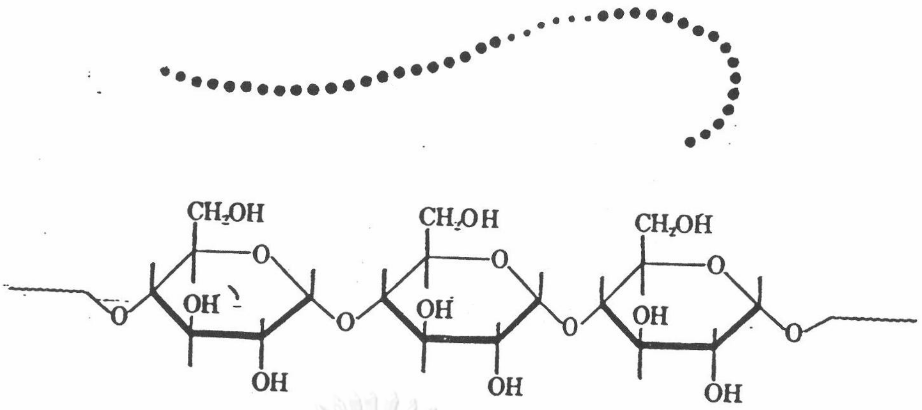


Figure 2.1 Chemical structure of amylose chain

Amylopectin is a highly branched polymer of α-D-glucopyranosyl units containing (1→4) links with (1→6) links at branch points. Each branch contains about 20 to 30 anhydroglucose units. Generally, starches contain about 75-86% amylopectin. A schematic diagram of the amylopectin molecule is shown in Figure 2.2.

Amylopectin is practically amorphous, and has a globular structure. It is capable of considerable expansion by hydration, where the side chains will cause considerable mechanical obstruction of the dissolved molecules, and a more or less extensive association takes place between the free ends of the molecular branches via hydrogen bonds. It is decomposed by β-amylase to only approximately 60% (9).

1.2 Gelatinized Starches

Native starches are insoluble in cold water, but imbibe water reversibly and swell slightly. This is a very important property, which enables

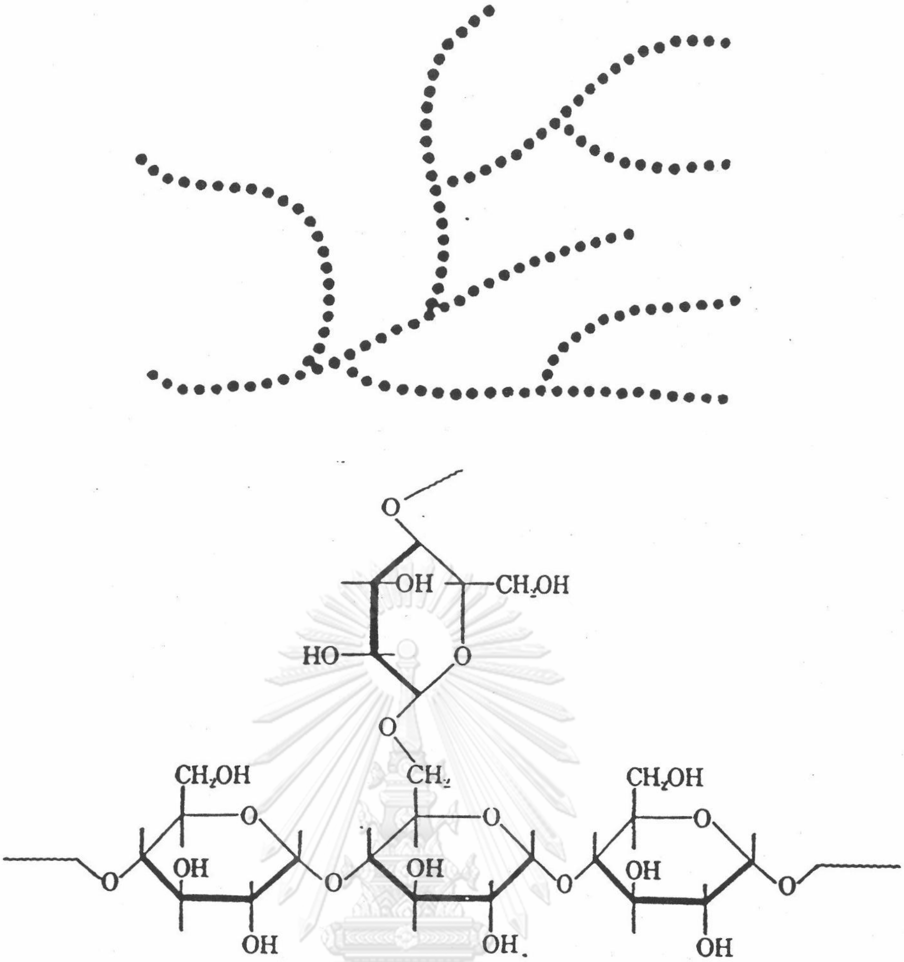


Figure 2.2 Branched structure of amylopectin

the starches granules to be easily extracted from the plant source in an aqueous system (10).

On continuous heating, starch granules absorb water, increase manifold in size, swell, and gelatinized. Concurrently, the initially thin, opaque starch suspension becomes at a certain point, viscous, semiopaque, and finally transparent. The temperature at which this drastic change occurs is usually termed the gelatinized or more correctly, the pasting temperature of starch (11).

During the swelling, the amylose tends to leach out of the granules and along with the amylopectin, becomes highly hydrated. The suspension begins to clarify and the viscosity of the suspension rises until it approaches a peak where the granules have approached their maximum hydration. As heating continued, the granules tend to rupture, collapse, and fragment, releasing the polymeric molecules and aggregates. Then the viscosity drops. During the process as the polymeric molecules are released, the sol often develops a cohesive, rubbery texture. As the resulting sol is cooled, the clarity decreases and the viscosity tends to rise, and in the case of regular cereal starches, such as, corn or wheat, the sol forms gel if the concentration is sufficiently high (10).

The gelatinization temperature is recorded as a temperature range in which the starch granules lose their birefringence. For cassava starch, initial and final gelatinizations occur at 60°C and 80°C, respectively. The point of gelatinization, to a certain extent, depends on granule sizes, the small granules being more resistant to swelling (11).

As result of cooking, starch forms a continuous system, referred to as starch cook or starch paste. In a starch cook or paste, there is a mixture of hydrated, swollen granule particle, held together by a typical maze of associative forces, as schematically presented in Figure 2.3.

