CHAPTER II

LITERATURE REVIEWS AND THEORITICAL CONSIDERATIONS

2.1 Cellulose as raw material of carboxymethylcellulose production

Cellulose is widely found in the plant kingdom and in some groups of fungi, bacteria and protists. The chemical structure of cellulose is a linear homopolymer composed of β-1,4- linked D-glucopyranosyl units (Richmon,1991). These residues form long chains with variable degree of polymerization (DP ; number of monomeric residues per polymer molecule). The DP values in higher plants appear to generally range between 7,000 to 14,000 or more for secondary walls, whereas they are as low as 500 for primary wall. These extended glucan chains strongly associate by hydrogen bonding and van der Waals forces (Haigler,1991). The cellulose from *Acetobacter xylinum* , DP value varied according to the age of culture, starting about 2,000 and increasing to nearly 4,000 at steady state growth (Richmon,1991).

Under electron microscopy, *Acetobacter* cellulose appears as a network of fibrils, among which bacteria are embedded and its characteristically takes the form of separate ribbon-like fibrils. In contrast, the cellulose from higher plants consist of bundles of microfibrils. The advantages of using bacterial cellulose for CMC production are : the bacterial cellulose are composed of pure cellulose which is devoid of lignin, hemicellulose and other substances (Yamanaka and Watanabe,1994).

2.1.1 Structure of cellulose

Cellulose is made up of anhydroglucose units linked through a β-1,4 glycosidic linkage. Each anhydroglucose unit contains three hydroxy groups, a primary hydroxyl at 6-position and two secondaries at 2 and 3 position, structural formula of cellulose is presented in Figure 2.1.

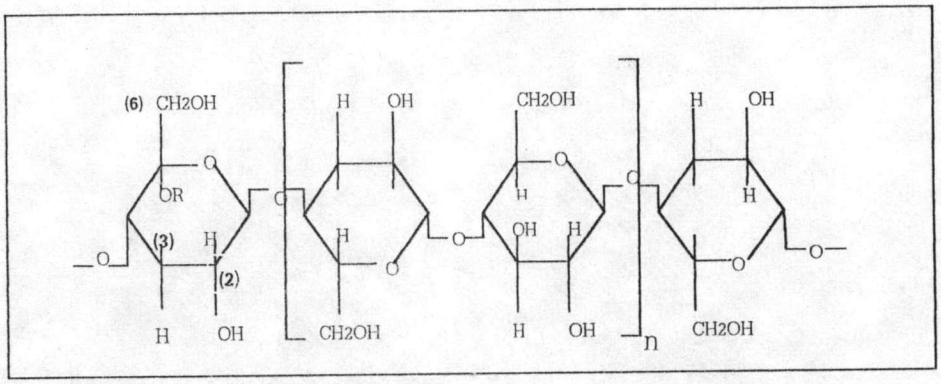

Figure 2.1 Structural formular for cellulose

Cellulose can exist at least in four different crystalline forms as determined by X-ray crystallography (Mueller and Brown,1980). The predominant native cellulose designated cellulose I. The Cellulose II is formed by mercerization process, which involves swelling cellulose in alkali, then washing in water. Although rare, there is evidence that cellulose II is produced in nature. Two other forms of cellulose, III and IV are reversibly produced by chemical treatments of either cellulose I and II (Richmon,1991).

2.1.2 Detection of cellulose

Preliminary indications of cellulose can be obtained by chemical tests such as the presence of anthrone-positive material after treatment with acetic-nitric reagent and staining with chlorozinc iodine(Ruben and Bokelman,1987). Degradation by purified cellulase with a yield of glucose and cellobiose further indicates cellulose. Recently, highly purified cellulase conjugated to colloidal gold were used as probes to identify cellulose by electron microscopy. The above chemical methods should be confirmed by crystallographicanalysis or by methylation analysis (Richmon,1991) with gas chromatrography/mass spectrometry or NMR spectroscopy to establish the presence of β-1,4 linked D-glucopyranosyl residues. By X-ray diffraction of polymer from *Acetobacter xylinum* yields reflections corresponding to those for celluloseI (Felcht,1985).

