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Chemical Modification of Polysaccharides Using Reactive Extrusion

Pratik Bhandari

University of Nebraska-Lincoln, pratikbhandari@gmail.com

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CHEMICAL MODIFICATION OF POLYSACCHARIDES USING REACTIVE EXTRUSION

By

Pratik N. Bhandari

A DISSERTATION

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CHEMICAL MODIFICATION OF POLYSACCHARIDES USING REACTIVE EXTRUSION

Pratik N. Bhandari, Ph.D.
University of Nebraska, 2012

Advisor: Milford A. Hanna

The objective of this dissertation was to study the use of reactive extrusion for the chemical modifications of starch and cellulose. A carboxymethyl derivative of starch and carboxymethyl and acetate derivatives of cellulose were prepared using reactive extrusion.

Carboxymethyl starch with rapid swelling properties in water was prepared using reactive extrusion. This was achieved by controlling the gelatinization and through the use of NaOH by controlling the water/ethanol ratio. The effects of NaOH, H₂O, temperature, ethanol, sodium mono chloro acetate, sodium tripolyphosphate, citric acid, epichlorohydrin and extruder screw configuration on the degree of substitution of carboxymethyl starch were studied.

The physical, chemical and morphological properties of sodium starch glycolate (cross-linked carboxymethyl starch) prepared using reactive extrusion were characterized and compared with those of VIVASTAR®P. The liquid (water and 0.1N HCl) uptakes by sodium starch glycolate prepared using reactive extrusion were lower than those by VIVASTAR®P. This may have been because of gelatinization of starch and insufficient cross-linking in sodium starch glycolate prepared using reactive extrusion.

Carboxymethyl starches with high degrees of substitutions also were prepared using
reactive extrusion. The effects of SMCA:starch ratio, aqueous ethanol:(starch+SMCA) and the extruder screw configuration on the degree of substitution and reaction efficiency were studied.

Further, the use of reactive extrusion for the chemical modification of a non-thermoplastic material like cellulose was demonstrated. The effects of water:ethanol ratio and amounts of NaOH used, on the degree of substitution, degree of crystallinity, cellulose-II crystalline fraction, saline uptake, saline absorption and surface morphology were studied. X-ray diffraction analysis revealed that carboxymethyl cellulose prepared using 100% H$_2$O had higher degree of crystallinity and cellulose-II crystalline fraction. Saline uptake by this carboxymethyl cellulose was comparable with that of sodium polyacrylate.

Acetate derivatives of cellulose also were prepared by a solvent-less reactive extrusion process with the use of iodine catalyst. The effects of the acetic anhydride:cellulose ratio and iodine concentration on the degree of substitution, crystalline structure, oligomer formations and surface microstructure were studied.
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DISSEMINATION FORMAT

This dissertation is a compilation of five chapters. Each chapter is written as a research paper that has either published, is in the process of being published or is intended for publication in a scientific journal. Each chapter consists of an abstract, introduction, materials and methods, results and discussion and conclusions. These chapters are preceded by an introduction which details the scope and objectives of the research.
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CHAPTER I

INTRODUCTION

Starch and cellulose are polysaccharides or polymers linked together by glycosidic bonds. Starch is a polymer with \( \alpha (1\rightarrow 4) \) linked glucopyranose units, containing amylose and amylopectin. Amylose is a straight chain polymer, whereas amylopectin is highly branched through \( \alpha (1\rightarrow 6) \) linkages. Cellulose is a straight chain polymer with \( \beta (1\rightarrow 4) \) linked glucopyranose units. Natural cellulose with cellulose-I crystalline structure is much more crystalline than starch.

Cellulose and starch are bio-materials that are renewable and produced in large amounts. Cellulose is the largest annually produced bio-material by volume. Bio-sourced products are experiencing renewed interest with diminishing fossil fuels and as researchers work on developing a bio-refinery based economy.

At present, chemical derivatives of starch and cellulose are prepared using batch reactors which use large amounts of solvents to suspend the reactants. These processes are also characterized by a large reaction time. Reactive extrusion could be an efficient method for chemical modifications of starch and cellulose. The use of solvents could be eliminated in this process. The use of a twin-screw extruder also allows efficient mixing of the reactants. Also, the use of kneading blocks in the extruder, results in large shear forces on the reactants and their efficient contact. The flexibility in using a desired barrel temperature permits efficient heat transfer to the reactants.
Reactive extrusion has been a popular technique for chemical modifications of starch. However, during extrusion the granule structure of starch is destroyed. Cross-linked carboxymethyl starch is a pharmaceutical excipient used for disintegration applications in solid-dosage forms. Highly gelatinized carboxymethyl starch impedes its disintegration functionality. The work in this dissertation attempts to prepare cross-linked carboxymethyl starch using reactive extrusion, but limit its gelatinization during the process, such that it is suitable for disintegrant applications. The physical, chemical and morphological properties of such material are compared with those of a commercial product, VIVASTAR®P. The use of reactive extrusion for preparation of highly substituted carboxymethyl starch also was explored.

Reactive extrusion has been sparingly used for chemical modifications of non-thermoplastic materials like cellulose. This work explored the carboxymethylation and acetylation of cellulose by reactive extrusion. Cellulose was acetylated using iodine catalyst which allowed for the complete elimination of solvents. The effects of reactant stoichiometry on properties of these cellulose derivatives prepared using reactive extrusion were studied.
CHAPTER II

A CONTINUOUS SOLVENT-LESS EXTRUSION PROCESS FOR PRODUCING SODIUM CARBOXYMETHYL STARCH SUITABLE FOR DISINTEGRANT APPLICATIONS IN SOLID DOSAGE FORMS

This research paper has been published as:

Abstract

Sodium carboxymethyl starch (CMS) was prepared using an extruder as a continuous solvent-less chemical reactor. The effects of NaOH, H₂O, temperature, ethanol, sodium mono chloro acetate (SMCA), sodium tripolyphosphate (STPP), citric acid, epichlorohydrin and screw configuration on the degree of substitution (DS) and swelling properties of CMS were studied. Using these factors, CMS with the DS required for pharmaceutical disintegrant applications (0.22-0.35) was prepared. By modifying the water/ethanol ratio in the formulations and by avoiding excess gelatinization caused by using NaOH solution, CMS with rapid swelling properties in an aqueous medium, essential for super-disintegrant applications in solid dosage forms, was obtained. Both corn and potato starches were used for preparing CMS. The carboxymethyl starch, with rapid swelling properties, was characterized using Fourier-transform infrared spectroscopy and scanning electron microscopy.

Keywords: Carboxymethyl starch; reactive extrusion; starch derivatives; sodium starch glycolate; starch ether; modified starch.
1. Introduction

Sodium salt of the carboxymethyl ether of starch, also known as carboxymethyl starch, was first prepared in 1924. Since then, carboxymethyl derivatives have been prepared from starches such as corn, amaranth, high amylose corn, potato, wheat, rice, mungbean (*Vigna radiata* L. Wilczek, Papilionaceae), Chinese yam (*Dioscorea opposita* thunb.), Leucaena glauca seed gum, Kudzu root starch and wastes from corn starch and potato flour. Carboxymethyl starch could be used in the textile industry as a sizing agent and as an eco-friendly, printing and finishing agent, as a thickening agent in foods, and also for personal care and surfactant applications. Partially cross linked carboxymethyl starch, called sodium starch glycolate, is used as a disintegrant by pharmaceutical companies. Carboxymethyl starches could be used in food extrusions as an extrusion aid. Recently, researchers have tried using cross-linked carboxymethyl starch for removing heavy metal ions from water. Apart from these, it also finds other uses in medical, adhesives, absorbents, paper and oil-well drilling applications.

Carboxymethyl starch (CMS) is prepared by the reaction of sodium monochloroacetate (SMCA) with starch, in the presence of NaOH. Usually carboxymethylation is carried out in a batch reactor with aqueous ethanol used as the solvent. The factors affecting the reaction are type of solvent, concentration of solvent, solvent to starch ratio, concentration of NaOH, concentration of SMCA, temperature and time.

An aqueous solvent generally is used during carboxymethylation of starch, since CMS becomes soluble in water at a DS of 0.1. Tijsen et al. compared the performance of 90% aqueous methanol, ethanol, n-propanol, isopropanol, n-butanol and secondary and tertiary butanol as solvents for carboxymethylation of starch and found isopropanol to
give the highest reaction efficiency. This also was demonstrated by Bhattacharyya et al.\textsuperscript{11} and Khalil et al.\textsuperscript{18}. The type of solvent used affects the solubility of reactants in it and its ability to swell the starch and facilitate the reaction. The concentration of the aqueous solvent affects the solubility of NaOH and the swelling of starch and hence the ability of the reactants to enter the starch granule. A 10\% aqueous isopropanol solution was found to give the highest reaction efficiency for potato starch\textsuperscript{4} and amaranth starch\textsuperscript{11} and 20\% aqueous isopropanol for corn starch\textsuperscript{11,18}. Increase in the solvent:starch ratio improved the swelling of starch and hence the absorption of the reactants on the starch granule which would result in a higher DS.\textsuperscript{11,18} However, at high solvent:starch ratios the dilution of the reactants resulted in a lower rate of reaction and hence lower degree of substitution.

Bhattacharyya et al.\textsuperscript{11} found an ideal starch to solvent ratio of 1:15 for carboxymethylation of corn starch. Similarly, increases in the SMCA and NaOH concentrations also increased the DS, but at larger concentrations the DS decreased due to the side reaction between SMCA and NaOH.\textsuperscript{6,11,18} The temperature of the reaction had to be set such that the starch did not gelatinize. Gelatinization of starch in the solvent caused problems in recovery and drying. For carboxymethylation of corn starch, the ideal temperature was found to be 65°F.\textsuperscript{11}

Several researchers have tried using alternate reactors for starch derivatives. Lammers et al.\textsuperscript{19} used static mixers to prepare hydroxypropyl ethers of starch pastes. Gelatinized starch pastes do not encounter the problems with recovery and drying that gelatinized starches in solvent systems do. However, starch paste completely loses its granular structure. Kuipers et al.\textsuperscript{20} used a stirred vibrating fluidized bed to produce hydroxyethyl ethers of potato starch. It formed starch ethers without losing the starch granular
structure, but it was essentially a solid-gas reaction system. Caldwell et al.\textsuperscript{21} made granular CMS by blending starch with NaOH and SMCA and storing it in a dry form for extended periods at room temperature or by heating it, in dry form, in a blender. However, this was an extremely slow process with reaction times ranging from a few hours to a few weeks. Thompson et al.\textsuperscript{22} reacted a dry blend of starch, NaOH and SMCA in a nitrogen fluidized bed at a very high temperature, but did not report the details with respect to the DS of CMS yield.

Extruders have been used for a long time for preparing ready-to-eat cereals.\textsuperscript{23} Several researchers have used reactive extrusion (RE) processes for preparing starch derivatives like oxidized starch\textsuperscript{24}, starch phosphates\textsuperscript{25,26}, low DS starch esters like maleate, phthalate and succinate\textsuperscript{27}, low DS starch acetate\textsuperscript{28}, starch-graft-poly acrylic acid and starch-graft-poly acrylamide\textsuperscript{29}, starch-graft-poly acrylonitrile\textsuperscript{30}, cationic starches\textsuperscript{31,32} and glycol glucosides\textsuperscript{33,34}. In many cases, using reactive extrusion, high reaction efficiencies were obtained for short reaction times compared to a conventional process. Gimmler et al.\textsuperscript{35} developed a systems analytical model to determine the influence of extrusion conditions on the efficiency of carboxymethylation of starch. Johnson et al.\textsuperscript{36} used reactive extrusion for preparing carboxymethyl starch of DS 0.17-0.74 useful for inhibiting scale formation in water handling equipments.

This study deals with preparation of carboxymethyl starch for super-disintegrant applications used in solid dosage forms, using a continuous and solvent-less extrusion process. Super-disintegrant applications require the carboxymethyl starch, when inserted in an aqueous medium, to absorb water and swell at a rapid rate. This rapid-swell ability of the carboxymethyl starch present in solid dosage forms, causes the tablet to
disintegrate and disperse due to the swelling force exerted against the tablet matrix, resulting in the release of the drug. Worldwide, pharmacopoeia specifications require the DS of carboxymethyl starch to be in the range of \(0.22 \text{–} 0.35\).

In this study, the effects \(\text{NaOH, ethanol, } \text{H}_2\text{O and cross-linking agents (citric acid, sodium tripolyphosphate (STPP) and epichlorohydrin)}\) in the reaction formulation and the extruder barrel temperature on the degree of substitution were determined. These factors were then optimized to obtain rapid water absorption and swelling properties of the carboxymethyl starch.