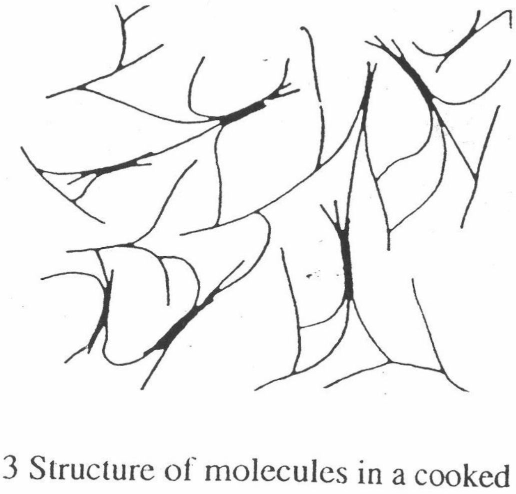


Figure 2.3 Structure of molecules in a cooked starch paste

2. Graft Copolymer and Copolymerization

A graft copolymer is a polymeric backbone with covalently linked polymeric side chains. In principle, both the backbone and side chains could be homopolymers or copolymers. Graft copolymers are of great interest in the field of thickener in a number of aspects. Grafting can be carried out in such a way that the properties of the side chains can be added to those of the substrate polymer without greatly changing the latter.

2.1 Method of Grafting Copolymerization

In this thesis, the backbone polymers for grafting are starch. The grafted side chains can be hydrophilic in nature, either cationic, anionic and nonionic. This can be prepared by a direct grafting such monomer of acrylic acid, or by grafting the monomer, methyl acrylate and hydrolyzing it to acrylic acid and its salts (12).

In principle, there are two general methods for the synthesis of a graft copolymer.

(a) The side chain polymer could be linked directly by a suitable chemical reaction to the backbone.

(b) The backbone polymer could have active sites such as free radicals or ions formed upon it. These can then be used to polymerize a suitable monomer to produce the side chain of polymer.

2.1.1 Free Radical Initiation

Radical can be produced by a variety of redox, thermal, photochemical, and irradiation methods. In order to function as a useful source of radicals, an initiator system should be readily available and stable under ambient or refrigerated conditions, which process at a practical rate of radical generation at temperatures that are not excessively high (13).

(a) Redox Initiation

A redox initiation is often an efficient method of initiating a graft polymerization. This reaction produces radicals that can be used to initiate polymerization.

A prime advantage of redox initiation is that radical production occurs at reasonable rates over a very wide range of temperatures, depending on the particular redox system including the initiation of the moderate temperature of 0-50°C and even lower (13).

A wide range of redox reactions, including both inorganic and organic components, either wholly or in part, may be employed for this purpose. Some redox systems involve a direct electron transfer between reductant and oxidant, while others involve the intermediate formation of reductant-oxidant complexes, the latter are charge-transfer complexes in some cases.

(b) Type of Redox Initiator

Peroxide, in combination with a reducing agent, is a common source of radicals, for example,

$$H_2O_2 + Fe^{2+} \rightarrow HO^- + HO^\bullet + Fe^{3+} \qquad (2.1)$$

$$HO^\bullet + Fe^{3+} \rightarrow HO^- + Fe^{2+} \qquad (2.2)$$

The $HO^\bullet$ radical further decomposes the H_2O_2 molecules.

$$HO^{\bullet} + H_2O_2 \rightarrow H_2O + HO_2^{\bullet} \tag{2.3}$$

$$HO_2^{\bullet} + H_2O_2 \rightarrow HO^{\bullet} + H_2O + O_2 \tag{2.4}$$

However, decomposition of H_2O_2 by $HO^{\bullet}$ radicals takes place to an appreciable extent only if H_2O_2 is present in large excess over Fe^{2+} ions.

Other reactants, such as, Cr^{2+}, V^{2+}, Ti^{2+}, Co^{2+} and Cu^{2+} can be in place of ferrous ion in many instances. Most of these redox systems are aqueous or emulsion systems.

Organic-inorganic redox pairs initiate usually polymerization, but not always by an oxidation of the organic component, for example, the oxidation of an alcohol by Ce^{4+}

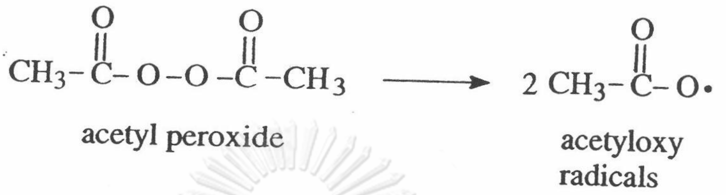
$$R\text{-}CH_2\text{-}OH + Ce^{4+} \rightarrow Ce^{3+} + H^{+} + R\text{-}^{\bullet}CH\text{-}OH \tag{2.5}$$

There are some initiator systems in which the monomer itself acts as one component of the redox pair. Examples are the pairs of thiosulfate plus acrylamide and methacrylic acid, and N,N-dimethylaniline plus methyl methacrylate.

(c) Thermal Decomposition of Initiators

The thermal, homolytic dissociation of initiators is the most widely used mode of generating radicals to initiate a polymerization. The number of different types of compounds that can be used as thermal initiators is rather limited. One is usually limited to compounds with bound dissociation energies in the range of 100-170 kJ/mole. Compounds with higher or lower dissociation energies will dissociate too slowly or too rapidly.

Only a few classes of compound, involving those with O-O, S-S, N-O bonds, process the desired range of dissociation energies. However, it is only the peroxides, which find extensive use as radical sources. The other classes of compounds are usually either not readily available or not stable enough. Several different types of peroxy compounds are widely used. These are acyl peroxides, such as, acetyl and benzoyl peroxides.



$$CH_3-\overset{O}{\overset{\|}{C}}-O-O-\overset{O}{\overset{\|}{C}}-CH_3 \longrightarrow 2\,CH_3-\overset{O}{\overset{\|}{C}}-O\cdot \qquad (2.6)$$

$$\varnothing-\overset{O}{\overset{\|}{C}}-O-O-\overset{O}{\overset{\|}{C}}-\varnothing \longrightarrow 2\,\varnothing-\overset{O}{\overset{\|}{C}}-O\cdot \qquad (2.7)$$

(d) Radiation

Grafting by irradiation of certain polymers either in the presence or absence of a vinyl monomer has been one of the most successful methods and has been applied to an enormous number of polymer-monomer systems.

The irradiation of organic macromolecules leads predominantly to the formation of free radicals. If the irradiation is carried out in air, and an effective free radical scavenger, peroxides and hydroperoxides, are formed within the polymer. If, however, the polymeric substrate is highly crystalline and in particular if the irradiation is carried out at low temperature and in the complete absence of air, the free radical can be trapped in the system and can remain *active* for a considerable time. The free radical, peroxide, and hydroperoxide formed or trapped in polymeric substrate, upon irradiation, can be used quite conveniently to initiate block and graft copolymerizations.

The two common γ sources available are the Co-60 and Cs-137, though the Co-60 is the one commonly used in radiation plants. The Co-60 source is relatively cheap, and has a useful half life of 5.25 years, the source itself is unaffected by high energy radiations from external source, and may be used up to a temperature of 1300 K.

Radiation is a very convenient source for graft initiation as it allows a considerable degree of control to be exercised over structural factors, such as, the number and length of the grafted chains by a careful selection of the dose and dose rate. Preirradiation and mutual irradiation are two different techniques used for radiation grafting (14).