2.1.3 Bacterial cellulose

The first observation of cellulosic gel was in vinegar fermentation, a gelatinous mat sometimes form on the surface of the broth in static cultures. The analysis of this gelatinous material revealed to be cellulosic material (Daimaguila, 1967). Under electron microscopy, the gelatinous material appears as a network of fibrils among which bacteria are embedded (Banzon, Gonzalez and de Leon, 1990)

In an attemp to examine the question of whether the fibers and ribbons were indeed the primary structural unit of cellulose itself, Muhlethaler (1949) performed the studies on the electron micrographs of bacterial cellulose. The results of his investigation showed that the cellulose built by *A. xylinum* occurs in the form of fibers having about the same diameter (ca 250 $^{\circ}$A) as that of the fibrils of cellulose from the cell walls of numerous plant (Banzon, Gonzalez and de Leon, 1990). Bacterial cellulose takes the form of separate ribbon-like fibrils as shown in Figure 2.2a. The microfibrils were excreted from many sites on the surface of *A. xylinum* and joined to form a ribbon structure. The width of the ribbon was estimated to be 3.2 x 133 nm ; the lenght varied with the duration of culture and estimated, from electron micrographs, to be over 10μm (Figure 2.2b).

The degree of polymerization (DP) depended on the ribbon lenght, that also varied with the age of culture. The DP of cellulose, starting about 2,000 and increasing to nearly 4,000. The velocity of ribbon growth was estimated to be about 2nm/min (Yamanaka and Watanabe, 1994). The cellulose from *Acetobacter xylinum* constitutes up to 4% of floc dry weight. Its compositions are shown in Table 2.1.