2. Experimental

2.1. Materials
Corn starch and potato starch were obtained from Tate & Lyle (Decatur, IL) and AVEBE (Veendam, Netherland), respectively. Citric acid, STPP and sodium hydroxide were obtained from Thermo Fisher Scientific (Hampton, NH). SMCA was obtained from Alfa Aesar (Ward Hill, MA). USP grade ethanol (200 proof) was obtained from Decon Labs (King of Prussia, PA).

2.2. Preparation of samples for extrusion
Five hundred grams of corn starch, having 8.8% moisture content on a dry basis, were uniformly sprayed with a NaOH solution in water with constant mixing using a Hobart C-100 (Hobart Corp. Troy, Ohio) planetary type mixer. It was important to spray the NaOH solution uniformly, since instant swelling of starch takes place in the presence of NaOH. The NaOH solution was prepared by dissolving the required amount of NaOH in the required amount of water. Further, powdered SMCA was added to the starch. As required
by the experimental design, citric acid, sodium tripolyphosphate, epichlorohydrin and ethanol also were added. The formulations were then mixed in a Hobart planetary mixer for 10 min. In some cases potato starch (11.75% moisture dry basis) was used. Also, in some cases the method of adding NaOH to the formulation was modified and has been indicated where relevant.

2.3. Extrusion parameters

A Brabender TSE-20 (C.W. Brabender Instruments Inc., South Hackensack, NJ) co-rotating twin screw extruder was used for the reactive extrusions. The starch-reactant formulation was fed using a Brabender PW40PLUS-0 (Brabender Technologie Inc., Ontoria, Canada) flex wall volumetric feeder. The screw configurations are detailed in Table 1. The extruder was operated without a die or nozzle. The extruder barrel temperature was set according to the experimental design. The extrudates were collected in trays and allowed to cool at 25°C for 30 min.

2.4. CMS purification

CMS extrudates were ground using a Krups GX4100 (Millville, NJ) grinder. Ground extrudates were purified to remove unreacted SMCA and the by-products NaCl and sodium glycolate. Two grams of ground extrudate were washed with 40 mL of 80% aqueous ethanol for 30 min. While washing the sample, the pH was neutralized using citric acid. After washing, the ethanol was decanted. This procedure was repeated twice. The purified CMS extrudates were washed with 95% aqueous ethanol and then dried overnight in a vacuum oven at 50°C. There was no observed loss of carboxymethyl starch during purification.
2.5. *NaCl measurement*

The *NaCl* content in the purified carboxymethyl starch was measured using the method described by the British Pharmacopoeia. In 100 mL distilled water, 0.5 g purified carboxymethyl starch was suspended along with 1 mL nitric acid. This suspension was then titrated potentiometrically with 0.1 M silver nitrate using a silver indicator electrode. One milliliter of 0.1 M silver nitrate is equivalent to 5.844 mg of *NaCl*.

The *NaCl* content was calculated as:

\[
\%NaCl = \frac{V_1 \times \frac{5.844}{1000}}{W_1} \times 100
\]

where \(V_1\) (mL) = the amount of 0.1 M silver nitrate added until the point of inflection was reached and \(W_1\) (g) = weight of CMS.

2.6. *Assay measurement*

The CMS was assayed according to the method presented by British Pharmacopoeia. The assay gave the weight % of Na present in CMS, in the form of sodium carboxymethyl ether. Purified CMS (0.7 g) was refluxed in 70 mL glacial acetic acid for 2 h and then allowed to cool to room temperature. Then, the refluxed sample was titrated potentiometrically using a 0.1 M solution of perchloric acid in glacial acetic acid. A Fisher Scientific Accumet® BASIC AB 15+ (Hampton, NH) pH meter with a glass, single junction electrode with silver/silver chloride reference was used to measure the potential difference during titration. One milliliter of 0.1 M perchloric acid is equivalent to 2.299 mg of Na. Measurements were replicated once and the average value was recorded.
The assay was calculated as:

\[
%Na = \frac{V_1 \times 2.299}{W_1 \times 1000} \times 100
\]

where \(V_1 \) (mL) = the amount of 0.1 M perchloric acid added until the point of inflection was reached.

\(W_1 \) (g) = weight of CMS used for determining the assay.

Then the assay was converted to DS as:

\[
DS = \frac{\left[\frac{Assay \times 162}{2300}\right]}{1 - \left[\frac{Assay \times 80}{2300}\right]}
\]

2.7. *Swelling ability in water*

In a beaker with 30 mL distilled water at 25°C, 1 g of purified CMS was added. The behavior of the CMS when added to water was observed.

2.8. *Fourier-transform infrared spectroscopy*

The Fourier-transform infrared (FTIR) spectra for native potato starch and carboxymethyl starch were obtained using a Smiths Detection SensIR (Danbury, CT) FTIR microscope, with an attenuated total reflectance (ATR) objective. Powdered carboxymethyl starch was pressed against the objective and analyzed directly. Thirty two scans were acquired per image at a resolution of 4cm\(^{-1}\).

2.9. *Microstructure of carboxymethyl starch*

The microstructure of carboxymethyl starch was examined using a Hitachi S-3000N (San Jose, CA) variable pressure scanning electron microscope. A small amount of powdered carboxymethyl starch was placed on a metal stub with a double sided adhesive tape. This
carboxymethyl starch was sputter coated with gold under vacuum to render them conductive. Microstructure image was then acquired at a 200X magnification and at a 1280×960 pixel resolution.

2.10. Statistical analyses

Several factorial designs, described in the Results and Discussion section, were used to determine the effects of the reaction variables on the degree of substitution. Experiments in the factorial designs were replicated once. The response variable (degree of substitution) was analyzed using ‘Proc Mixed’ procedure of SAS version 9.1 (SAS Institute Inc., Cary, NC) with a significance level of $\alpha \leq 0.05$. The statistical significance was reported using P values, which is the probability of error in accepting the result as true.

3. Results and Discussion

3.1. Reactive extrusion

In an extruder, kneading blocks exert a large shear force on the reactants because of which there is more intimate contact between the reactants. The pre-exponential constant $(k_0)$ in the Arrhenius equation $k = k_0 \cdot e^{-\frac{E}{RT}}$ which is a function of the degree of contact between the reactants, increases under such conditions, resulting in a higher reaction rate. Also, since no solvents were present during the reactive extrusions, the reactants present were not diluted. A higher concentration would result in higher reaction rate.

For this study, the extruder was used without a die and nozzle to limit the gelatinization of starch. When a die and nozzle are used in the extruder, extremely high shear forces and
pressures are generated, molten starch is formed, and the granular identity of the starch is lost. Such extruded starch would have undergone sever shearing and de-polymerization. Brümmer et al.\textsuperscript{39} showed that the starch molecular weight decreased exponentially with an increase in the specific mechanical energy input.

The effects of NaOH, water (H\textsubscript{2}O) and extruder barrel temperature on the DS were studied using a 3×2×2 factorial design with 3 levels of NaOH (10 g, 15 g and 20 g), 2 levels of H\textsubscript{2}O (40g and 50g) and two extruder barrel temperature profiles (80-85-85°C and 95-90-85°C). The results are summarized in Table 2. The effect of the amount of NaOH on the DS was found to be significant (P<0.0005). Examination of the simple main effects revealed that the DS with 15 g NaOH was significantly higher than that at 10 g NaOH (P<0.05) and also DS with 20 g NaOH was found to be significantly higher than that at 10 g NaOH (P<0.0005). However, no statistical difference was detected between the DS with 15 g and 20 g NaOH. However, it seems that NaOH may have limited the DS, since the DS obtained were similar to the molar ratios of NaOH and starch. The DS of CMS was measured only after making sure the NaCl content in them was below 0.1 wt\%, since large residual NaCl amounts affected the DS measurements.

As discussed in the introduction section, several researchers reported that for the batch process, the DS increased with an increase in the concentration of NaOH until it reached a peak and then decreased. But, this was only when the reaction was allowed to go to completion. Tijsen et al.\textsuperscript{4} showed that for NaOH concentrations above the peak value, although the final DS obtained was lower, the initial rate of reaction was much faster. Hence for shorter reaction times where the reaction is not reaching completion, as is the case with reactive extrusion, higher amounts of NaOH should give higher DS,
irrespective of the peak concentration of NaOH. The maximum DS obtained was 0.14 at a reaction efficiency of 0.39. For this study, reaction efficiency was defined as the ratio of the number of moles of SMCA that had reacted to form CMS to the total number of moles of SMCA used. The maximum DS obtained here was less than the pharmacopoeia requirements.

CMS is partly cross-linked for use as a disintegrant in solid dosage forms. STPP was used previously to cross link starch. Citric acid, a trifunctional reagent, also is used industrially for preparing cross-linked CMS for super-disintegrant applications. It was interesting to determine whether these cross-linking agents had an effect on the carboxymethylation reaction in the extruder. This was investigated using a 2×2 factorial design involving STPP (0 g and 2 g) and citric acid (0 g and 2 g). The reaction conditions and the resultant DS are summarized in Table 3. The effects of both STPP and citric acid were found to be significant (P<0.005). The use of STPP may have catalyzed the reaction and increased the DS whereas the use of citric acid seemed to reduced the rate of reaction and lowered the DS.

In the presence of water, the carboxymethyl groups on the starch absorb water molecules and form a viscous gel. It was thought that as the starch derivative was being formed during the reactive extrusion process, it would start to form a gel in the presence of water. That would retard the mass transfer of the reactants to the starch granule and would result in a lower reaction rate, and hence, lower DS. CMS does not form a gel in the presence of ethanol and its presence could hypothetically give a higher DS. To check this hypothesis, a 2×2 factorial design with 2 levels of ethanol (0 g and 20 g) and 2 levels of extruder
barrel temperatures (80-85-85°C and 95-90-85°C) were used. The reaction conditions and the resultant DS are summarized in Table 3. The effect of ethanol was found to be significant (P<0.01), and its use during extrusion gave a higher DS. Hence, the presence of ethanol prevented the retardation of mass transfer of the reactants during extrusion and could be used in the formulation for higher reaction efficiency.

For pharmaceutical applications, CMS with a DS 0.22 to 0.35 is required. The maximum DS obtained so far was 0.2. Since it was observed earlier that the amount of NaOH used had a significant effect on the DS, and the effect of the amount of H_2O used seemed to be approaching significance, these variables were used to obtain a higher DS. Earlier, the maximum amount of NaOH used was 20g. In this case, the effects of using 40g and 50g of NaOH and a H_2O content of 50g and 70g H_2O were determined in a 2×2 factorial design. For a higher DS, the amount of etherifying agent, SMCA, also was increased to 170g and 3 kneading blocks were used. The reaction conditions and the resultant DS are summarized in Table 4. The effects of both NaOH and H_2O were found to be significant. Increasing NaOH from 50g to 70g increased the DS (P<0.005), and increasing H_2O from 50g to 70g decreased the DS (P<0.05). Presence of higher moisture content may have retarded the mass transfer to the reaction sites due to the formation of a viscous gel, resulting in a lower DS. A maximum DS of 0.27 was obtained when 50g H_2O and 50g NaOH were used at a reaction efficiency of 0.57. This DS was within the pharmacopoeia specifications.

The swelling behaviors of these purified carboxymethyl starches with higher DS (0.2-0.27) were observed. All the above samples formed lumps when added to water and no swelling behavior was observed. This was because the rapid rate of absorption of water
by CMS, after it adding to water, resulted in the formation of a gel-like surface. The uncontrolled water uptake in this case may have been because of excess gelatinization of starch during the extrusion. The gel surface formed a viscous barrier which prevented any further uptake of water by CMS. This resulted in the formation of knots with a gel-like surface encapsulating dry CMS powder inside. This phenomenon is called gel-blocking. The swelling behavior of CMS in water is critical for its application as a disintegrant in solid dosage forms.

To reduce the gelatinization of starch, the use of fewer kneading blocks was investigated. Using fewer kneading blocks would result in less amount of shear on the starch and would decrease the extent of gelatinization. The effect of epichlorohydrin on the DS and swelling behavior of CMS also was examined. Epichlorohydrin is a popular cross-linking agent. Cross-linking of CMS results in controlled uptake of water and prevents gel blocking. Since epichlorohydrin is insoluble in water, it was dissolved in ethanol and added to the formulation. The reaction conditions and the resultant DS are summarized in Table 5. When epichlorohydrin was used a higher DS was obtained. But this may have been because of the effect of the accompanying ethanol. The swelling behaviors of these carboxymethyl starches, in water, were observed. In spite of fewer kneading blocks and a cross-linking agent, gel blocking was still present. Extrusions with 2 kneading blocks and even higher amounts of epichlorohydrin were then performed. Gel blocking was still prevalent at 5, 10, 15 g of epichlorohydrin.