(e) Chain Transfer and Copolymerization

An active site capable of initiating a graft copolymerization may be formed on a polymer via a *chain transfer* step in vinyl addition polymerization. For an initiation by chain transfer, the efficiency of the grafting reaction is dependent on the tendency of a propagating radical to transfer to a polymer (15).

In this method, radicals are created on the polysaccharide backbone by the following reactions:

$$R^{\bullet} + \text{Polysaccharide (PS)} \rightarrow PS^{\bullet} + RH \tag{2.8}$$

$$PS^{\bullet} + \text{Monomer}^{\bullet} \rightarrow \text{Graft copolymer} \tag{2.9}$$

$R^{\bullet}$ can be the growing chains of polymer formed by the polymerization with a radical initiator in the presence of the polysaccharide, or by the primary radical from the initiator itself. The efficiency of this type of grafting reaction is also greatly improved by increasing the ratio of polysaccharide to monomers, such as, by using a simple swollen system or with the correct choice of swelling agents. With the primary radical approach, the initiator can be absorbed first into the polysaccharide.

3. Thickener and Thickening Agents

Thickening is colloids which are usually prepared from thickening agents and which are either highly viscous solution of gel-like structure (9). The prime function of thickening agent in textile printing is to enable the dyestuff or pigment to be transfered to the fabric at the printing stage. Immediately following printing, the thickening agent must contain the dye within the printed area during drying. Furthermore, the thickening agent must break down during steaming or any other fixation process to such an extent the color *bleeding* occurs.

Viscosity of thickening must not change, easily soluble in water. After printing and drying, the thickening must be produced to a satisfactory adhesion of the print paste on the fibre, but the dry film should not be so brittle that it tends to peel. During the fixation on the prints, there should be a maximum degree of transfer of the dyestuffs or chemicals from the thickening to the fibre. The thickening should not react with dyestuff, nor should it react with chemicals in any undesirable way. This condition must not be imposed to prevent any impairment of the color strength of the prints, and also because the thickening agents must be easily and completely removed from the fibre after fixation of the dyestuffs, thus preventing hardening of the handle or stiffening in the prints areas and it must not be too expensive.

3.1 Selection of Thickening Agents

Colors printed on the fabrics mixed with thickener, which acts as a carrier of coloring matter, chemicals and solvents that it brings into close contact with the textile material during the printing process. Thickeners have various types, which may have similar or different qualities to produce satisfactory adhesion in the printing block, viscosity and chemical stability. Some thickener may be suitable for only one type of color but not the others, or can be used with various types of color, but it has the difference in the case of steam process or heating dry. So there is not one thickener that can be used for every task.

Thickeners are divided into four types, as the following:

3.1.1 Natural products are divided by the sources of the product (1).

- Plant seed which contain starch or similar products as a food reserve and may be cultivated for industrial use, such as, guar gum.
- Seaweed, the only source of the alginate.
- Plant gum exudates, of which the best known are gum arabic and gum tragacanth.

3.1.2 Modified Natural Products

To improve the solubility, the viscosity performance and the resistance to aging of native starch and of solutions, thickening agent prepared from both chemical and physical methods are used. In this manner, the following substances are obtained (9):

(a) Modified Starches

The following kinds of starches are known as modified starches :

- Pre-swelled starches, which have been prepared with partial decomposition are suitable for the printing of sensitive fibre types, or for printing with fine engravings.

- Acid-modified starches can be obtained by the treatment with diluted acids. This treatment will result in the limited formation of reductive end groups. The starch granules will disintegrate into smaller particles when they are boiled in water. This increases the solubility of the acid-modified starches.

- Oxidized starches. The oxidative decomposition of starch has the advantage of the formation of fewer reductive end groups compared with other decomposition process. The colloidal solutions of the soluble starches produced in this manner will tend to form gel either very slowly or not at all; and their behavior in respect of subsequent thickening is in line with this property. As a result, they are less viscous than native starches (9).

- Cross-bonded starches. These are chemical reaction products of starch by esterification or etherification. The property of these cross-bonded starches are much better than those of the original starch. Solutions of cross-bonded starches, which like the highly esterified hydroxyalkyl starches, are obtained by the reaction of starch with ethylene or propylene oxide, do not decompose on cooling down, and process with constant viscosity

(b) Starch derivatives

They are obtained by esterification or etherification of native starches. Secondary reactions will then lead to chemically cross-linked starch products. Starch ethers are preferable as thickening agents.

Starch ethers and starch esters (alkyl and acyl starches) (1). The etherification or esterification of the OH groups present in the glucose units will result in a signification change of the properties of starch. It prevents the association of the starch molecules, which are kept at a distance from each other by the substituents. Sometime, the solubility and washing properties of the thickening agents are considerably improved, and the stability of the starch solution is increased. Ethers are more stable than esters, because esters will tend to hydrolize or saponify more easily. Examples of starch derivatives are:

- Carboxymethyl starches (starch glycolates). These are produced from natural or decomposed starch reaction by chloroacetic acid in the presence of alkaline:

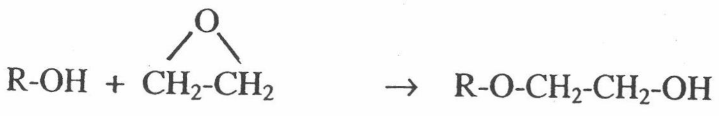
$$R\text{-}ONa + CH_2Cl\text{-}COOH \rightarrow R^{\bullet}\text{-}O\text{-}CH_2\text{-}COOH + NaCl \qquad (2.10)$$

($R^{\bullet}$ = starch radical)

- Methyl starches are produced by a chemical reaction of starch with dimethylsulfate in the presence of alkaline (caustic soda):

$$2\ R\text{-}ONa + (CH_3)_2SO_4 \rightarrow 2\ R\text{-}O\text{-}CH_3 + Na_2SO_4 \qquad (2.11)$$

- Hydroxylethyl starches (oxyethyl starches). These starch derivatives are obtained by the action of ethylene oxide on the native starch or more or less decomposed starches of different degrees of etherification:



$$\text{R-OH} + \overset{\overset{O}{\diagup\ \diagdown}}{CH_2\text{-}CH_2} \rightarrow \text{R-O-}CH_2\text{-}CH_2\text{-OH} \qquad (2.12)$$

(c) Decomposition Products of Starches

Highly molecular decomposition products of native starches, namely, Dextrin and British gum.

Dextrins. These are obtained by roasting starch with an acid addition, whereby highly decomposed products are obtained (white and yellow dextrins), with a pronounced reducing capacity. They can dissolve in water and are sometimes used as a 50% solution in the preparation of highly alkaline print pastes.

British gum. These thickening agents are produced by roasting maize starch or other kinds of starch, usually without any additions, but in some cases after adding acid or alkaline substances. Depending on the degree of decomposition, the yield of the individual products may differ. Thickenings will usually contain 30-50% dry substance, and are used both alone and in mixture with other thickening agents (9).