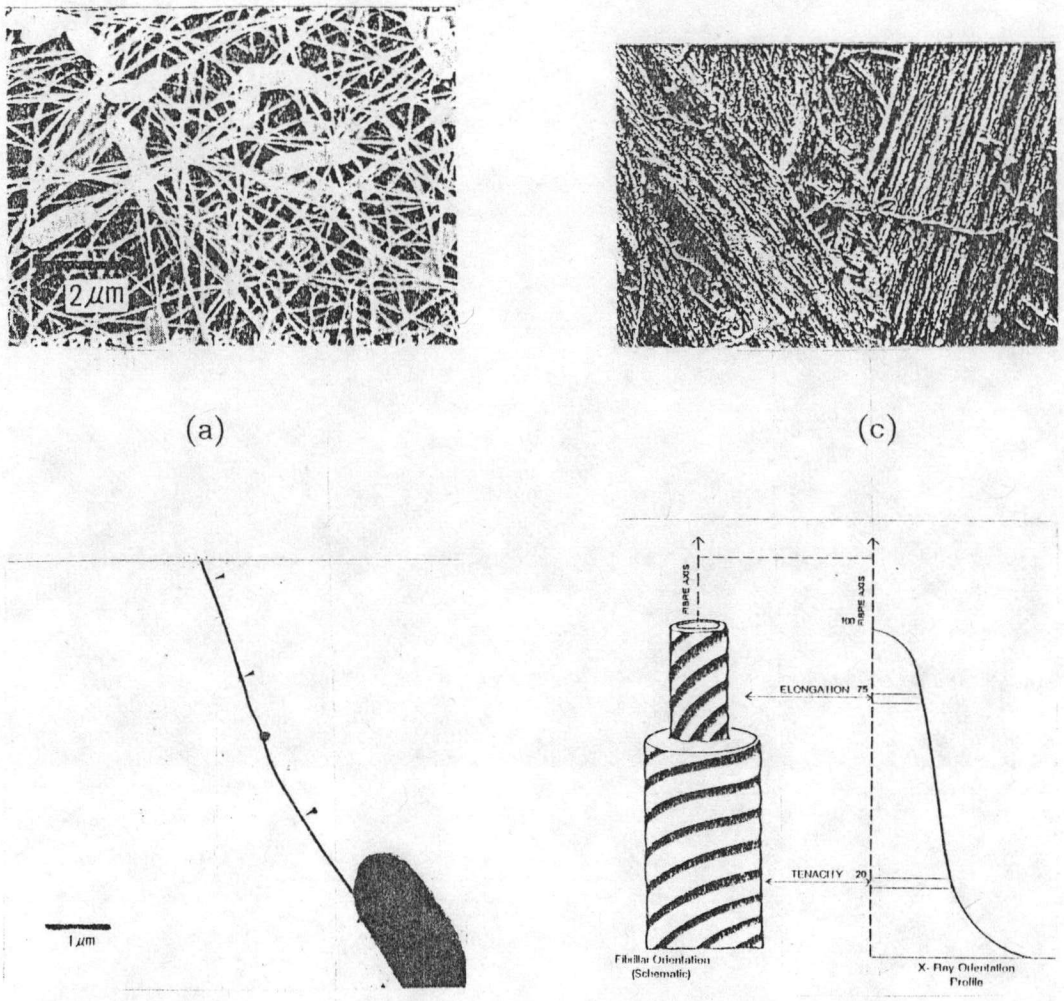

Figure 2.2 (a) Scanning electron micrograph of a network of ribbons in gelatinous material, *Acetobacter xylinum* are entrapped with the network. (Yamanaka and Watanabe,1994)

(b) Transmission electron micrograph of bacterial cellulose ribbon produced by *Acetobacter xylinum*(Yamanaka and Watanabe,1994)

(c) Microfibrillar stucture of cotton as revealed under Transmission electron microscope (Felcht,1985)

(d) Fibrillar model (Warrier and Chidambareswaren,1993)

Table 2.1Composition of cellulosic gel from *Acetobacter xylinum* (Arunoros, 1976)

carbohydrate	3.00 %	calcium	34.50 mg
fat	0.05 %	Ferrous	0.20 mg
fiber	1.10 %	phosphorus	22.00 mg
protein	0.68 %	vitaminB1	0.10 mg
ash	0.77 %	vitaminB2	0.02 mg
H_2O	94.40 %	niacin	0.22 mg

2.2 Nata Cellulosic gel production by *Acetobacter xylinum*

2.2.1 *Acetobacter xylinum* as cellulose producer

Acetobacter xylinum has long been considered the most successful model for studies on the mechanism of cellulose biogenesis. *A. xylinum* cells are aerobic rod-shaped, occuring singly, in pairs or in chains. Young cells are gram-negative, becoming gram variable as the cultures get older. The X-ray diffraction of its cellulose reflections corresponding to cellulose I. Its cellulose is unusual, however, in not being synthesized as a wall component. Instead it is secreted into medium from pores along the length of the cell, a single ribbon being produced from a single cell (Legge, 1990). The ribbon, which is a composite of hundreds of glucan chains extruded along the row of the pores, is formed parallel to the longtitudinal cell axis. The carbon compounds utilized by the organisms are glucose, galactose, sucrose, ethylene, glycol, maltose, lactose and mannitol. Pentoses and trisacchrides are not acted upon (Banzon, Gonzalez and de Leon, 1990). The bacterial cell take up glucose from the substrate and combine it with a fatty acid to form a precursor inside the cell membrane. The precursor is then excreted into the cellulose outside the cell walls. In sugar-grown, carbohydrates are oxidized by way of a pentose cycle. (Shramm, Gromet and Hestrin,1957), and organic acid and ethanol are oxidized by way of TCA cycle which also participates in the sugar oxidation. It could be assumed that the probable chemical pathways participating in cellulose formation from various substrates by *A. xylinum*.