Starch is gelatinized in an extruder by the action of moisture, heat, shear and pressure. As discussed before, decreasing shear by reducing the number of kneading blocks from 3 to
did not eliminate the gel blocking. Also, the use of epichlorohydrin as a cross-linking agent did not achieve the desired results. The temperature during extrusion cannot be reduced since it would affect the reaction rate. Also, decreasing the amount of H$_2$O would result in a dry formulation which would plug the extruder. It was then postulated that if a higher amount of ethanol was used, the ethanol would bind the H$_2$O, making it less available for gelatinization. Also, the earlier technique of spraying concentrated NaOH solution on the starch may have caused excess gelatinization even prior to the extrusion. Hence, instead of spraying the NaOH solution, only H$_2$O was used and 40 mesh NaOH beads were added separately to the formulation right before the extrusion. The number of kneading blocks was reduced to 1. For 500 g potato starch, 170 g SMCA, 50 g NaOH beads and ethanol and H$_2$O combinations of 45 + 45 g, 30 + 60 g and 10 + 80 g were used and extruded using screw configuration 4 at 80-85-85°C. For the formulation with 45 g ethanol and 45 g H$_2$O, when 1 g of purified carboxymethyl starch was added to 30 mL of distilled water, no gel blocking was observed. The rapid water uptake resulted in rapid swelling of the sample. The DS of this sample was 0.31. For the other two formulations, no gel blocking was observed but no significant swelling was observed either.

High reaction efficiencies for carboxymethylations in slurry reaction processes are obtained for relatively large reaction times. Volkert et al.$^{42}$ obtained a reaction efficiency of 0.7 for DS of 1 and reaction time of 30 min. Khalil, et. al.$^{18}$ obtained a reaction efficiency of 0.3 for carboxymethylation of corn starch to a DS of 0.51 with a reaction time of 120 min. Bhattacharyya, et. al.$^{11}$ obtained a reaction efficiency of 0.1 for carboxymethylation of corn starch to a DS of 0.17 with a reaction time of 90 min. Tijsen
et al.\textsuperscript{4} obtained a reaction efficiency of 0.45 for a DS of 0.49 with a reaction time of 100 min. In this study CMS with a DS of 0.38 was prepared with a reaction efficiency of 0.81. The reaction time was less than 2 min.

3.2 \textit{Fourier-transform infrared spectroscopy}

The FTIR spectra for the carboxymethyl potato starch with DS 0.31 and rapid swelling properties and native potato starch, for the wavelengths from 4000 to 650 cm\textsuperscript{-1}, are shown in Figure 1. Carboxymethylation of starch was confirmed by the peak at a wavelength of 1600 cm\textsuperscript{-1} which corresponded to the carbonyl group.\textsuperscript{8} The peak at 1600 cm\textsuperscript{-1} is absent in the spectra for potato starch.

3.3 \textit{Carboxymethyl starch microstructure}

The microstructures of native potato starch and carboxymethyl potato starch with DS 0.31 and rapid swelling properties, obtained using scanning electron microscope, are shown in Figures 2 and 3, respectively. The microstructure of potato starch shows granules with a smooth surface and size in the range of 20-80µ. The granules, around 20µm in size, were mostly spherical in shape, whereas, the granules with sizes ranging from 40-80µm were oval or distorted spherical in shape. The microstructure of carboxymethyl starch shows particles in the range of 20-100 µm in size along with a small amount of fines (<20 µm in size). The carboxymethyl starch particles were irregularly shaped with jagged surfaces, indicating that the granular structure of the starch was destroyed during the reactive extrusion.
4. Conclusions

This study showed that the effects of NaOH, H₂O and ethanol on the DS of CMS were significant. STPP, used during extrusion, may have had a catalytic effect on the carboxymethylation reaction, whereas citric acid may have had a retarding effect on the reaction. The effects of STPP and citric acid on the carboxymethylation reaction need to be investigated further. Using only 1 kneading block, avoiding gelatinization of starch due to the use of concentrated NaOH solution and using a cross-linking agent, carboxymethyl starch with rapid swelling properties was obtained. Using reactive extrusion, reaction efficiency of up to 68% was obtained. Also, the reaction time in the extrusion process was extremely short as compared to the conventional process. The amount of ethanol used in the formulation in this study was about 4% of the weight of starch. The amount of solvent used in the conventional slurry batch process is up to 15 times the weight of starch. Hence in comparison, the extrusion process can be said to be a “solvent-less” process. Reactive extrusion presents a fast, efficient and convenient continuous process for preparing cross-linked carboxymethyl starch with rapid swelling properties, suitable for application as a disintegrant pharmaceutical excipient.
References


(21) Caldwell, C. G.; Martin, I. Cold Water Soluble Starch Ethers. U.S. Patent 2,802,000, **1957**.


Table 1. Elaboration of screw profiles used in this study.

<table>
<thead>
<tr>
<th>Screw profile no.</th>
<th>No. of kneading blocks</th>
<th>Configuration (From die end)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screw profile 1</td>
<td>0</td>
<td>9.(20R)+8.(30R)</td>
</tr>
<tr>
<td>Screw profile 2</td>
<td>3</td>
<td>9.(20R)+3.(KC)+6.(30R)</td>
</tr>
<tr>
<td>Screw profile 3</td>
<td>2</td>
<td>9.(20R)+1.(30R)+2.(KC)+6.(30R)</td>
</tr>
<tr>
<td>Screw profile 4</td>
<td>1</td>
<td>9.(20R)+3.(30R)+1.(KC)+5.(30R)</td>
</tr>
</tbody>
</table>

30R=30mm convey segment, 20R=20mm convey segments with short pitch, KC=20mm kneading convey segment
Table 2. Effect of NaOH, H2O and temperature on the degree of substitution.

<table>
<thead>
<tr>
<th>NaOH (g)</th>
<th>H2O (g)</th>
<th>T (°C)</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>40</td>
<td>80-85-85</td>
<td>0.09</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>80-85-85</td>
<td>0.1</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>80-85-85</td>
<td>0.12</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>80-85-85</td>
<td>0.11</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>80-85-85</td>
<td>0.12</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>80-85-85</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>95-90-85</td>
<td>0.07</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>95-90-85</td>
<td>0.12</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>95-90-85</td>
<td>0.14</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>95-90-85</td>
<td>0.08</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>95-90-85</td>
<td>0.12</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>95-90-85</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Reaction conditions: Corn starch=500g, SMCA=130g, screw configuration 1.
Table 3. Effects of citric acid, STPP, ethanol and temperature on the degree of substitution.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>Citric acid (g)</th>
<th>STPP (g)</th>
<th>Ethanol (g)</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>95-90-85</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0.13</td>
</tr>
<tr>
<td>95-90-85</td>
<td>0</td>
<td>2</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>95-90-85</td>
<td>2</td>
<td>0</td>
<td>-</td>
<td>0.11</td>
</tr>
<tr>
<td>95-90-85</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>0.13</td>
</tr>
<tr>
<td>80-85-85</td>
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<td>-</td>
<td>0</td>
<td>0.08</td>
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<tr>
<td>80-85-85</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>0.16</td>
</tr>
<tr>
<td>95-90-85</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0.11</td>
</tr>
<tr>
<td>95-90-85</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Reaction conditions: Corn starch=500g, SMCA=130g, NaOH=40g, H₂O=40g and screw configuration 1.
Table 4. Effects of NaOH and H2O on the degree of substitution.

<table>
<thead>
<tr>
<th>NaOH (g)</th>
<th>H2O (g)</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>50</td>
<td>0.22</td>
</tr>
<tr>
<td>40</td>
<td>70</td>
<td>0.2</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>0.27</td>
</tr>
<tr>
<td>50</td>
<td>70</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Reaction conditions: Corn starch=500g, SMCA=170g, temperature 80-85-85°C, screw configuration 2.
Table 5. Reaction conditions for obtaining CMS with rapid swelling properties

<table>
<thead>
<tr>
<th>Epichlorohydrin (g)</th>
<th>Kneading Blocks</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>0.38</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.32</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Reaction conditions: Potato starch=500g, SMCA=170g, NaOH=50g, H₂O=45g, temperature 80-85-85°C, screw configuration no.2 for 3 kneading blocks, screw configuration no.3 for 2 kneading blocks.
Figure 1. FTIR spectra for carboxymethyl starch and native potato starch
Figure 2. Microstructure of native potato starch obtained using scanning electron microscope at 200X magnification.
Figure 3. Microstructure of carboxymethyl starch with DS 0.31 and rapid swelling properties, obtained using scanning electron microscope at 200X magnification.
CHAPTER III

CHARACTERIZATION OF SODIUM STARCH GLYCOLATE PREPARED USING REACTIVE EXTRUION AND ITS COMPARISONS WITH VIVASTAR®P.

This research paper is in review for the journal:

Industrial Crops and Products
CHARACTERIZATION OF SODIUM STARCH GLYCOLATE PREPARED USING REACTIVE EXTRUSSION AND ITS COMPARISONS WITH VIVASTAR\textsuperscript{®}P.

Abstract

Reactive extrusion is a popular technique for chemical modification of starches. In this study, the physical, chemical and morphological properties of sodium starch glycolate, prepared using reactive extrusion (SSG), were compared with those of VIVASTAR\textsuperscript{®}P. As measured by sieve analysis, VIVASTAR\textsuperscript{®}P had a larger weight fraction in the particle size range of 0-38µm compared to SSG. The sodium assays of VIVASTAR\textsuperscript{®}P and its particle size fractions (0-38µm and 38-75µm) were found to be significantly lower than those of SSG and its particle size fractions (0-38µm, 38-75µm and 75-106µm). The NaCl content, pH, settling volume, bulk density, tap density and Carr index for VIVASTAR\textsuperscript{®}P and SSG were measured. X-ray diffraction patterns indicated substantial loss of crystallinity of starch in both VIVASTAR\textsuperscript{®}P and SSG. The water uptakes of the 0-38µm and 38-75µm fractions of VIVASTAR\textsuperscript{®}P, measured at 20 and 180 s, were significantly higher than those for the 0-38µm and 38-75µm fractions of SSG. However, no differences were found between the water uptakes of both the VIVASTAR\textsuperscript{®}P fractions and the 75-106µm fraction of SSG at 20 s. However, the water uptake of the SSG 75-106µm fraction leveled off as time progressed. When, 0.1N HCl was used, at an interval of 20 s, the SSG 75-106µm and 38-75µm fractions had significantly higher liquid uptake than VIVASTAR\textsuperscript{®}P and its 0-38µm fraction. However, no significant differences were found between the liquid uptakes of the SSG 75-106µm and 38-75µm fractions and the VIVASTAR\textsuperscript{®}P 38-75µm fraction. After a time interval of 180 s, the liquid uptakes of the
SSG 75-106µm and 38-75µm fractions were significantly lower than those for VIVASTAR®P and its 0-38µm fraction. The liquid uptake of the VIVASTAR®P 38-75µm fraction was higher than that of the SSG 38-75µm fraction but not significantly different from the SSG 75-106µm fraction. The microstructure of VIVASTAR®P revealed the presence of spherical and ovoid shaped particles with smooth surfaces. This was in contrast with the irregular shaped particles of SSG with jagged surfaces.

**Keywords:** Sodium starch glycolate, reactive extrusion, super-disintegrants, VIVASTARP, pharmaceutical excipients.
1. Introduction

Disintegrants are substances present in tablet formulations that promote their dispersion and result in the release of the drug. Native starch has historically been used in disintegrant applications in oral solid dosage forms (Shah and Augsburger, 2002). However, at the required amounts, native starch does not achieve required tablet compactibility (Augsburger et al., 2007; Bolhuis et al., 1984). To improve the efficiency of disintegration, a new class of ‘superdisintegrants’ were developed. These superdisintegrants include products like sodium starch glycolate, crospovidone and croscarmellose sodium.

Sodium starch glycolate is a popular disintegrant, prepared by cross-linking sodium carboxymethyl starch. Sodium carboxymethyl starch is prepared by substituting the hydroxyl groups in the anhydroglucose units of starch molecules with sodium carboxymethyl groups. The sodium carboxymethyl substitution is obtained by reacting starch with sodium mono chloro acetate in the presence of NaOH. Impurities formed during the reaction, like sodium glycolate, sodium chloride and sodium citrate, are partially removed from the product after the reaction.