3.1.3 Synthetic Products

Copolymers of acrylates have been used more widely. Relatively low-cost copolymers of methacrylic acid and ethyl acrylate, for example, give low-viscosity dispersions in water in which the molecules are randomly coiled. Synthetic thickeners have good solubility in water (1).

3.1.4 Emulsion Thickening Agent

In 1937 it was shown that water-in-oil emulsions could be used to thicken pigment printing paste. Albumen was one of the best materials giving a flexible film with good pigment binding properties, but it was rather expensive. By using emulsions of water in white spirit, only the emulsifier remained when the thickener film was dried.

In 1950, attention was turned to oil-in-water emulsion. The large volume of white spirit makes it more essential to remove the vapor efficiently while drying, in order to avoid the build-up of explosive concentration in air at the baking stage.

3.2 Print Paste Rheology

The viscosity, η, of the liquid is defined as the ratio of the applied shearing stress $S = (F/A)$ to the rate of shear produced, $D = (dv/dx)$, also known as the velocity gradient (eq 2.13).

$$\eta = S/D \tag{2.13}$$

The viscosity of a liquid that requires a shearing stress of 1 $N.m^{-2}$ to produce a rate of shear of s^{-1} is 1 Pa.s (10 poise).

The viscosity of liquid is often determined by measuring the velocity of flow through a capillary tube, a calculation being based on a simple extension of the Newtonian analysis given above. The velocity will be high in the middle and low near the walls, a phenomenon readily appreciated by any who has rowed against the current in a river. Further, the velocity gradient will not be constant, but is high near the wall and zero in the middle. For a simple Newtonian liquid such as, water, glycerol, sugar solutions and many oils, the velocity is independent of the rate of shear. Typical print pastes are described as non-Newtonian, because their viscosities change as the rate of shear is changed. Measurement of their viscosities, therefore, is not straightforward. Information is required, nevertheless, because the viscosity of print paste during the shearing that occurs in the printing process will determine the quality of the print.

3.3 Non-Newtonian Flow

From the flow curves thus obtained, it is found that the behavior of a viscous liquid can be classified into four categories, shown diagrammatically in Figure 2.4.

A few material, such as, gum arabic paste, poly(vinyl alcohol) solutions, and silicone oils, are essentially Newtonian (curve A in Figure 2.4), when its viscosity is independent of shear. Most print pastes show shear-thinning properties (curve B), with a significant reduction in apparent viscosity as the shearing stress is increased. When the change is reversible, the viscosity increases immediately while shearing is reduced. A significant number of paste do not flow at all units when the shear stress exceeds a minimum value, known as the yield value (curve C in Figure 2.4). Beyond this value, they may show either linear or nonlinear flow properties. The former has been called Bingham systems. Bingham, who defined the term *plastic viscosity* as the ratio of shear stress to the rate of shear. The concept of plastic viscosity has been extended to shear-thinning print pastes in which flow curves that approach linearity at a high shear, by extrapolating the linear section of the curve back to the stress

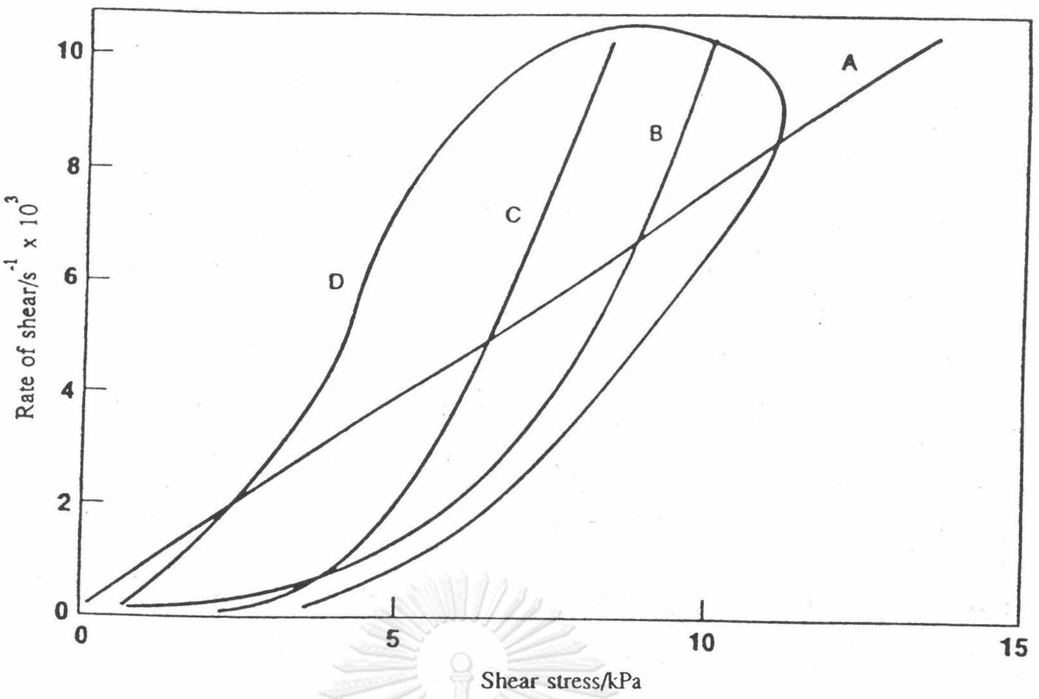


Figure 2.4 Typical flow curves demonstrating behavior of viscous liquids: A =Newtonian properties, B = Shear-thinning properties, C =Plastic viscosity, D = Thixotropy.

axis to define a yield value.

A fourth type of behavior, found in paste with high pigment concentrations, has been called dilatant, but it is better described as *shear thickening* to avoid the assumption that a volume expansion necessary occurs. This is the phenomenon seen on wet sand, when pressure causes particles to prevent their movement into expanded spaces. Free liquid is drawn into these spaces, making the system look dry. Dilatancy is the change from low to high viscosity, solidification (9).

Finally, there is the phenomenon known as thixotropy that is the reduction of viscosity (liquefaction) caused by a mechanical movement (shear stress). Thixotropy, shown by gelatin solutions and bentonite suspension, for example (curve D). In this case, the measured values of viscosity depend on the duration of shear as well as on the rate of shear.

3.4 Alginates (1)

Discovery of these substances is attributed to E. C. Stanford in 1883, who found that many of the brown seaweeds contained a viscous matter, which on treatment with sodium carbonate and subsequently with mineral acid, produced a new compound that was called alginic acid. This is a reserve product of cellular metabolism.

Alginic acid is a complex polyuronide, which is very stable to hydrolysis. It has been shown to be comprised mainly of β-1,4-linked D-mannuronic and L-guluronic acid units as shown in Figure 2.5.

Figure 2.5 Structure of D-mannuronic and L-guluronic acids

The alginic acid itself is insoluble in water, but has a high capacity for absorbing water. Its Na, K, NH_4, Mg, Fe(ferrous) salts are soluble in water, giving high viscosity, whereas the Ca, Al, Zn, Cu, Cr, Fe(ferric) and Ag complexes yield an insoluble compound.