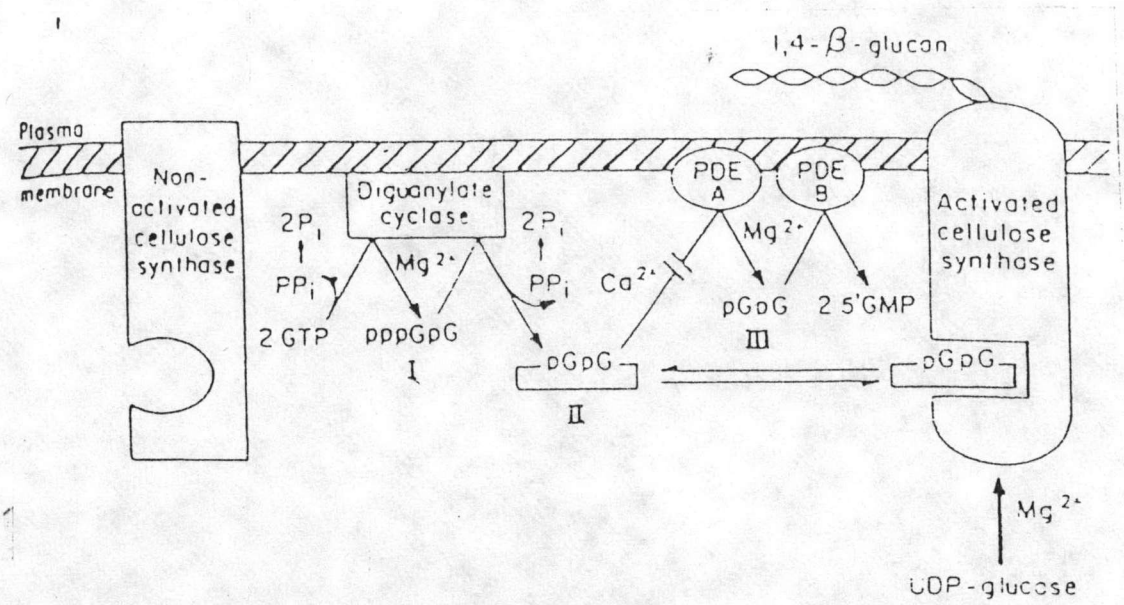

Figure 2.3 Propose regulatory control pathway of cellulose synthase (Ross, Mayer and Benziman,1991)

2.2.2 Culture medium for *Acetobacter xylinum*

The cellulosic gel producer, *A. xylinum*, are widely distributed in nature. It may obtained from fermenting sweet plant juices or rotting sugar fruits and vegatables (Lapuz, Gallardo and Palo,1967). The cellulosic gel formation by *A. xylinum* could be produced on coconut water, coconut milk and synthetic media. Some of synthetic media were shown in Table 2.2. The carbon compounds utilized by *A. xylinum* were the hexose sugars or substances which the organism can readily convert into hexoses. The nitrogen source utilizations; casein, yeast extract, and ammoniumphosphate have been reported to give high yield of cellulosic gel (Daimaguila, 1963).

Table 2.2 Some of synthetic media formulars for *Acetobacter xylinum*

medium formular / **substrate**	**Hestrin and Schramm (1954)**	**Alaban (1962)**	**Forang et al. (1989)**	**Okiyama et al. (1992)**
C-source	glucose 2.0 %	sucose 10.0 % ethanol 2.5 %	glucose 2.0 %	sucrose 5.0%
N-source	yeast extract 0.5 % peptone 0.5 %	yeast extract 0.25 % $(NH_4)_2SO_4$ 0.06 %	NH_4Cl 0.1 %	yeast extract 0.5 % $(NH_4)_2SO_4$ 0.5 %
trace elements	Na_2PO_3 0.27% citrate 0.12%	KH_2PO_4 0.5 % $MgSO_4.7H_2O$ 0.02 %	Na_2HPO_4 0.27% $MgSO_4.7H_2O$ 0.025% KCl 0.01% citric acid 0.115%	KH_2PO_4 0.3 % $MgSO_4.7H_2O$ 0.2 %

2.3 Structure and properties of carboxymethylcellulose

The structure of carboxymethylcellulose was shown in Figure 2.4. Each anhyhydroglucose unit of cellulose contains three hydroxyl groups. The average number of hydroxyl groups which have been substituted per anhydroglucose unit to form any cellulose derivatives is designated as the degree of substitution (DS). The carboxymethylcellulose shown in Figure 2.4 presented the DS of 1.0.