Disintegration of orally administered solid dosage forms generally takes place in the gastro-intestinal tract. There is no single mechanism which can explain the disintegration behavior of sodium starch glycolate. The important proposed mechanisms of disintegration include liquid wicking, swelling and deformation recovery. Liquid wicking is considered to be a crucial first step for tablet disintegration. For sodium starch glycolate, a larger rate and extent of water uptake has been observed to have resulted in
faster disintegration (Augsburger et al., 2007). Swelling is the most important
disintegration mechanism for sodium starch glycolate. Sodium starch glycolate is a
hygroscopic substance that absorbs gastrointestinal fluids and swells. The swelling of the
sodium starch glycolate particles against the tablet matrix generates a bursting force.
Caramella et al. (1984) found that measurable forces were generated only after significant
particle swelling and then tablet disintegration took place. It is important that the tablet
matrix does exhibit a plastic deformation at this stage, which may accommodate the
generated stress and result in incomplete disintegration. The presence of large internal
porosity also may, similarly, result in ineffective disintegration. Generation of a swelling
force at a rapid rate also is essential (Augsburger et al., 2007). Caramella et al. (1984)
found a log-log relationship between the disintegration time and the rate of development
of the disintegration force.

Caramella et al. (1984), in a study of various disintegrants (maize starch, sodium starch
glycolate, microcrystalline cellulose, low substitution hydroxypropyl cellulose,
croscarmellose sodium, low substitution carboxymethyl cellulose, cross-linked
polyvinylpyrrolidone, potassium salt of methacrylic acid and polymethylmethacrylate),
suggested that factors other than the extent of swelling may also be responsible for the
disintegrating force. Other mechanisms proposed included air expansion due to heat of
wetting, particle-particle repulsion and capillary pressure. However, none of those have
been verified experimentally (Caramella et al. 1989).

Other factors that may affect disintegration performance include pH of the disintegrating
media, particle size of the disintegrant, molecular weight, tablet matrix solubility,
presence of lubricants and the method of incorporation in granulation (Augsburger et al., 2007; Bolhuis et al., 1984; Rudnic et al., 1983; Lerk et al., 1982). List et al. (1979) found that the magnitude of the disintegration force increased with particle size, for various disintegrants. Rudnic et al. (1983) and Shotton et al. (1976) found that varying the intragranular and extragranular sodium starch glycolate concentration affected the tablet disintegration.

Sodium carboxymethyl starch is traditionally prepared using a slurry batch process. Starch is reacted with sodium mono chloro acetate in an aqueous alcohol suspension in the presence of NaOH. In this process large amounts of aqueous ethanol are used as a solvent (Bhattacharyya et al., 1995; Hebeish and Khalil, 1988). We previously developed a reactive extrusion process for the preparation of sodium starch glycolate which used much smaller amounts of solvent (Bhandari and Hanna, 2011a). Reactive extrusion also presents a convenient continuous method for the preparation of carboxymethyl starch. However, in the reactive extrusion process, the high reaction temperature and the accompanying shear forces, gelatinize the starch resulting in the loss of granular structure. The properties of sodium starch glycolate prepared using this process for disintegration applications have not been studied. This project compared the physical, chemical and morphological properties of a commercial brand of sodium starch glycolate, Vivastar®P (JRS PHARMA GmbH), with those of sodium starch glycolate prepared using reactive extrusion.
2. Experimental

2.1. Materials

Sodium starch glycolate was prepared by extruding a reaction mixture of potato starch (AVEBE, Veendam, Netherland), sodium mono chloro acetate (Alfa Aesar, Ward Hill, MA), NaOH (Thermo Fisher Scientific, Hampton, NH), epichlorohydrin (Thermo Fisher Scientific, Hampton, NH) and aqueous ethanol (King of Prussia, PA). A Brabender TSE-20 (C.W. Brabender Instruments Inc., South Hackensack, NJ) co-rotating twin screw extruder was used. The process has been described in detail elsewhere (Bhandari and Hanna, 2011a). Vivastar®P was obtained from JRS PHARMA GmbH (Pirna, Germany) and used as is.

2.2. Methods

2.2.1. NaCl Content and Assay

The NaCl content and the sodium carboxymethyl were determined by potentiometric titration using 0.1M silver nitrate and 0.1M perchloric acid respectively, as described by Bhandari and Hanna (2011a). Each measurement was duplicated.

2.2.2. Sieve Analysis

A W.S. Tyler vibrating sifter (model RX-29, Mentor, OH) was used to determine particle size distribution. Forty grams of sample were placed on a stack of 12 sieves. The sieves used were Tyler standard series numbered 8, 10, 14, 20, 28, 35, 48, 65, 100, 150, 200 and 400. The sifter was run for 5 min and then the weights of the powders retained on every sieve and also of that passing through the number 400 sieve were measured. The recorded
weights of the samples were used to plot the particle size distribution and the cumulative
percent undersize.

2.2.3. Bulk and Tap Density
A pre-weighed amount of sodium starch glycolate powder was added to a dry measuring
cylinder and tapped lightly so that the material settled down and there was no powder
sticking to the glass walls. The volume occupied by the powder was observed and the
bulk density was calculated. The measuring cylinder was then manually tapped 200
times. The new volume was noted and the tap density was calculated. Each observation
was replicated twice and the average value was used. Carr index was determined from the
bulk and tap density (Carr, 1970).

\[
\text{Carr Index} = 1 - \left( \frac{\text{bulk density}}{\text{tap density}} \right)
\]

2.2.4. Settling Volume Study
One gram of sodium starch glycolate was added to 75 mL of distilled water, in a
measuring cylinder, in small portions. The mixture was shaken vigorously after each
addition and then diluted to 100 ml. The volume of the gel that had settled down was
observed after 12 hr.

2.2.5. Surface area, pore volume and pore size
The surface area, pore volume and pore size were measured using Quantachrome
Autosorb 1-C (model AS1-OT-11, Quantachrome Instruments, Boynton Beach, FL) by
\( \text{N}_2 \) adsorption using BJH method (Stasiak and Jamroz, 2009). Each measurement was
duplicated.
2.2.6. X-ray diffraction

X-ray diffraction patterns were obtained using a Rigaku D/Max-B diffractometer (Rigaku Americas, TheWoodlands, TX) as described by Bhandari and Hanna (2011b). Diffraction patterns were obtained for an angular range (2θ) of 5° to 45°, with a scanning speed of 5° (2θ)/min and a step size of 0.05° (2θ).

2.2.7. Liquid Uptake Study

The setup prepared for measuring the liquid uptake consisted of a 2.5 L glass bottle with an outlet near the bottom of the bottle. The outlet near the bottom was connected through a tube to a Millipore (Billerica, MA) 47 mm filter setup consisting of a fritted glass filter plate. The glass bottle was placed on an Ainsworth CR-12001 weighing balance. The filter height was adjusted so that it was at level with the liquid in the glass bottle. The kinks present in the tube and the air bubbles present in the tube and under the filter were removed. After zeroing the balance, 0.2 g of sample were added on the glass filter. The sodium starch glycolate sample uptakes liquid through the fritted glass filter. The weight of liquid uptake by the sample was registered by the weighing balance and also recorded on the computer connected to the weighing balance using a LabVIEW VI (National Instruments Corporation, Austin, TX). The weights were recorded after a time interval of 10 s. Each observation was the average obtained using 2 duplicates. The liquid uptake study was done using distilled water and 0.1N HCl.
2.2.8. Scanning Electron Microscopy

The morphological properties of sodium starch glycolate were examined using a variable pressure scanning electron microscope (Model S-3000N, Hitachi High Technologies America Inc., San Jose, CA) as described elsewhere (Bhandari and Hanna, 2011b).

2.2.9. Data Analyses

The data on the sodium assay of VIVASTAR®P and SSG, water and 0.1N HCl uptakes and porosity measurements were analyzed using ‘Proc Mixed’ procedure of SAS version 9.1 (SAS Institute Inc., Cary, NC) with a significance level of α≤0.05.

3. Results and Discussion

3.1. Sieve Analysis

Figure 1 shows the particle size distribution for VIVASTAR®P and sodium starch glycolate prepared using reactive extrusion (SSG). It can be observed that 51.7% of the total amount of VIVASTAR®P had a particle size of 0-38µm, as compared to 24.5% for SSG. Only 0.4% of the total weight of VIVASTAR®P was present in the 75-106µm range, as compared to 8.2% for SSG. VIVASTAR®P had a larger weight fraction of material with a smaller particle size, compared to SSG.

3.2. Bulk and Tap Density

Table 1 gives the bulk density, tap density and the Carr index for VIVASTAR®P and SSG. VIVASTAR®P has a higher bulk and tap density and a lower Carr index. Carr index is a measure of the ability of the powder to flow freely. A Carr index of less than
0.15 is indicative of good flowability (Lea and Febiger, 1986). VIVASTAR®P, with a Carr index of 0.12, has better flowability than SSG which has a Carr Index of 0.21.

3.3. Assay, NaCl content and settling volume
Table 1 gives the sodium assay and NaCl content for the sodium starch glycolate samples. VIVASTAR®P and SSG had assays of 2.9% and 4.2% and NaCl contents of 2.4% and 0.03%, respectively. In terms of the assays and NaCl contents, both samples were US Pharmacopoeia compliant. VIVASTAR®P had a settling volume of 35 ml whereas SSG had a settling volume of 34 ml.

Table 2 lists the assays of the 0-38µm, 38-75µm and 75-106µm fractions of SSG and 0-38µm and 38-75µm fractions of VIVASTAR®P. Statistical analysis revealed that the 0-38µm fraction of SSG had a significantly higher assay as compared to its 38-75µm fraction (P=0.0025) and 75-106µm fraction (P=0.0034). However no significant difference was found between the assays of the 38-75µm and 75-106µm fractions (P=0.6953). Also, no significant difference was found in the assays of 0-38µm and 38-75µm fractions of VIVASTAR®P (P=0.4442). The assays of 0-38µm (P<0.0001, P<0.0001), 38-75µm (P=0.0002, P=0.0003) and 75-106µm (P=0.0001, P=0.0002) fractions of SSG were higher than those for the 0-38µm and 38-75µm fractions of VIVASTAR®P.

3.4. Surface area, pore volume and pore size
Table 3 lists the surface areas, pore volumes and pore sizes for the various particle size fractions of VIVASTAR®P and SSG. The surface areas of SSG 38-75µm and 75-106µm fractions were lower than those for the SSG 0-38µm fraction (P<0.0001). Similarly, the
surface area of the VIVASTAR® P 38-75µm fraction was lower than that of the VIVASTAR® P 0-38µm fraction ($P=0.0021$). The surface area of the SSG 0-38µm fraction was higher than that of the VIVASTAR® P 0-38µm fraction ($P<0.0001$). However, no difference was found between the surface areas of the 38-75µm fractions of SSG and VIVASTAR® P ($P=0.2041$).

Similarly, the pore volumes of the SSG 38-75µm and 75-106µm fractions were lower than that of the SSG 0-38µm fraction ($P<0.0001$) and the pore volume of VIVASTAR® P 38-75µm fraction was lower than that of the VIVASTAR® P 0-38µm fraction ($P=0.0043$). The pore volume of the VIVASTAR® P 0-38µm fraction was lower than that of the SSG 0-38µm fraction ($P<0.0001$), whereas, no differences were found between the pore volumes of VIVASTAR® P and SSG 38-75µm fractions ($P=0.2858$).

No differences were found between the pore sizes of the 0-38µm, 38-75µm and 75-106µm fractions of SSG ($P=0.3499$, $P=0.6282$ and $P=0.6282$). The pore sizes of VIVASTAR® P 0-38µm fraction were smaller than that of its 38-75µm fraction ($P<0.0001$). The pore sizes of the SSG 0-38µm and 38-75µm fractions were larger than those of VIVASTAR® P 0-38µm and 38-75µm fractions ($P<0.0001$, $P=0.0091$).

3.5. X-ray diffraction studies
X-ray diffraction patterns for VIVASTAR and the 75-106µm fraction of SSG are presented in Figure 2. Potato starch has B-type crystallinity whose X-ray diffraction pattern is characterized by peaks around 5°, 15°, 17°, 19°, 22° and 23° (Hulleman et al., 1999). The X-ray diffraction pattern of VIVASTAR® P also was observed to consist of a broad peak from 16° to 24°. The X-ray diffraction pattern of SSG is also observed to
have a similar broad peak, besides a peak at 12°. The X-ray diffraction patterns of both VIVASTAR® P and SSG were devoid of the characteristic peaks of B-type crystalline starch. This is indicative of the considerable loss of crystallinity in both the VIVASTAR® P and the SSG.