Sodium alginates have become very important for print paste thickening because of their ready solubility, even after high-temperature fixation treatments. They are especially important for pastes of reactive dyes, because the extent of interaction is very small. This is due to the absence of primary hydroxyl groups and to the repulsion of dye anions by the ionized carboxyl groups of the polymer under alkaline conditions.

The viscosity of sodium alginate paste will depend on the r.m.m., concentration, method of preparation (high temperatures should be avoided to maintain the r.m.m.), shear stress and the presence of other ions. Some high-viscosity products contain a significant concentration of calcium ions, which allow the user to modify the paste properties. The crosslinking effect of the calcium ions increases the viscosity and the pseudoplasticity of the paste, but additions of controlled amounts of polyphosphate reduce these effects (Figure 2.6).

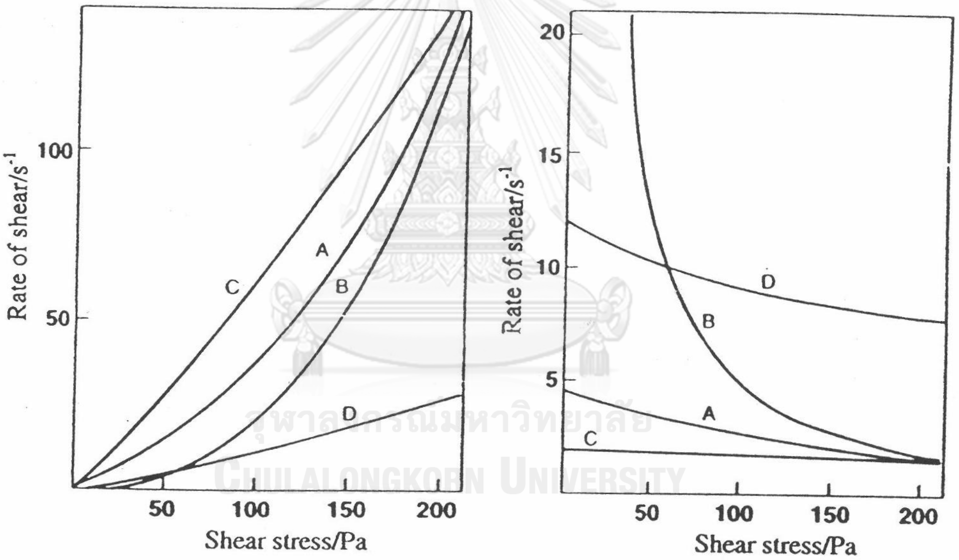


Figure 2.6 Effect of r.m.m., concentration and calcium ions on the viscosities of sodium alginate paste: A: high r.m.m., concentration 2%, Ca sequestered; B: high r.m.m., concentration 2%, 12%of alginate as Ca salt; C: low r.m.m., concentration 6%, Ca sequestered; D: low r.m.m., concentration 8.3%, Ca sequestered.

The stability is good between pH 4 and pH 10, but the gel is formed above pH 11.5 and below pH 3.5. Buffered alginates are sold for use in strongly alkaline conditions. Alginate pastes are compatible with a wide range of materials, including starch, cellulose ethers, tragacanth, and galactomannan gums.

4. Terminology and Definition

Before proceeding to the experimental part, several technical terms need to be clarified for the better understanding of readers.

4.1 Percent add-on

It is referred to as the weight percent of synthetic polymer in the graft copolymer and is determined as follows:

$$\%\text{ add-on} = \frac{\text{weight of polymer grafted} \times 100}{\text{weight of the grafted copolymer}} \qquad (2.14)$$

It is calculated after the acid hydrolysis method. The grafted polymers are separated from the starch backbone by heating the graft copolymer under reflux in a dilute acid solution.

4.2 Grafting Efficiency

It is a term often used to describe graft polymerization reactions and is defined as the percentage of the total synthetic polymer formed that has been grafted to starch. High grafting efficiencies are desirable since a polymerization of the low grafting efficiency would afford mainly a physical mixture of starch and homopolymer. It can be then calculated as follows:

$$\%\ \text{grafting efficiency} = \frac{\text{weight of polymer grafted} \times 100}{\text{weight of polymer grafted} + \text{weight of homopolymer}} \qquad (2.15)$$

4.3 Percent Conversion

It is used to describe the degree of polymerization of the monomer, which gives graft copolymer and homopolymer. It can be evaluated by the following expression.

$$\%\ \text{conversion of monomer} = \frac{\text{weights of polymers formed} \times 100}{\text{weight of monomer charged}} \qquad (2.16)$$

4.4 Grafting Ratio

It is a term used to describe a graft copolymerization, which is defined as the percentage ratio of the polymer grafted starch with respect to the starch, and can be calculated by the following expression.

$$\%\ \text{grafting ratio} = \frac{\text{weight of polymer grafted} \times 100}{\text{weight of starch}} \qquad (2.17)$$

4.5 Grafting Frequency

It is defined as an average number of anhydroglucose units (AGUs) per high-molecular weight graft that can be calculated by the following expression:

$$\text{grafting frequency (AGU/chain)} = \frac{\text{glucose (mole)}}{\text{PAA (mole)}} \qquad (2.18)$$

where

$$\text{PAA (mole)} = \frac{\text{\% add-on}}{\text{molecular weight}} \tag{2.19}$$

$$\text{glucose (mole)} = \frac{100 - \text{\% add-on}}{\text{glucose molecular weight (162)}} \tag{2.20}$$

5. Size Exclusion Chromatography (SEC)

This technique is different from all the other LC (Liquid Chromatography) methods because separations are based on a physical sieving process, not on chemical attractions and interactions. Therefore, the purpose of the mobile phase is merely to act as a solvent for the sample (16).

SEC has its origin in to separate groups of workers and was called by two separate names. Beginning in 1959, the name *gel filtration chromatography* was used by those using dextran gel to separate biochemical polymers using aqueous mobile phases, while, a few year later, the name *gel permeation chromatography* was coined by polymer chemists separating synthetic organic polymers on polystyrene gels using nonaqueous mobile.

SEC is a method for separating molecules based on their size by using stationary phases with pore size of discriminating among the analytes in a sample. Its primary application to the analysis of polymers, and it is commonly used to get the molecular weight distribution of polymers. The terms of gel filtration and gel permeation chromatography generally represented the use of hydrophillic and lipophilic gels for the separation of water-soluble and water-insoluble compounds, respectively.

Gel Filtration Chromatography (GFC) is a chromatographic technique, which separates products according to their moecular size using hydrophilic macrogels swelled in aqueous system (17).

5.1 Types of macrogel

5.1.1 Carbohydrate-derived gels. The most popular materials used for GFC are the Sephadex G-types gels. They are based on dextran, which is α-1,6-linked polymer of glucose. Sephadex gels are formed by cross-linking dextran with epichlorohydrin. Sephadex G-10, G-15, G-25, G-75, G-100 and G-200 are commercially available with molecular weights ranging from 700 to 200,000 for dextrans.