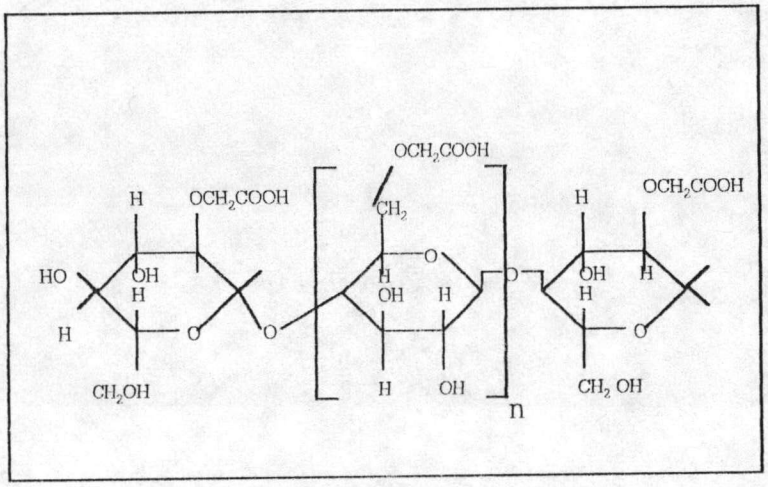

Figure 2.4 The structure of carboxymethylcellulose molecule (Dieckman, Jarrell and Voris,1953)

The CMC is a white or yellowish odorless and tasteless powder which may or may not contain fibrous residues. It browns upon the heating at 180°C to 225°C and chars when heating to 210°C to 250°C (Brown and Houghton,1941). The CMC is soluble only in aqueous sodiumhydroxide when the DS equals 0.05 to 0.2. As the DS is increased, the solubility in water is improved. The CMC with the DS of 0.6-0.8 gives the good water solubility (Savage, Young and Maasberg,1954). In addition, the CMC with the DS of 0.4-1.4 is mostly used in various industries. The low substitution types (DS ≤ are insoluble in water but soluble in alkali. Water solubility is achieved with DS greater than 0.4 (Green, 1963).

The wood cellulose with the DP value between 300 to 5000 were widely used for CMC production (deButts, Hudy and Elliott, 1957). The viscosity of aqueous solution is depend upon the DP of cellulose. As the DP is increased, viscosity is improved. The viscosity of CMC solution is classified to three types as follows, low viscosity, 25-50 cP in 2% CMC solution; medium viscosity, 60-400 cP in 2% CMC solution; high viscosity, 1500 cP or above in 1% CMC solution (Thai Cellulose Products Ltd., 1995).

The CMC solution is pseudoplastic-viscosity. Its viscosity is decreases as shear rate is increased. The pseudoplastic flow occurs in solution where the carboxymethylcellulose has the minimum uniformity of carboxymethyl substitution.

Like other cellulose derivatives, sodiumcarboxymethylcellulose may be degraded by the action of heat, radiation, acids and oxidizing agents. This degradation represents hydrolytic cleavage of the acetal linkages of the cellulose. The CMC is degraded by air oxidation under strongly alkali conditions(Batdorf,1959). Other properties of the CMC are shown in Table 2.3.

Table 2.3 Typical properties of Na-CMC (Dupont Company, 1982)

moisture content (%Max)	10
browning range* (^{o}C)	190-210
charring temperature* (^{o}C)	235+
pH at 1% solution	6.5-8.5
bulking value, insoluble, (gal/lb)	0.0659
refractive index of 1% solution	1.333

* After one minute on Parr bar

2.4 Uses and applications of carboxymethylcellulose

The CMC is useful in aqueous solutions because of its thickening, suspending and stabilizing properties (Hollabaugh, Burt and Walsh.,1945). Applied from solution to form films is useful as an adhesive and as a durable hydrophilic coating (Batdorf,1959). In addition, the CMC is usually used in the form of its sodium salt. The nonpurified grades of CMC, containing significant amounts of sodiumchloride and sodiumglycolate, may be used in any of the industrial applications where the impurities are not objectionable. The important of CMC applications are in various industries ; food, adhesives, detergents, pharmaceuticals and cosmetics, paper, etc.

2.4.1 Foods

CMC can be applied in various kind of food products such as ice cream, soft drink, milk, cheese product, dressing, sauces, confectionery, bakery product, low calories foods, instant foods and product containing protein. Toxicity testing by extensive feeding studies have been made on rats, pigs, dogs and human being. Metabolism test have also been conducted with rats and rabbits using CMC. Such studies have also been made on rats with a radioactive form (Hollabaugh, Burt and Walsh,1945). Moreover, the hydrophilic properties of CMC make it suitable for use in a broad range of food products. Some important properties of CMC utilized in different food applications are presented in Table 2.4.