3.6. Liquid uptake
Figure 3 illustrates the water uptake profiles for VIVASTAR® P, SSG and their particle size fractions. No differences were found between the extents of water uptakes by the VIVASTAR® P 0-38µm and 38-75µm fractions at time intervals of 20 s ($P=0.7563$) and 180 s ($P=0.4407$). It can be observed that the rates and extents of water uptakes obtained for VIVASTAR® P and its particle size fractions were much higher than that those obtained for SSG and its fractions. The extents of water uptakes by VIVASTAR® P 0-38µm and 38-75µm fractions were significantly higher than those for the SSG 38-75µm fraction at time intervals of 20 s ($P=0.0025$, $P=0.0051$) and 180 s ($P<0.0001$, $P<0.0001$), and also was higher than the water uptakes observed for the SSG 0-38µm fraction at 20 s ($P<0.0001$, $P<0.0001$) and 180 s ($P<0.0001$, $P<0.0001$). However, no differences were found between the water uptakes of both fractions of VIVASTAR® P and the SSG 75-106µm fraction after a time interval of 20 s ($P=0.1722$, $P=0.2838$). The similarities in the water uptakes of the SSG 75-106µm fraction and both fractions of VIVASTARP can be observed in Figure 3. The water uptake profiles were similar up to period of 70 s.

Subsequently, however, the water uptake of SSG 75-106µm fraction leveled off. At 180 s, the water uptakes of both fractions of VIVASTAR® P were significantly higher than that of SSG 75-106µm fraction ($P<0.0001$, $P<0.0001$). The water uptakes of the 0-38µm fraction of SSG were significantly lower than those of SSG 38-75µm and 75-106µm.
fractions at intervals of 20 s (P=0.0025, P<0.0001) and 180 seconds (P<0.0001, P<0.0001).

Bolhuis et al. (1986) observed that a lower NaCl content resulted in a higher rate and extent of water uptake, provided a high degree of cross-linking was present. Insufficient cross-linking in the SSG sample may have resulted in its lower water uptake. Also, gelatinisation during extrusion may have further contributed to the lower water uptake.

It also can be observed from Figure 3 that the larger particle size fractions of both samples, SSG and VIVASTAR®P, had higher rates and extents of water uptakes. However, other researchers found the smaller particle size fractions of sodium starch glycolate from other manufacturers (NF Explotab, (Penwest, NY) and Primojel (AVEBE Chem., Foxhol, Netherlands)) had higher rates and extents of water uptakes. They attributed the higher rates and extents of water uptake by the smaller particle size fractions to their larger surface areas (Shah and Augsburger, 2002). However, in this study, SSG and VIVASTAR®P fractions with larger surface areas (SSG 0-38\(\mu\)m and VIVASTAR®P 0-38\(\mu\)m) were observed to have lower water uptakes compared to their other particle size fractions.

Figure 4 shows the liquid uptake profiles, using 0.1N HCl, for VIVASTAR®P, SSG and their various particle size fractions. The performance of disintegrants in an acidic medium is important, since a majority of the rapid release tablets are intended to disintegrate in the gastrointestinal tract where the conditions are acidic. The liquid uptake of SSG 0-38\(\mu\)m fraction was lower than VIVASTAR®P and both of its fractions and SSG 38-75\(\mu\)m and 75-106\(\mu\)m fractions at time intervals of 20 s and 180 s (P<0.0001). At a
time interval of 20 s, SSG 75-106µm and 38-75µm fractions had higher extents of liquid uptake than that of VIVASTAR®P \((P<0.0006, P<0.0006)\) and also than that of VIVASTAR®P 0-38µm fraction \((P=0.0340, P=0.0340)\). Also, no differences were found between the SSG 75-106µm and 38-75µm fractions and the VIVASTAR®P 38-75µm fraction \((P=0.2722, P=0.2722)\). At 180 s, the SSG 75-106µm and 38-75µm fractions had lower liquid uptakes than that of VIVASTAR®P \((P=0.0097, P<0.0001)\) and VIVASTAR 0-38µm fraction \((P=0.0097, P<0.0001)\). No significant difference was found between the SSG 75-106µm fraction and VIVASTAR®P 38-75µm fraction \((P=1.0000)\). However, the SSG 38-75µm fraction had lower liquid uptake than that of VIVASTAR®P 38-75µm fraction \((P=0.0097)\). Hence, the SSG 75-106µm and 38-75µm fractions had high rates of liquid uptake initially, but tended to level off as time progressed. In general, the liquid uptake performance of VIVASTAR®P was better than that of SSG, however, there was less difference between their uptakes under acidic conditions. The liquid uptake of SSG increased with its particle size, with the liquid uptake profile of the SSG 75-106µm fraction of SSG being similar to those of the VIVASTAR®P fractions.

By comparing Figure 3 and Figure 4, it can be noted that the overall rates and extent of liquid uptakes in an acidic medium, were lower than those in water. Similar behavior was reported by Shah and Augsburger (2002) and Zhao and Augsburger (2005). This can be ascribed to the conversion of the sodium carboxymethyl groups to carboxymethyl groups in an acidic medium. The acidified carboxymethyl groups formed has lower capacities to absorb water. Since, the initial assay of SSG was higher than that of VIVASTAR®P; SSG may have had a larger number of sodium carboxymethyl groups present in the acidic medium. This may have helped SSG in obtaining a better liquid uptake profile that was
comparable to VIVASTAR®P. It also was noted that, for both SSG and VIVASTAR®P, the particle size fractions with larger surface areas and pore volumes had initial liquid uptakes in acidic conditions.

3.7. Scanning Electron Microscopy (SEM)
Figure 5 illustrates the microstructures of sodium starch glycolate prepared using reactive extrusion and VIVASTAR®P at 150x (A and B) and 1200x (C and D) magnifications. The smaller granules of VIVASTAR®P, around 20µm in size, appear to be almost perfectly spherical, whereas, the larger granules seem to be ovoid or pear-shaped. The surfaces of the VIVASTAR granules were smooth. In contrast to VIVASTAR®P, the granules of sodium starch glycolate prepared using reactive extrusion were irregular in shape with extremely jagged surfaces. This indicates that the starch granules were partially damaged due to gelatinization during extrusion. A large amount of fines also were observed for this sample.

4. Conclusions
Water uptakes of all particle size fractions of VIVASTAR®P were higher than those of SSG. In acidic conditions, the liquid uptakes of the SSG 38-75µm and 75-106µm fractions were lower than that of VIVASTAR®P and its 0-38µm fraction. The lower liquid uptake of SSG, in spite of a higher assay and lower NaCl content, may have been due to lower cross-linking. The microstructure of sodium starch glycolate prepared using reactive extrusion revealed irregularly shaped particles with jagged surfaces, indicating the gelatinization of starch granule during extrusion. This may have contributed to its lower liquid uptakes. It was further observed that the particle size fractions of both
VIVASTAR®P and sodium starch glycolate prepared using reactive extrusion with higher surface areas and pore volumes had lower water uptakes and lower initial liquid uptakes in acidic conditions.

Acknowledgement

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References


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Table 1. Sodium starch glycolate (SSG and VIVASTAR®P) and US Pharmacopoeia specifications

<table>
<thead>
<tr>
<th></th>
<th>SSG</th>
<th>VIVASTAR</th>
<th>USP32-NF27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay (%)</td>
<td>4.2</td>
<td>2.9</td>
<td>2.8-4.2</td>
</tr>
<tr>
<td>NaCl (%)</td>
<td>0.03</td>
<td>2.4</td>
<td>7</td>
</tr>
<tr>
<td>pH</td>
<td>6.3</td>
<td>5.8</td>
<td>5.5-7.5</td>
</tr>
<tr>
<td>Settling volume (mL)</td>
<td>35</td>
<td>34</td>
<td>-</td>
</tr>
<tr>
<td>Bulk Density (g/cm³)</td>
<td>0.66</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>Tap Density (g/cm³)</td>
<td>0.84</td>
<td>0.91</td>
<td>-</td>
</tr>
<tr>
<td>Carr Index</td>
<td>0.21</td>
<td>0.12</td>
<td>-</td>
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</tbody>
</table>
Table 2. Sodium assays for sodium starch glycolate (SSG and VIVASTAR®) according to particle size fractions

<table>
<thead>
<tr>
<th>Particle Size Fraction</th>
<th>Assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSG (0-38µm)</td>
<td>4.61</td>
</tr>
<tr>
<td>SSG (38-75µm)</td>
<td>4</td>
</tr>
<tr>
<td>SSG (75-106µm)</td>
<td>4.05</td>
</tr>
<tr>
<td>VIVASTAR (0-38µm)</td>
<td>2.93</td>
</tr>
<tr>
<td>VIVASTAR (38-75µm)</td>
<td>3.02</td>
</tr>
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</table>
Table 3. Surface areas, pore volumes and pore sizes

<table>
<thead>
<tr>
<th>Particle Size Fraction</th>
<th>Surface Area (m²/g)</th>
<th>Pore Volume (cm³/g)</th>
<th>Pore Size (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSG (0-38µm)</td>
<td>2.89</td>
<td>1.57E-03</td>
<td>10.80</td>
</tr>
<tr>
<td>SSG (38-75µm)</td>
<td>0.28</td>
<td>1.49E-04</td>
<td>10.81</td>
</tr>
<tr>
<td>SSG (75-106µm)</td>
<td>0.29</td>
<td>1.23E-04</td>
<td>10.81</td>
</tr>
<tr>
<td>VIVASTAR (0-38µm)</td>
<td>0.53</td>
<td>2.74E-04</td>
<td>9.59</td>
</tr>
<tr>
<td>VIVASTAR (38-75µm)</td>
<td>0.20</td>
<td>1.09E-04</td>
<td>10.77</td>
</tr>
</tbody>
</table>
Figure 1. Particle size distributions using sieve analysis.
Figure 2. X-ray diffraction patterns for VIVASTAR and the 75-106µm fraction of SSG.
Figure 3. Water uptakes for sodium starch glycolate (SSG and VIVASTAR®P).
Figure 4. Liquid uptakes for sodium starch glycolate (SSG and VIVASTAR®) using 0.1N HCl.
Figure 5. Microstructures of sodium starch glycolate prepared using reactive extrusion and VIVASTAR®P 150x (A and B) and 1200x (C and D) magnifications.
CHAPTER IV

PREPARATION OF HIGHLY SUBSTITUTED CARBOXYMETHYL STARCH USING A TWIN-SCREW EXTRUDER

This research paper has been published as:

Abstract

Carboxymethyl starch was prepared by reacting corn starch with sodium mono chloro acetate (SMCA) in a Brabender TSE-20 co-rotating twin screw extruder. The effects of the SMCA:starch ratio (theoretical degrees of substitutions 2.73, 3.62 and 4.53), aqueous ethanol:(starch+SMCA) ratio (0.13 and 0.25) and the screw configuration (0, 1 and 2 kneading blocks) on the degree of substitution and reaction efficiency were studied. A 3 × 2 factorial design with 3 levels of the starch:SMCA ratio and 2 levels of the aqueous ethanol:(starch+SMCA) ratio were used. This 3 × 2 factorial design was blocked, with the screw configuration as the blocking variable. The effects of the SMCA:starch ratio and the screw configuration were found to be significant. The carboxymethylated starches were characterized using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and X-ray diffraction. The microstructure of the carboxymethylated starches, studied using SEM, revealed that the integrity of the starch granules was partially preserved at low aqueous ethanol levels when no kneading block was used. However, for all other reaction conditions either granule agglomeration or fusion was observed. X-ray diffraction analysis indicated substantial loss of crystallinity in the carboxymethylated starches, irrespective of the damage to the granule structure.

Keywords: High degree of substitution, modified starch, reactive extrusion, scanning electron microscopy, sodium carboxymethyl starch.
1. Introduction

Carboxymethyl starch is prepared industrially for various applications. However, the preparation of carboxymethyl starch is restricted to low degrees of substitutions (DS) because the gelatinization temperature of carboxymethyl starch decreases with increase in DS. Gelatinization of starch in the aqueous solvents typically used, cause problems in product handling and processing. Highly substituted carboxymethyl starch could find applications as thickening, suspension and colloidal agents in the food, pharmaceutical and cosmetic industries. They also could be used to replace non-biodegradable polyacrylates in detergents [1]. Ragheb et al. found that high DS carboxymethyl starch (0.65-0.9) was suitable for textile printing since it gave color strength comparable to the conventionally used sodium alginate [2]. Holst et al. investigated the use of highly substituted and cross-linked carboxymethyl starch for absorption applications [3].