5.1.2 Polyacrylamide gels. Crosslinked polyacrylamide gels are vailable from Bio-Rad Laboratories under the trade name of Bio-Gel. Bio-Gels P-2, P-4, P-6, P-10, P-30, P-150, P-200 and P-300 have fractionation ranges varying progressively from 200 to 400,000 for globular proteins. The excluded limit of this gel are roughly estimated by the number following the letter P multiplied by 1,000. The gels are extremely hydrophilic and compatible with a dilute organic acid, 8M urea and even organic solvents to the extent of about 20% in water.

5.1.3 Inorganic gels. The TSK-SW and TSK-PW series of gels (Toyo Soda Co., Tokyo, Japan) have been introduced specifically for HPLC. This exact structure is unknown but it is understood that they contain a silica or styrene divinylbenzene co-polymer core with the surface modified by a layer of hydrophobic bonded phase or hydroxylated polyether. The G-1000SW and G-2000SW are suitable up to molecular weight of 25,000 and 100,000, respectively, for globular proteins, while the TSK G-3000SW and G-4000SW have fractionation ranges of 10,000 to 600,000. TSK G-1000PW to G-6000PW series have progressively increasing exclusion limits extending to higher than 2,000,000.

5.2 The separation mechanisum of GFC

- The molecular seive properties of macrogels.
- Adsorption.
- Ion Exchange.
- Ion Exclusion.

For product separation one can utilize open columns with diameters of 1-5 cm and lenghts of 20-200 cm or automatic commercially produced chromatographs with close columns.

5.3 The main application of GFC

- Separation of macromolecules from low molecular weight salts.
- Buffer exchange in macromolecular solutions.
- Fractionation of macromolecules, especially for biopolymers, in aqueous solutions.
- The study of molecular association, e.g. to measure bound small molecules in macromolecular solutions.
- The study of equilibrium constant in macromolecular solutions and polyelectrolytes.

Literature Survey

Joseph, A., and Radhakrishnan, G. (2) studied grafting of poly(butyl acrylate) onto gelatin with H_2O_2-ascorbic acid redox system. It was found that grafting efficiency increase with backbone concentration initially since more backbone radicals would be produced with high concentration of gelatin resulting in more grafting. With a further increase in backbone (BB) concentration, for the ratio of [BB]/[M]>1, GE decreases. Grafting efficiency increases initially with initiator concentration as the number of sites grafted increases with a further increase in initiator concentration. The homopolymerization is also increased with reduction in grafting efficiency. GE decreases with an incsease in temperature due to the decrease in monomer and initiator concentrations in the swollen phase as the rate of consumption of the monomer due to homopolymerization is also higher at higher temperatures.

Dipak, K., Ranjan, G., and Vithal, S. (18) prerared grafting of methyl methacrylate onto gurgum (GG) by hydrogen peroxide initition. It was found that a higher percent conversion was observed with higher concentrations of initiator. The decrease in percent add-on at higher concentrations of H_2O_2 indicated an enhancement in the rate of homopolymerization. Even after a polymerization period of 3 h, only 33% add-on has been achieved at $20x10^{-3}M$ H_2O_2, while 52% add-on has been achieved by $5x10^{-3}M$ H_2O_2. The amount of GG was increased from 2 to 10 g, whereas percent add-on decreased from 36.5 to 25.9% in the same range. The percent add-on showed a decreasing tendency with increasing the amounts of GG. This might be explained as the increase in amount of GG charged decreased the monomer to GG ratio.

Bayazeed, A., Elzairy, M. R., and Hebeish, A. (19) prepared graft copolymerization of maize starch with acrylic acid using potassium persulphate as free radical initiator. The graft yield is almost directly related to acrylic acid concentration up to 150% (based on the weight of starch). As the monomer concentration increases above this value, the graft yield tends to decrease. That is, the acrylic acid concentration of 150% constitutes the optimum concentration for grafting under the condition used. Starch/water ratio examined ranges from 1:3.5 to 1:23.5. It is observed that the maximum graft yield is obtained when a starch/water ratio of 1:6 was used. Higher or lower that this ratio brings about lower grafting. At the polymerization at 25°C, the graft yield very poor. Rising the polymerization temperature from 25°C to 40°C enhances the extent of grafting. When the polymerization was carried at temperature higher than 43°C the graft yield was decreased.

Okieimen, E. F., and Ebhoaye, J. E., (20) studied graft copolymers of acrylonitrile and of acrylic acid on cellulose materials obtained by ceric ion initiator. It can be seen that, at a constant initiator concentration, both the graft level and efficiency of grafting increase with time, tending to level off at longer durations of polymerization. Although an increase in ceric ion concentration is accompanied by an increases in graft level, the efficiency of grafting decreases with increase in initiator concentration. Any unreacted or unadsorbed ceric ion in the polymerizing system could initiate homopolymerization by a transfer to monomer.

The effect of temperature on the grafting of acrylic acid on the holocellulosic substrate is shown. The results show a decrease in the level of incorporation of poly(acrylic acid) grafts onto the backbone between 35°C and 40°C the graft level remaining virtually of grafting is more significant, decreasing from 54% at 35°C to 60°C

Vazquez, B., Goni, I., Gurruchaga, M., Valero, M., and Guzman, G. M. (21) studied graft copolymerization of methacrylic acid (MA) on potato starch using H_2O_2/Fe^{2+} redox system. The variations of %GE and %G with hydrogen peroxide concentration, decrease at beginning with increasing H_2O_2 concentration. The decreasing in %GE means that the amount of homopolymer produced is higher than that of graft copolymer as the $[H_2O_2]$ increases. The percent grafting efficiency and percent grafting increase when polymerization takes place during the first 2 h of the reaction and the rate then decreases gradually, because of the depletion in monomer and initiator concentrations as well as in available active sites on the substrate backbone. Rising the polymerization temperature from 60°C to 70°C brings about a significant increase in the %PMA grafted but not in the percent grafting.

Fanta, G. F., Burr, R. C., and Doane, W. M. (22) showed that the molar ratio of corn starch:CAN was varied between 10 anhydroglucose units (AGU) per $Ce^{4+}$and 1000 AGUs per Ce^{4+}. With granular starch, it appeared that ceric ion concentrations ranging from 1 mol/10 AGUs to 1 mol/500 AGUs yielded starch-*g*-PAN with about the same % add-on. Lowering the amount of CAN initiator to 1000 AGUs, Ce^{4+} produced starch-*g*-PAN with only 8% add-on by doubling the concentrations of starch and monomer in water, a 33% add-on was achieved at this starch:ceric ion ratio. Reactions run with gelatinized corn starch afforded starch-*g*-PAN with 55-56% add-on when 50, 100, and 200 AGUs per Ce^{4+} were used. As expected, graft polymerization to gelatinized starch gave higher graft molecular weights than did polymerization of granular starch.

Graft polymerizations were carried out by portionwide addition of initiator. It is apparent that portionwise addition of initiator gives granulars that are more highly grafted at the surface (from scanning electron micrographs), probably because each small amount of added ceric ion reacts rapidly with polysaccharide near the granule surface before it has a chance to diffuse to the granule interior.