2.4.2 Adhesives

Savage, Young and Massberg (1954) described a specially dried and powdered water soluble carboxymethylcellulose or its sodium salt as an adhesive. A viscous aqeuous solution containing 3 to 8% of CMC, alone or with soluble starches, was reported as forming adhesives suitable for pasting wall paper or any paper products such as boxes.

2.4.3 Detergents

The addition of 0.3-1.0% CMC to synthetic detergents greatly improves their efficiency in washing cotton clothing. It is beleived that CMC function is a soil suspending antiredeposition agent (Niewenhuis,1961).

Table 2.4 Some important properties of CMC utilized in different food applications (Kloow, 1985)

Application / Properties	ice cream	soft drink	milk drink	cheese product, dressing and sauces	confec tionery	bakery product	low calorie foods	Instant foods	product containing protein
thickening	■	■	■	■			■	■	■
stabilizing		■	■	■	■	■			
water retention	■		■		■	■	■	■	■
protective colloid action		■		■					■
gel formation			■		■				
complex formation			■						
crystallization inhibition action	■				■			■	

2.4.4 Paper applications

Paper such as boxboard, is highly porous. When paperboard is coated with CMC, a gloosy finish is obtained. The surface is much more suitable for subsequent printing than the porous uncoated surface ; the printing is sharper (Barber, 1961).

2.4.5 Pharmaceuticals and cosmetics

CMC is used as a suspending agent in lotions and as a thickener for jellies and ointment (Batdorf, 1959). In addition, Anon (1988) reported that medicaments, such as capsules, tablets, and powders surrounded by an enteric film or layer of carboxymethylcellulose, were insoluble in the stomach acids but soluble in alkaline intestinal fluids. Because of its swelling action, it is used as a bulk laxative.

2.4.6 Miscellaneous

Miscellaneous proposed used of CMC include clarifying agents for liquids, can-sealing compounds, crystallization inhibitors, making films for wrapping, stabilizer and emulsifier etc.

2.5 Carboxymethylcellulose production

The cellulose-derived hydrocolloid most widely used in the food industry is the water soluble sodiumcarboxymethylcellulose (Na-CMC). In CMC preparation, the cellulose is treated with sodiumhydroxide and is then reacted with chloroacetic acid or sodium chloroacetetate. The etherification reaction (eq. 2.1) involved is

$$[C_6H_7O_2(OH)_3]_n \xrightarrow{NaOH} [C_6H_7O_2(OH)_2ONa]_n \xrightarrow{ClCH_2COONa} [C_6H_7O_2(OH)_2\text{-}O\text{-}CH_2COONa]_{n(2-1)} \quad (2.1)$$

cellulose — alkali cellulose — sodiumcarboxymethylcellulose

(McLaughlin and Herbst, 1950)

The chemical reaction, the two principal steps are ; (i) the reaction between cellulose and sodiumhydroxide to form alkali cellulose, and (ii) the reaction of alkali cellulose with dry sodiumchloroacetate to form sodiumcarboxymethylcellulose and sodiumchloride. In addition, the conversion of sodiummonochloroacetate to sodiumglycolate as side reaction, occurs simultaneously is shown in equation 2.2

$$ClCH_2COONa + NaOH \xrightarrow{H_2O} HOCH_2COONa + NaCl \quad (2.2)$$

(McLaughlin and Herbst, 1950)

The efficiency of the carboxymethylation is increased and the side reaction is decreased as high sodiumhydroxide concentrations. However, enough water must be present to insure adequate swelling of the cellulose so that etherification occurs uniformly (McLaughlin and Herbst, 1950). The CMC purification for removing sodiumglycolate and sodiumchloride, CMC is treated with alcohol-water mixture.

The industrial CMC production is shown in Figure 2.5. Firstly, raw material is milled and, then activated with NaOH to form alkali cellulose. This reaction is called as mercerization reaction. The mercerized cellulose is slightly lower in density than the untreated cellulose and caused to get the increase of water absorption, the better dye ability, the lower tensile strength, and the higher extensibility (Krassis and Kitchen, 1961).

The next step is the carboxymethylation reaction of alkali cellulose with dry sodiumchloroacetate to form sodium carboxymethylcellulose. The reaction mixtures, composed of cellulose, sodiumhydroxide and sodiumchloroacetate, remain in a particulate form until the DS is great enough to impart water solubility to the product. If the water soluble stage is attained, the product is transformed into a heavy dough which is difficult to handle. This doughty stage can be avoid by stopping the mixing and allowing the reaction to continue in bins or by adding enough of a water-miscible liquid such as ethanol, to prevent the crude CMC from going to doughty stage (Ganz,1973).