Carboxymethyl starch has been prepared traditionally by a slurry batch process. Starch is suspended in an aqueous alcohol solvent along with NaOH and sodium mono chloro acetate (SMCA). Usually a starch to liquor ratio of 1:15 and a temperature of around 65°C is used for the reaction [4]. Ragheb et al. prepared carboxymethyl starch with DS of 0.65-0.9 using native maize starch and oxidized maize starch [2]. A mixture of ethanol and toluene was used as a solvent and the reaction was allowed to continue overnight. Tijsen et al. obtained a DS up to 1.34 in a single step carboxymethylation reaction. The reaction time was very large (~500 min), and was preceded with at least a 10 h pretreatment with NaOH [5]. They also were able to obtain carboxymethyl starch with a DS of 2.2 using a three step carboxymethylation reaction that lasted 26.5 h. Tijsen
et al., in a different study, designed a continuous slurry carboxymethylation process which gave a product with a DS of 1.5 with a total reaction time of 8.1 h [6].

Reactive extrusion offers a simple process with lower amounts of solvents and a shorter reaction time for preparing highly substituted carboxymethyl starch. Researchers have investigated the use of extruders for preparing several starch derivatives like starch-graft-poly acrylic acid and poly acrylamide[7], starch-graft-poly acrylonitrile[8], cationic starch[9,10], glycol glucosides[11,12]. Gimmler et al. prepared a model to study the effect of extrusion conditions on low DS carboxymethylated starch [13]. Johnson et al. patented a process for the preparation of carboxymethyl starch (DS 0.14 - 0.74) using an extruder, for preventing scale formation in equipments handling aqueous solvents [14].

The objective of this study was to investigate the preparation of highly substituted (theoretical degrees of substitution 2.73, 3.62 and 4.53) carboxymethyl starch using reactive extrusion. Theoretical degree of substitution (DS, ) is the degree of substitution obtained if all the SMCA present reacts, to form the carboxymethyl derivative of starch. The influence of the amount of 50% aqueous ethanol in the reaction formulation and the screw configuration on the DS, reaction efficiency and microstructure of the carboxymethylated starch also were examined.

2. Experimental

2.1. Materials

Corn starch was obtained from Tate & Lyle (Decatur, IL). Sodium hydroxide beads (140 mesh) were obtained from Thermo Fisher Scientific (Hampton, NH). SMCA
was obtained from Alfa Aesar (Ward Hill, MA) and pure anhydrous ethanol (USP grade) was obtained from Decon Labs (King of Prussia, PA).

2.2. Methods

2.2.1. Extrusion

Corn starch with 8.8% moisture (dry basis) was mixed with 50 wt% aqueous ethanol using a liquid spray bottle. The 50% aqueous ethanol/(SMCA+starch) ratios used were based on weight of starch with 8.8% moisture. SMCA was added to the formulation and mixed using a Hobart C-100 (Hobart Corp., Ohio) planetary mixer. The amounts of SMCA used in the formulations corresponded with the selected DS. NaOH beads were added to the formulation immediately before the extrusion such that the NaOH/SMCA ratio of 0.74 was constant for all formulations. A Brabender TSE-20 (South Hackensack, NJ) co-rotating twin screw extruder was used as a reactor for the carboxymethylation reaction. The extruder was operated at a screw speed of 70 rpm without a die. The reaction formulation was fed using a Brabender PW40PLUS-0 (Brabender Technologies Inc., Ontoria, Canada) flex wall volumetric feeder at a feed rate of 20 g/min. The extruder barrel temperature was set to 80-85°C.

2.2.2. Purification and measurement of NaCl content

Extruded products were ground to a fine powder using a Krups GX4100 (Millville, NJ) grinder. Ground carboxymethylated starches were purified by, first, washing with anhydrous ethanol. This was followed by washing the substituted starch thrice with 90% aqueous ethanol (20:1 w/w) for 2 hr each. This was followed by washing again with anhydrous ethanol. The pH of the sample was neutralized during the ethanol
washings using acetic acid. After the ethanol washings, samples were dried in a vacuum oven. The dried samples were ground to pass through 100 mesh sieve. The NaCl content was then measured according to the method described by British Pharmacopoeia [15]. In a beaker, 0.5 g of carboxymethyl starch were mixed with 100 mL of distilled water along with 1 mL of nitric acid. The mixture was titrated with 0.1 M silver nitrate and the end point was determined potentiometrically. One milliliter of 0.1 M silver nitrate corresponds to 5.844 mg of NaCl.

2.2.3. Degree of substitution

The degree of substitution was measured according to the method described by British Pharmacopoeia [15]. Carboxymethyl starch (0.7 g) was refluxed in 70 mL glacial acetic acid for 2 h and then allowed to cool to room temperature. The refluxed sample was titrated using a 0.1 M solution of perchloric acid in glacial acetic acid and the end point was determined potentiometrically. One milliliter of 0.1 M perchloric acid is equivalent to 2.299 mg of Na. The analysis was replicated twice and the average of three measurements was used.

2.2.4. Fourier-transform infrared spectroscopy

The Fourier-transform infrared (FTIR) spectra for the carboxymethylated starches was obtained using a Smiths Detection SensIR (Danbury, CT) FTIR microscope. The FTIR microscope was used with an attenuated total reflectance (ATR) objective. The sample powder, placed on a glass slide, was pressed against the objective and analyzed directly. Sixteen scans were acquired per image at a resolution of 4cm⁻¹.
2.2.5. Microstructure of carboxymethyl starch

The microstructures of the carboxymethylated starches were examined using a Hitachi S-3000N (San Jose, CA) variable pressure scanning electron microscope. A small amount of powder was placed on a metal stub with a double sided adhesive tape. Excess powder was removed from the stub using compressed air. The carboxymethylated starches were sputter coated with gold under vacuum to render them conductive. Images of the samples were then acquired at various magnifications and at a 1280x960 pixel resolution.

2.2.6. X-ray diffraction analyses

The X-ray diffractograms for the carboxymethylated starches indicated the effects of the reactive extrusion on the crystalline structure of the starches. The crystalline structure of the carboxymethylated starches was studied using a Rigaku D/Max-B X-ray diffractometer (The Woodlands, TX). X-rays were generated using a 2 kW copper target. The diffracted beam was focused on a monochromator which removed all radiations except Cu Ka wavelengths (about 1.544 Å). The detector and sample were rotated at angles of θ and 2θ respectively, with reference to the incident beam. Diffraction patterns were recorded from a diffraction angle (2θ) of 5-40° at a scan speed of 7°/minute and step size of 0.02°.

2.2.7. Experimental design and data analyses

There are six treatment combinations (3 levels of theoretical DS × two levels of 50% aqueous ethanol:(starch+SMCA) ratio). These six treatment combinations were blocked. The numbers of kneading blocks used during extrusion were the blocking variables with three blocking levels (no kneading blocks used, 1 kneading block and 2
kneading blocks). These blocks were treated as ‘fixed blocks’. The response variables, the DS and reaction efficiency, were analyzed using ‘Proc Mixed’ procedure of SAS version 9.1 (SAS Institute Inc., Cary, NC) with a significance level of $\alpha \leq 0.05$.

3. Results & Discussion

3.1. FTIR spectroscopy

The FTIR spectra for a single sample of carboxymethyl starch, prepared using no kneading blocks at DS$_t$ 3.62 and high level of ethanol, for the wavelengths from 4000 to 650 cm$^{-1}$ are summarized in Figure 1. The peak at a wavelength of 1600 cm$^{-1}$, corresponding to the carbonyl group, confirms the carboxymethylation of starch.

3.2. Degree of substitution and reaction efficiency

During the carboxymethylation reaction, NaCl is formed as a by-product. Also, sodium glycolate is formed in a side reaction of NaOH and SMCA. These by-products along with the excess reactant were removed by washing with ethanol. The NaCl content was checked to determine the extent of purification. The DS was determined only after the NaCl content was found to be less than 0.1% by weight of the carboxymethylated starch, since it affected the DS measurements.

The DS of the carboxymethylated starches and the corresponding reaction efficiencies are summarized in Table 1. Here, reaction efficiency is defined as the fraction of SMCA that had reacted with starch to form carboxymethyl starch. The main effects of the number of kneading blocks and SMCA:starch ratio on the DS and reaction efficiencies were found to be significant. The DS at DS$_t$ 3.62 was higher than the DS at DS$_t$ 2.73. As expected, an increase in the amount of SMCA available to starch increased
the DS. However, the DS at DS$_t$ 4.53 was lower than that at DS$_t$ 3.62. In this case, increase in the amount of SMCA caused a decrease in the DS. The large amount of SMCA and NaOH present, relative to the amount of starch, in this case, may have resulted in a higher selectivity for the SMCA reacting with NaOH to form sodium glycolate. Consumption of the SMCA in the side reaction instead of the carboxymethylation reaction may have resulted in the low DS. Similar effects of increasing the NaOH and SMCA concentrations have been reported in slurry batch reactions [4, 16]. The DS at DS$_t$ 4.53 was higher than that for DS$_t$ 2.73. The reaction efficiency for DS$_t$ 4.53 was found to be lower than that for DS$_t$ 2.73 and DS$_t$ 3.62. No distinction could be found between the reaction efficiency at DS$_t$ 2.73 and DS$_t$ 3.62. The DS and reaction efficiency obtained by using 1 and 2 kneading blocks was higher than that obtained when no kneading block was used. No distinction was found between the use of 1 and 2 kneading blocks. The effects of the aqueous ethanol:(starch+SMCA) ratio on the DS and reaction efficiency were not significant. The highest DS and reaction efficiency obtained were 1.54 and 0.42, respectively.

3.3. Carboxymethyl starch microstructure

Tamaki et. al., in their study on the effect of ball mill treatment on the structures of maize starch granules, found that even after 80 h of treatment the starch granules still retained their initial structures [17]. Hence, it seems safe to say that the milling treatment in this study was unlikely to have dramatically affected the microstructure of carboxymethyl starch in cases where the granule structure was not destroyed during extrusion. The microstructures of the carboxymethylated starches, extruded with no kneading blocks at DS$_t$ 2.73 and at both aqueous ethanol levels are shown in Figure 2. The microstructure of
the carboxymethylated starch extruded at the lower aqueous ethanol level shows particles appearing to have deformed spherical shapes and relatively smooth surfaces. Whereas, the microstructure of the carboxymethylated starch obtained at at the same DS, and zero kneading blocks, but at a higher level of added aqueous ethanol, showed completely deformed granules with extremely jagged surface.

For no kneading blocks and a DS of 3.62, the microstructure of the carboxymethylated starch at lower aqueous ethanol level consisted of fused spherical particles with relatively smooth surfaces, as shown in Figure 3. In contrast, at higher aqueous ethanol levels, the microstructure revealed more deformed particles with irregular surfaces. Similarly, as observed in Figure 4, with no kneading blocks, DS of 4.53 and lower ethanol level the microstructure showed deformed spheres with smooth surfaces fused together, but at higher ethanol level more jagged particle surfaces were observed. These observations suggest that for no kneading blocks, at high aqueous ethanol levels, more extensive melting of the starch occurred, which gave more deformed carboxymethylated starch granules. The additional melting of starch may have been due to the additional moisture available to the starch when higher aqueous ethanol was used.

The microstructures of the carboxymethylated starches that were prepared with 1 kneading block (Figure 5) and 2 kneading blocks (Figure 6) showed completely deformed granules for both aqueous ethanol levels. This may have been due to the extensive melting of starch caused by the additional mechanical energy imparted by the kneading blocks.
Thus, the observed carboxymethyl starch microstructures can be classified as granule damage without agglomeration or fusion, granule agglomeration and complete granule fusion. Granule damage without agglomeration or fusion occurs at the mildest reaction and extrusion conditions, as observed in Figures 2A and 2B, with no kneading blocks, lowest DS, and low amount of aqueous ethanol. Granule agglomeration occurred for higher DS, but otherwise similar conditions as before. This can be seen in Figures 3A and B and Figures 4A and B with higher DS, but no kneading block and low amounts of aqueous ethanol. In contrast to the first two classifications, granule fusion occurred with the use of higher amounts of aqueous ethanol, irrespective of the number of kneading blocks and DS (Figures 1-4 C&D). However, it was more predominant with the use of kneading blocks (Figures 5 & 6), which promoted melting of the starch granules. The melting and fusion of starch granules resulted in the formation of chunks in which the starch granules were no longer discernible. It also could be noted that in cases where the granules were completely destroyed, a broad particle size distribution and large number of fine particles were observed. These fragments may have been caused by the milling operation.