Hebeish, A., and EL-kashouti, M.A. (23) cyannoethylated rice starch by reacting with acrylonitrile in the presence of sodium hydroxide at diffirent concentrations of acrylonitrile and various reaction temperatures. The effect of cyanoethylation on the rheological and solubility properties of starch was examined. It was found that the extent of cyanoethylation reaction, expressed by 25% N and the degree of substitution (DS), increased by increasing [AN] provided that the latter was not less 8 cm^3 acrylonitrile per 10 g starch. A temperature of 50°C constituted the optimum temperature for this reaction. Examination of rheological properties of the modified starch revealed that, cyanoethylated starches are characterized by pseudoplastic behavior of particular interest were the results of the viscosity. Cyanoethylated starch having smaller amounts of cyanoethyl groups had a higher viscosity than those of relatively larger amounts. On the other hand, the cyanoethylated starches were soluble in water, regardless of the extent of cyanoethylation.

Fanta, G. F., Burr, R. C., and Doane, W. M. (24) carried out a series of graft copolymerizations onto starch and cellulose at different stirring speeds using acrylonitrile and methacrylate monomer. Polymerizations were initiated with ceric ammomiun nitrate. This experiment showed no large effect of stirring speed on polysaccharide graft copolymerization which seems to be highly dependent on reaction condition. The polymerization might be rapid enough (with AN and MA) to produce high conversions, even under the most adverse stirring conditions. The important conclusion is that the stirring speed plays a minor role on the graft polymerization.

Burr, R. C., Fanta, G. F., Russell, C. R., and Rist, C. E. (25) determined accurately how the degree of swelling and the extent of granule disruption would influence the composition of graft copolymer prepared from gelatinized wheat starch with AN under the constant condition. They obtained a more frequently graft copolymer whose grafted chain and moleculars weights were on the order of 150,000. In contrast, the grafting AN to gelatinized wheat starch has afforded a product with few numbers of grafted chains, but high in molecular weight (about 800,000).

Taylor, N. W., Bagley, E. B. (26) prepared a starch-*g*-PAN copolymer with subsequent hydrolysis of PAN moiety to a mixture of carboxylate and carboxamide. This mixture behaved like polyelectrolyte solutions, but dilution experiments revealed that linear reduced vicosity-concentration plot could be obtained even under isoionic dilution conditions. These results combinded with a determination of gel constant from centifugation studies, led to the conclusion that the high viscosity of HSPAN was mixtures consisting of swollen, deformable gel particles closely packed in an intimate contact. Under a high dilution or at a high ionic strength condition, the gel particles no longer are tightly packed, solvents are present in excess, the viscosity drops precipitously, and thickening action effectively disappears.

Bazvaye, A., Okieimen, F. E., and Said, O. B. (27) prepared starch-*g*-PAN copolymer by varying the amount of initiator, and monomer. The results showed that, at each monomer concentration, the maximum graft level was attained at 10.0 m.mol Ce^{4+}/g starch. The ceric ion concentration at which the maximum graft level was attained was considered to indicate the ability and extent of the backbone polymer to reduce Ce^{4+} to Ce^{3+}.

Abraham, T. E. (28) found that cassava starch had poor paste stability during prolonged cooking. The starch was modified by heat treatment. A premoistured starch (18-24% moisture) was subjected to heat treatment for 3-16 h to bring about paste stability. Different types of heat treaments like moist pressure heating, dry heating and microwave heating were tried. The optimum heat treatment to bring about the paste stability was found to be 18-21% premoistured starch, which was heated at 110°C per 16 h. The modified starch granules were intact and had comparatively increased sedimentation volume, oil binding capacity, amylose susceptibility, solubility and decreased crystallinity, water binding capacity, solubility and paste translucency. The freeze-thaw stability was excellent with modified cassava starch.

Fanta, G. F., Burr, R. C., and Doane, W. M. (29) investigated the method by which methanol was added to precipitate saponified stach-*g*-PAN (HSPAN) from an alkaline saponification mixture that would influence the particle size of the polymer and thus the properties of the absorbent. The amount of methanol added to saponificate before dewatering and the method of methanol addition both would influence the particle size of the resulting HSPAN. For example, when 100 cm^3 of methanol was blended in to saponificate and the methanol was added rapidly than it could be adsobed, the resulting mass consisted of millimeter-sized lump of swollen HSPAN separated by a more fluid, methanol rich phase. Dewatering of HSPAN with excess methanol thus gave a granular product with good absorption. When methanol was added more slowly, and the addition was accompanied by thorough mixing to ensure complete absorption of methanol, it was precipitated individualy with micron-sized gel fragments of HSPAN gel and it would yield product with tiny or minute particle size to produce a smooth, free-flowing gel dispersion when added to water. This product was perhaps more useful as a thickener for aqueous systems than as a water absorbent.

Hebeish, A., El-Zaire, M. R., El-Rafie, M. H., Higazy, A., and El-Sisy, F. (30) prepared poly(acrylic acid)-starch composites by polymerizing acrylic acid with maize starch using $KMnO_4$/citric acid as a redox initiator system. These composites were used as pastes for printing cotton fabrics with reactive dyes. It is used to produce prints for comparing with those of sodium alginate which is a universally accepted thickener for printing of reactive dyes. It is seen that the color strength depends on the nature of the dye and time allowed before printing. With sodium alginate, the value of color strength is somewhat lower than those of pastes thickened with poly(AA)-starch composite. The print for sodium alginate and poly(AA)-starch composite show that cotton samples printed using the composite thickener acquire better fastness properties to rubbing, washing and perspiration as compared with those printed using sodium alginate.

Guion, T. H. (31) examined synthetic resin such as ethylene maleic anhydride copolymer (EMA91) and carboxymethyl cellulose (CMC) mixture for shear thinning behavior and salt sensitivity. The CMC makes the resin more resistant to loss in viscosity on addition of electrolytes. CMC must be added at a concentration of 2% or greater. The equivalent concentration of the cation of the electrolyte governs the loss in viscosity, three common sodium salts showing identical behavior. At low concentrations (0.004N), the calcium and magnesium ions responsible for *water hardness* have the same as sodium ions. At high concentrationes, the divalent metal exerts a stronger influence on viscosity loss.

Mixing CMC with resin EMA91 also reduces the high shear thinning behavior of the synthetic thickener, making it more like natural gums in this regard. The different grades of CMC tested were alike, however, in their ability to reduce the salt sensitivity of EMA91.

Reyes, et al. (32) prepared the graft copolymer of starch with unsaturated vinyl type compounds. It further showed that the saponification of the starch vinyl type copolymers provided products whose dilute aqueous solutions exhibited greatly increased viscosities that rendered the products highly useful as flocculents, sizing for paper, and thickening agent for textile.