At the end of carboxymethylation, the reaction mixture contains a slight excess of sodiumhydroxide. The pH is generally nuetralized to 7-7.5. If the pH of reaction mixture is adjusted to 6.0 or less, the dried CMC have low water solubility. If the pH of reaction mixture is 4 or below, the dried product is insoluble in water.

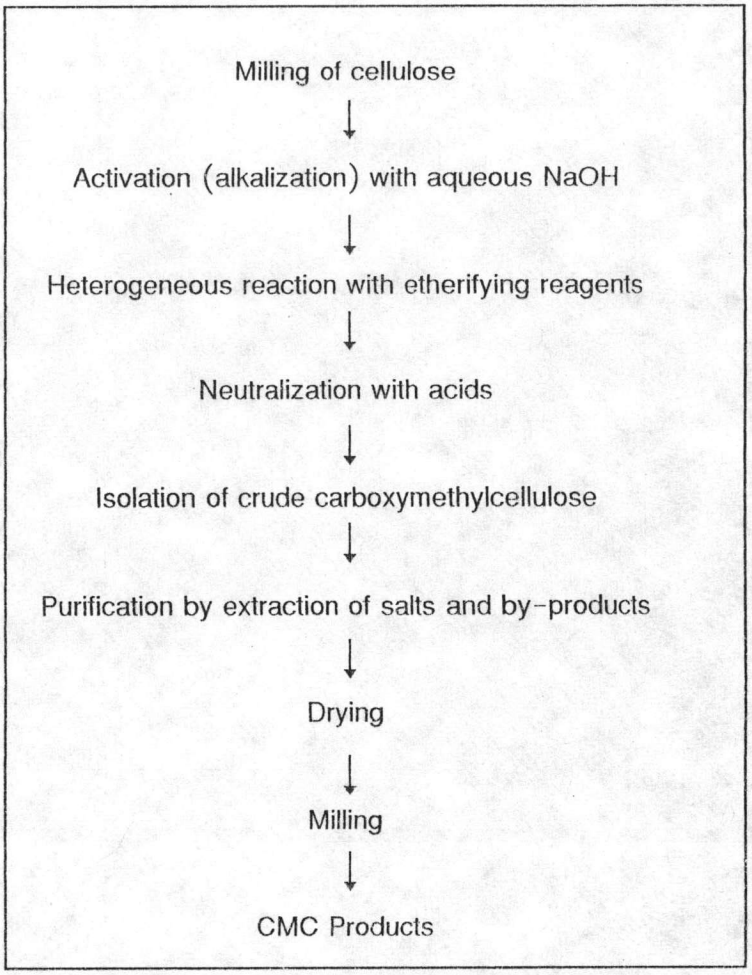

Figure 2.5 Principal steps in technical production of carboxymethylcellulose (Felcht,1985)

In commercial CMC manufacture, several different processes are being used. Alkali cellulose is prepared by steeping sheeted cellulose in aqueous sodumhydroxide, pressing out the excess base, and masticating in Warner-Pfeidere type of mixer. Monochloroacetic acid or its sodium salt is then added and mastication is continued at the room temperature or an elevated temperature of 50°C -75°C (Klug, 1963). In a similar process, the steeping and pressing operation are omitted and the ingredients are combined directly in a mixer shredder. Some procedures have been described in which the cellulose is treated with monochloroacetic acid before the sodium hydroxide is added (Klug,1963).

The improvement of DS of CMC have been studied by various research groups. Theoritically, the extent of etherification is determined by the reaction variables such as reaction temperature and the relative proportions of cellulose, alkali, etherifying agent and water in the reaction mixture. In 1965, Nakamura and Watanabe introduced a technique for CMC production with DS higher than 1.0 by using multistep of etherification. In addition, David (1974) reported that the addition of inert solvent into reacting solution eliminated dough formation and increased the DS of product. It could be assumed that DS of CMC could be increased if the influence of reaction variable on the DS is in the state of optimization.