3.4. X-ray diffraction patterns

The X-ray diffraction patterns for the native corn and carboxymethylated starches are presented in Figure 7. The X-ray diffraction patterns for native corn starch (Figure 7e) shows peaks at 15°, 17°, 18° and 23° (2θ) [18, 19]. These peaks are characteristic of A-pattern crystallinity observed in cereal starches. The X-ray diffraction patterns of carboxymethylated starches obtained using 1 kneading block (Figure 7a) and 2 kneading blocks (Figure 7b) shows the presence of a single peak around 20° and absence of the
characteristic peaks observed for native corn starch. The absence of the characteristic peaks in the carboxymethylated starches indicates the substantial loss of crystallinity during the reactive extrusion process [19]. The loss of crystallinity in carboxymethylated starches when 1 and 2 kneading blocks were used is not surprising, considering their extensive granule destruction, observed using scanning electron microscopy. However, even when no kneading blocks were used and at the lowest DS, of 2.73, for which there was relatively less granule damage, the X-ray diffraction patterns (Figures 7c & 7d) show the absence of the characteristic peaks. This indicates a dramatic loss of crystallinity even when there was no extensive granule damage. Kittipongpatana et al observed a similar loss of crystallinity without extensive granule damage in sodium carboxymethyl mungbean starches [20].

4. Conclusions

Corn starch was reacted in a twin-screw extruder. The DS obtained increased from DS, 2.73 to DS, 3.62, but decreased at DS, 4.53. This decrease in the DS may have been due to the consumption of SMCA in a side reaction. Also, the reaction efficiency at DS, 2.73 and 3.62 was higher than that at DS, 4.53. The DS and reaction efficiency when 1 and 2 kneading blocks were used was found to be higher than that for 0 kneading blocks. No significant effect of the use of aqueous ethanol on the DS was found. Also, experimental conditions strongly influenced the product morphology. The microstructure of the carboxymethylated starch revealed that for no kneading blocks when lower amount of aqueous ethanol was used, the granular structure of starch was better preserved. For
other conditions granule agglomeration or granule fusion was observed. Overall, the maximum DS and reaction efficiency obtained were 1.54 and 0.42, respectively.
References


Table 1. Degree of substitution of carboxymethyl starch

<table>
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<tr>
<th>DST</th>
<th>50% Aq. ethanol/ (SMCA+starch)</th>
<th>Kneading blocks</th>
<th>Degree of substitution</th>
<th>Reaction efficiency</th>
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<td>0.18</td>
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<td>0.68</td>
<td>0.25</td>
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</tr>
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<td>1.09</td>
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</tr>
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</tr>
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<td>2</td>
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Figure 1. FTIR spectra for carboxymethyl starch.
Figure 2. Microstructure of carboxymethyl starch obtained with 0 kneading blocks, DST 2.73 and (A and B) 50% aqueous ethanol: starch+SMCA ratio of 0.13 (700X and 2500X magnifications, respectively) and (C and D) 50% aqueous ethanol:starch+SMCA ratio of 0.25 (200X and 1200X magnifications, respectively).
Figure 3. Microstructure of carboxymethyl starch obtained with 0 kneading blocks, DST 3.62 and (A and B) 50% aqueous ethanol: starch+SMCA ratio of 0.13 (200X and 1200X magnifications, respectively) and (C and D) 50% aqueous ethanol:starch+SMCA ratio of 0.25 (200X and 1200X magnifications, respectively).
Figure 4. Microstructure of carboxymethyl starch obtained with 0 kneading blocks, DST 4.53 and (A and B) 50% aqueous ethanol: starch+SMCA ratio of 0.13 (200X and 1200X magnifications, respectively) and (C and D) 50% aqueous ethanol:starch+SMCA ratio of 0.25 (200X and 1200X magnifications, respectively).
Figure 5. Microstructure of carboxymethyl starch obtained with 1 kneading block, DSt 4.53 and (a and b) 50% aqueous ethanol: starch+SMCA ratio of 0.13 (200X and 1200X magnifications, respectively) and (c and d) 50% aqueous ethanol:starch+SMCA ratio of 0.25 (200X and 1200X magnifications, respectively).
Figure 6. Microstructure of carboxymethyl starch obtained with 2 kneading blocks, DSt 3.62 and (a and b) 50% aqueous ethanol: starch+SMCA ratio of 0.13 (200X and 1200X magnifications, respectively) and (c and d) 50% aqueous ethanol:starch+SMCA ratio of 0.25 (200X and 1200X magnifications, respectively).
Figure 7. X-ray diffraction patterns for 1 kneading block, DSt 4.53 and high ethanol level (a), 2 kneading blocks, DSt 2.73 and high ethanol level (b), 0 kneading blocks, DSt 2.73 with low ethanol level (c) and high ethanol level (d) and native corn starch (e).
CHAPTER V

CARBOXYMETHYLATION OF CELLULOSE USING REACTIVE EXTRUSION

This research paper has been published as:

CARBOXYMETHYLATION OF CELLULOSE USING REACTIVE EXTRUSION

Abstract

Carboxymethyl cellulose was prepared using a continuous, reduced solvent, reactive extrusion process with a short reaction time. The effects of the amounts of NaOH (30g, 40g and 50g), water:ethanol ratio (100%, 70%, 50%, 30% and 10% H2O) and their interactions on the physical, chemical and morphological properties of carboxymethyl cellulose were studied. Experiments were conducted using a 5×3 blocked factorial design. X-ray diffraction analyses revealed higher degrees of crystallinity and fractions of cellulose-II crystalline structure when 100% H2O was used as compared to that for 70%, 50%, 30% and 10% H2O and a commercially available brand of carboxymethyl cellulose, AQUASORB A500. Statistical analysis revealed a significant interaction between the effects of NaOH and H2O on the degrees of substitutions. The degrees of substitutions decreased with increasing amounts of NaOH and tended to increase with increasing alcohol concentrations. Liquid uptake measurements revealed that the extent of saline uptake, measured at intervals of 1 min, 5 min and 10 min, by carboxymethyl cellulose prepared with 100% H2O, especially when 40g and 50g NaOH was used, was higher than that for 70%, 50%, 30% and 10% H2O and AQUASORB A500. This may have been because of the higher crystallinity in carboxymethyl cellulose prepared with 100% H2O. Carboxymethyl cellulose prepared with 70% H2O and 30g and 50g NaOH had the highest saline absorption, using the soak method, before and after centrifugation respectively. Scanning electron microscopy for carboxymethyl cellulose prepared with
100% and 10% H₂O, through images at 120X magnification, revealed fibers 100µm to >800µm in length and 0.8-3.3µm in breadth. Some non fibrous particles, 0.8-6.7µm in dimensions, also were observed for 100% H₂O. Images at 900X magnification revealed partially damaged fiber surfaces.

**Keywords:** Carboxymethyl cellulose, Cellulose crystallinity, Reactive extrusion, Scanning electron microscopy, X-ray diffraction.
1. Introduction

Cellulose ethers were described theoretically, first, in the year 1905 and their preparation was first studied and published by Chowdhury in 1924 (Chowdhury, 1924; Ott, Harold, Spurlin, & Grafflin, 1954). The sodium salt of carboxymethyl cellulose is a water soluble cellulose ether also known as cellulose gum, sodium cellulose glycolate or only carboxymethyl cellulose (Ott et al., 1954). Carboxymethyl cellulose has colloidal, binding, thickening, absorbing, stabilizing and film-forming properties, because of which it finds applications in the food, personal care, detergent, cosmetics, pharmaceutical, oilfield, printing and dyeing, and paper industries (Ott et al., 1954; Yang & Zhu, 2007). Carboxymethyl cellulose is used in the biomedical field for applications such as preventing postoperative adherences and epidural scarring (Barbucci, Magnani, & Consumi, 2000). Tomanová et al. (2008) recently found partially esterified carboxymethyl cellulose an environmentally friendly alternative to surfactants.

Carboxymethyl cellulose is obtained by reacting cellulose with sodium mono chloro acetate (SMCA) in the presence of NaOH. Sodium chloride and water are the by-products. Although, generally, alkali cellulose is formed separately by treating cellulose with a NaOH solution and this alkali cellulose is then reacted with SMCA.

\[
\text{Cell-(OH)}_3 + \text{ClCH}_2\text{COONa} + \text{NaOH} \rightarrow \text{Cell-(OH)}_2\text{OCH}_2\text{COONa} + \text{NaCl} + \text{H}_2\text{O}
\]

Also, sodium glycolate is formed by a side reaction.

\[
\text{ClCH}_2\text{COONa} + \text{NaOH} \rightarrow \text{HOCH}_2\text{COONa} + \text{NaCl}
\]
Production of carboxymethyl cellulose is carried out commercially on a large scale, exclusively, by the slurry process (Heinze & Koschella, 2005). In the slurry method, cellulose is suspended in an alcohol-water-NaOH system, which has an excess of alcohol (Mann, Kunze, Loth, & Fink, 1998). In this process the liquid phase acts as a solvating agent which dissolves the NaOH and distributes it uniformly to the cellulose hydroxyl groups. The aqueous NaOH penetrates the crystalline structure of cellulose, solvates the hydroxyl groups in this region and makes them available for etherification by breaking the hydrogen bonds (Ott et al., 1954). This process of treating cellulose with NaOH is termed mercerization. Further, this alkali cellulose is then reacted with SMCA to form carboxymethyl cellulose ethers.

Several factors affect the carboxymethylation of cellulose and the resultant properties, such as the type of solvent (Barai et al., 1997; Pushpamalar et al., 2006; Olaru, Olaru, Stoleriu, & Ţîmpu, 1998), concentration of solvent (Pushpamalar et al., 2006; Zhang, Li, Zhang, & Shi, 1993), amount of H$_2$O, amount of SMCA (Mann et al., 1998), concentration of NaOH (Heinze & Pfeiffer, 1999), reaction time (Hedlund & Germgård, 2007), temperature (Xiquan, Tingzhu, & Shaoqui, 1990) and additional ingredients like borax (Majewicz, 1981) and cobalt (Stigsson, Kloow, Germgård, & Andersson, 2005) used during the reaction.

Researchers have experimented with several methods for preparing cellulose ethers, such as homogeneous carboxymethylation (Heinze, Liebert, Klufers, & Meister, 1999), rotating drum technique (Swinehart & Allen, 1950), fluidized bed technique (Durso, 1981), sheet carboxymethylation (Collings, Freeman, & Anthonisen, 1942), Werner-
Pfleiderer type mixers (Tokimatsu & Yamashita, 1969) and also a solvent-less method using a double screw press (Eichenseer & Kletschke, 1971; Edelman & Lindroos, 1990) and a paddle reactor (Holst, Lask, & Kostrzewa, 1978).

Reactive extrusion is a popular technique for chemical modification of starches (Moad, 2011). However, extruders also have been used as chemical reactors for non-thermoplastic polysaccharides, such as in the acid hydrolysis of cellulose to glucose (Rugg & Stanton, 1982; Green, Kimchie, Malester, Rugg, & Shelef, 1988), partial hydrolysis of cellulose to microcrystalline cellulose using mineral acids (Hanna et al., 2001) and hydrogen peroxide (Kopesky & Ruszkay, 2006) and alkaline extraction of alginates from seaweeds (Vauchel et al., 2008). Carlborn & Matuana (2002) used reactive extrusion for the surface esterification of wood particles with maleated polyethylene and maleated polypropylene. In this case, although, the esterification agents used were thermoplastic and formed a melt phase in the extruder, their amounts, used along with wood particles were small (5-20 wt%).