Masuda, F., Nishida, K., and Nakamura, A. (for Sanyo Chemical Industries) (33) prepared the starch graft poly(acrylic acid) copolymers by stirring rice starch in water at 55°C for 1 h while purging with nitrogen gas. After the addition of calcium oxide, L(+)-ascorbic acid, and acrylic acid-hydrogen peroxide mixture were incorporated. The reaction mixture was subjected to the polymerization while stirring at 35°C for 3 h to obtain an elastically white gel which was neutralized with 30% NaOH, dried and pulverized.

Bazuaye, A., Okieimen, E. F., and Said O. B. (34) prepared the graft copolymer of AN on bleached holocellulose and starch at 29°C using the continuous initiation and depleted initiator methods. They found that, the extent of graft copolymer formation (grafting level and moleculer weight of grafted chains) on starch was higher than on bleached holocellulose. The extent of graft copolymer formation using the depleted initiator method was higher than that using the continuous initiator method but grafts from the latter method were more frequent on the polymeric substrates.

Rodehed, C., and Ranby, B. (35) prepared starch-*g*-PAN copolymers. Both granular native starch and water swollen gelatinized starch were used as substrates. Conversion of monomer, grafting ratio and percent add-on tended to increase when increasing the amounts of monomer charged. The amount of homopolymer formed was extremely low at approximately 1%. The efficiency, measured as conversion, was consistently higher with gelatinized starch as a substrate. The molecular weight of the grafts on geltinized starch was approximate eight times higher than those of granular starch.

The determination of molecular weight was carried out by gel permeation chromatography (GPC), by Prazink, W., Burdicek, G., and, R.H.F. (36). In this research, gel type used are dextran or agarose gel or porous glass. For the separation of potato starch, a system consisting of two columns, Sephacryl gels S-500 and S-1000, each with 90x1.6 cm I.D. was used. Chromatographic separations were performed at room temperature with 0.005M NaOH containing 0.002% sodium azide as eluent, flow rate of 25 cm^3/h and reflective index detector. From the number- and weight-average molecular weights of potato starch and maize starch were $0.32x10^6$, $1.05x10^6$, $0.27x10^6$, and $0.63x10^6$, respectively.

Aqueous gel chromatography for poly(ethylene oxide), pullulan and dextran was carried out, by Tadaya Kato, Tadashi Tokuya, and Akari Takahashi (37), in 0.1M aqueous sodium chloride solution at 25°C using a crosslinked hydrophilic vinyl polymer gel. From the result obtained, log M_w vs $V_{r,w}$ calibration curves for both poly(ethylene oxide) and pullulan were linear, but the calibration for dextran was non-linear. In conclusion, either PEO or pullulan is suitable as a polymer standard for aqueous gel chromatography. PEO in aqueous solution was less stable than pullulan, while the addition of sodium azide (0.02% w/v) to aqueous solvent was recommanded in order to prevent the bacterial degradation of pullulan. So pullulan is an important polymer standard based on the analogy of its chemical structure and properties with other linear polysaccharides. The dextran standard may be used as a standard for branched polymers as well.

The decomposition of starch-*g*-polyacrylamide (S-*g*-PAM), carried out by Dennenberg, R. J., and Abbott, T. P. (38), was modified to compensate for solubility problems of the PAM side chains in the glacial acetic acid-perchloric acid mixture. Ninety cubic centimeters of glacial acetic acid and 10 cm^3 $HClO_4$

(60%) were heated to 70-80°C. Two grams of the finely divided S-*g*-PAM was added to the hot reagent. After 20 minutes, the reaction mixture was poured into a 1:1 water-ethanol solution. The product was washed with ethanol, followed by vacuum drying. The swelling of the graft in a nonaqueous solvent like glacial acetic acid permits a rapid attack on the starch by $HClO_4$. For this reason, the $HClO_4$-glacial acetic acid treatment is a useful tool in analytical studies of the starch graft copolymer.

Gurruchaga, M., Goni, I., Valero, M., and Guzman, G.M. (39) studied of the acid hydrolysis of starch graft hydroxylic methacrylates and checked the extent of hydrolysis by characterizing the acrylic chains using the ^{13}C-NMR spectroscopy. The ^{13}C-NMR spectra of the poly(2-hydroxyethyl methacrylate) and poly(2-hydroxypropyl methacrylate) were obtained after the glacial acetic acid-perchloric hydrolysis. Both spectra showed the absence of amylopectin, but it showed a new band. This has led to a study about secondary reactions that might occur, and in this way to investigate the viability of the acid hydrolysis methods to be used with the hydroxylic methacrylates. The other reactions might occur during the utilization of perchloric acid. The chemical shifts of carbonyl groups in the NMR spectra are ester chemical shifts. The hypothesis that the new ester can be produced by transesterification in intermolecular with acetic acid solvent was postulated. The ^{13}C chemical shift at 20 ppm was due to the methyl group of the acetic acid. To check this hypothesis, they carried out the hydrolysis in a formic acid medium. The NMR spectrum does not show the methyl group (at 20 ppm). Then this hypothesis was proven true.

Wiwatwarrapan, C. (40) prepared the gelatinized cassava starch in water at 80°C for 1 h while purging with nitrogen gas. After the addition of calcium oxide, L(+)-ascorbic acid, and hydrogen peroxide solution were incorporated to the acrylic acid-starch mixture. The reaction mixture was subjected to the polymerization while stirring at 35°C for 3 h to obtain an elastically white gel which was then saponified with a 25% aqueous solution of KOH. The product was precipitate in methanol, dried and pulverized. The graft copolymer could absorb distilled, deionized water at 234 g/g.

A comparative study on the effect of monovalent salt on the rheology of water-soluble polymer was carried out by Tam, K.C., and Tiu, C.(41). In this research, the three commercial water-soluble polymers used are polyacrylamide, poly(ethylene oxide) and xanthan gum. The steady shear viscosity was examined. The relationship between the rheological properties of the polymers and the salt concentration is governed by the charged characteristic of the polymer chains. They found that, the effect of salt additions on the viscosity of polyacrylamide solutions were strongly influenced by the presence of cations such as Na^+. Such effects are consequence of the ionic charges present on the polymer chains and in the case of anionic polyacrylamide, in the form of $R\text{-}CO\text{-}NH_2$. It is evident that the presence of salt ($1x10^{-}$M to 0.5M) significantly reduces the viscosity of polyacrylamide. For poly(ethylene oxide), negligible effects are evident. Addition of NaCl does not change the viscosity at all. This highlights the inert nature of nonionic, whereby their rheological properties are not affected by the change in the solvent environment. In the case of xanthan gum, addition of salt initially reduces the viscosity but negligible effect was observed at $c_s > 5x10^{-2}$M. Xanthan gum is a polysaccharide consisting of a glucose backbone with charged trisaccharide side chains. Due to the bulky side chain, the polymer exhibits as a semirigid polymer. Addition of salt to solutions of xanthan causes a collapse of the side chains toward the backbone due to screening of charges, thereby resulting in a rodlike conformation. This causes a decrease in the solution viscosity upto c_s of $5x10^{-2}$M.