The objective of this project was to prepare carboxymethyl cellulose using reactive extrusion and study the effects of the water:ethanol ratio, amounts of NaOH and their interactions on the properties of carboxymethyl cellulose. NaOH and ethanol concentrations were found to be important factors in the slurry process of preparation of carboxymethyl cellulose, affecting the reaction rate and hence DS (Heinze & Pfeiffer, 1999; Barai et al., 1997), degree of polymerization and viscosity (Zhang et al., 1993) and crystalline structure (Zhang et al., 1993).
2. Experimental

2.1. Extrusion

Cotton linter pulp sheets (Grade UVE, DP\textsubscript{w} = 7077 and viscosity = 14,000 s), Buckeye Technologies Inc., Memphis, TN) were cut, using a sheet cutter, into 15×15 mm pieces and then shredded to a fluff using a blender (Model 31BL92, Waring Products Limited, New Hartford, CT). NaOH (Thermo Fisher Scientific, Hampton, NH) solution in aqueous ethanol (USP grade, Decon Labs, King of Prussia, PA) or distilled water, cooled to 15°C, was added to the cellulose fluff under a nitrogen atmosphere and then shredded in the blender for 5 min. For high ethanol concentrations, NaOH was not completely soluble and the suspension was used as is. Powdered SMCA (Alfa Aesar, Ward Hill, MA) was then added to the alkali cellulose and the formulation was again shredded in the blender, under a nitrogen atmosphere. The formulation was then mixed in a planetary mixer (model C-100, Hobart Corp., OH) and sealed in plastic bags, with nitrogen maintaining an inert atmosphere, and stored at 25°C for 7 h, for mercerization.

A co-rotating twin-screw extruder (model TSE-20, Branender Technologies Inc., South Hackensack, NJ), with four heating zones and an injection port in the barrel, was used as a reactor for carboxymethylation of cellulose. The extruder was operated without a nozzle and die because of the fibrous and non-thermoplastic nature of cellulose. The screw configuration used consisted of four kneading blocks and nine compression screws and is detailed in Table 1. The formulation was force fed into the second zone of the extruder screw using a plastic plunger. Nitrogen was injected in the extruder barrel through the injection port. The barrel temperature for the feed zone was set at 100°C and the temperatures for both the subsequent zones were set at 150°C. On the die end of the
extruder, an 80×80×900 mm steel tube was placed perpendicular to the extruder barrel. The extrudates leaving the extruder, dropped through the steel tube in which a nitrogen atmosphere was maintained, and were collected in a plastic bag attached to the lower end of the steel tube. The extrudates were sealed in the plastic bags and stored under inert conditions until further processing.

2.2. Extrudate purification
The extrudates were ground using a blender (model F203, Krups USA) and separated from the by-products by centrifuging an 80% aqueous ethanol suspensions using a Baxter MEGAFUGE (model 2.0R, Heraeus Instruments Inc., South Plainfield, NJ) at 4000 rpm. The aqueous ethanol was periodically replaced. The pHs of the extrudates were neutralized by adding glacial acetic acid to the ethanol. The extrudates were centrifuged until the NaCl content was found to have been reduced below 0.1 wt%. The NaCl content was measured by potentiometric titration with 0.1 M silver nitrate and has been described elsewhere (Bhandari & Hanna, 2011). Finally, the extrudates were washed with anhydrous ethanol and dried overnight in a vacuum oven. The purified extrudates were ground to pass through a 70 mesh Tyler Standard Series sieve.

2.3. Fourier-transform infrared spectroscopy
The Fourier-transform infrared (FTIR) spectrum for carboxymethyl cellulose was obtained using an Avatar 360 FT-IR E.S.P. spectrometer (Thermo Nicolet, Madison, WI). Sixteen scans were acquired per image at a resolution of 4 cm⁻¹.
2.4. X-ray diffraction

X-ray diffraction studies were conducted using a Rigaku D/Max-B diffractometer (Rigaku Americas, TheWoodlands, TX). X-rays, produced using a 2 kW copper target, were converged into a monochromator which removed all radiations except the Cu Ka wavelength (~1.544 Å). The samples were sprinkled uniformly on glass slides covered with vacuum grease (Dow Corning, Midland, MI) which were then inserted in the diffractometer. The sample and the detector were rotated at angles $\theta$ and $2\theta$ with respect to the incident beam. Diffractograms were registered for the angular range ($2\theta$) of 5° to 35°, with a scanning speed of 5° ($2\theta$)/min and a step size of 0.02° ($2\theta$). The degree of crystallinity was determined using the equation $X = n\times I_k/I_o$, $n=0.75$. Here, $I_o$ is the intensity of the maximum diffraction from the baseline and $I_k$ is intensity obtained by subtracting the base level from $I_o$ (Zhang et al., 1993). The fraction of cellulose-II crystalline form present in the carboxymethyl cellulose was calculated using the equation $X_{II}=X(1-C_{II})$, where $C_{II}$ is the fraction of cellulose-II in the crystalline aggregation and is calculated as $C_{II}=I_{12}/[I_{12} + 0.5(I_{14.7} + I_{16.1})]$. Here, $I_{12}$, $I_{14.7}$ and $I_{16.1}$ are the intensities at 2θ angles of 12°, 14.7° and 16.1° respectively, in the diffractogram, and they were determined as indicated by Zhang et al. (1993).

2.5. Degree of substitution

The degrees of substitutions were determined using the method described by US Pharmacopoeia NF24. In a crucible, which was previously ignited at 600°C for 30 min, then cooled in a desiccator and weighed, 1 g of carboxymethyl cellulose was added. The sample was moistened with 1 mL of dilute sulfuric acid (50 wt%) and then the crucible was heated on a flame until the cellulose was completely charred. After the sample had
cooled down, it was again moistened using 1 mL sulfuric acid and ignited in a muffle furnace at 600°C for 3 h. The crucible was cooled in a desiccator, weighed and the percentage of residue was calculated. The moistening with sulfuric acid and igniting was repeated until constant percentage of residue was obtained. The Na in sodium carboxymethyl cellulose was assayed using the equation \(\% \text{Na} = 0.3238A\), where A is the percentage of sulfated ash residue obtained on ignition. The \%Na assay was converted to DS using the equation:

\[
DS = \frac{(%Na/2300) \times 162}{1 - ((%Na/2300) \times 80)}
\]

The measurements were replicated once.

2.6. Capillary liquid uptake
The set-up for capillary liquid uptake measurements consisted of a graduated glass burette that was connected, through a tube, to a Millipore (Billerica, MA) 47 mm filter setup consisting of a fritted glass filter plate. The position of the fritted filter plate was adjusted so that the test liquid (1 wt% NaCl solution) in the graduated burette was level with it and the liquid barely touched the fritted plate. Any kinks present in the tube and any air bubbles present in the tube or under the fritted plate were removed. The powdered extrudate (0.2 g) was spread uniformly on the sintered glass plate. The test liquid passed through the tube and fritted plate by capillary action and was absorbed by the extrudates. The liquid level in the burette was noted periodically and the rate of water uptake by the extrudates was determined. The measurements were replicated once. Similarly, water uptakes were also determined for AQUASORB A500 (carboxymethyl cellulose, Ashland
Aqualon Functional Ingredients, Wilmington, DE) and cross-linked sodium polyacrylate (The Ark Enterprises, Inc., MO).

2.7. Water absorption

2.7.1. Flood method
Carboxymethyl cellulose (0.2 g) was immersed in 20 mL of 1% NaCl solution, in a beaker. After soaking for 30 min, the suspension was filtered using a 60 mesh (Tyler standard series) sieve and the filtrate was collected in a measuring cylinder. The unabsorbed liquid was measured and the absorbency (ml of liquid absorbed) was calculated. The measurements were replicated once. Water absorptions also were determined for AQUASORB A500 and sodium polyacrylate.

2.7.2. Centrifuge method
Carboxymethyl cellulose (0.2 g) was immersed in 20 ml of 1% NaCl solution for 30 min and then centrifuged using a Baxter MEGAFUGE 2.0R (Heraeus Instruments Inc., South Plainfield, NJ) for 2 min at 1800 g. The suspension then was filtered using a 60 mesh sieve and the absorbency was determined. Using the absorbency before and after centrifugation, the % retention after centrifugation also was calculated. The measurements were replicated once. Water absorptions, using this method, also were determined for AQUASORB A500 and sodium polyacrylate.

2.8. Scanning electron microscope
A variable pressure scanning electron microscope (model S-3000N, Hitachi High Technologies America Inc., San Jose, CA) was used to determine the product
microstructures as described by Bhandari & Hanna (2011). Images were obtained at 1280×960 pixel resolution and 120X and 900X magnifications.

2.9. Experimental design and data analyses
There were fifteen treatment combinations (5 levels of water:ethanol ratio × 3 levels of NaOH). These fifteen treatment combinations were blocked and replicated twice. The data on the degrees of substitutions, capillary water uptakes and water absorptions using flood and centrifuge methods were analyzed using ‘Proc Mixed’ procedure of SAS version 9.1 (SAS Institute Inc., Cary, NC) with a significance level of α≤0.05.

3. Results and discussions

3.1. FTIR Spectroscopy
The FTIR spectra for purified carboxymethyl celluloses for the wave-numbers 850-3500 cm\(^{-1}\) are summarized in Figure 1. The peaks corresponding to the backbone of the cellulose molecule were observed at 3432 cm\(^{-1}\) (broad absorption band due to stretching of –OH groups and intermolecular and intramolecular hydrogen bonds), 2920 cm\(^{-1}\) (C-H stretching), 1420 cm\(^{-1}\) (-CH\(_2\) scissoring), 1320 cm\(^{-1}\) (-OH bending) and 1060 cm\(^{-1}\) (CH-O-CH\(_2\) stretching) (Pushpamalar et al., 2006). The peak at 1600 cm\(^{-1}\) confirmed the carboxymethylation of cellulose.

3.2. X-ray Diffraction
The x-ray diffractogram for cotton linter pulp and carboxymethyl cellulose are presented in Figures 2 and 3, respectively. The x-ray diffractogram of cotton linter pulp, illustrated in Figure 2, shows the characteristic peaks of cellulose-I crystalline structure at 14.7°, 16.1°, 22.4° and 34.2°. While, in the x-ray diffractograms of carboxymethyl cellulose and
AQUASORB A500, illustrated in Figure 3, the peaks at 14.7°, 16.1° and 22.4° disappeared and peaks at 12°, 20° and 21.5°, characteristic of cellulose-II crystalline structure, appeared (Zhang et al., 1993). That indicated that during mercerization, prior to the carboxymethylation process, destruction of cellulose-I crystalline structure and formation of cellulose-II crystalline structure took place. During such metamorphosis, the NaOH solution penetrates the amorphous regions interspaced between the crystalline regions which results in the formation of Na cellulose-I with anti-parallel chains, effecting a gradual reduction of cellulose-I crystallinity which then leads to the formation of Na cellulose-I crystallites. Then, Na cellulose-I is able to absorb more alkali and is converted to Na cellulose-II which, after washing and drying, is converted to cellulose-II (El Oudiani, Chaabouni, Msahli, & Sakli, 2010). Thus, cellulose-II crystalline form is regenerated from amorphous cellulose. During this regeneration, small amounts of cellulose-I also is regenerated (Yokota, 1985).

Table 2 summarizes the degrees of crystallinity (X) and the cellulose-II crystalline fraction in the sample (X_{II}) for carboxymethyl cellulose, AQUASORB A500 and cotton linter pulp. Cotton linter pulp has a high degree of crystallinity (0.7), which also was apparent from the high intensity of the peak at 22.4° in the diffractogram (Figure 2), and no cellulose-II crystalline aggregation. AQUASORB A500 has a degree of crystallinity and fraction of cellulose-II crystalline aggregate of 0.37 and 0.12, respectively. Carboxymethyl cellulose prepared using reactive extrusion had degrees of crystallinity ranging from 0.18 to 0.51 and cellulose-II crystalline fraction ranging from 0.14 to 0.36. Carboxymethyl cellulose prepared with 100% H₂O seemed to have the highest degree of crystallinity and cellulose-II crystalline fraction, ranging from 0.43 to 0.51, and 0.27 to
Kumar, Luz Reus-Medina, and Yang (2002), in the process of modification of the crystalline structure of microcrystalline cellulose using NaOH concentrations (5N, 7.5N and 10N) similar to those used for 100% H₂O in this study, obtained products with similar degrees of crystallinity (46.99%, 49.3% and 47.58%) after 4 h of treatment.

The average degrees of crystallinity and cellulose-II fraction at 70% H₂O (0.36 and 0.18), 50% H₂O (0.25 and 0.19), 30% H₂O (0.26 and 0.21) and 10% H₂O (0.38 and 0.21) were less than that for 100% H₂O (0.47 and 0.31). Mansikkamäki, Lahtinen, and Rissanen (2007) found that for wet mass mercerization (aqueous ethanol:cellulose ratio = 6, v/w basis), the degree of mercerization (C-II % in crystalline fraction) decreased as the concentration of ethanol increased because of the reduced solubility of NaOH at the high alcohol concentrations. In this study too, at high alcohol concentrations, 2-phase solutions and insoluble NaOH were observed which may have resulted in lower crystallinity at higher alcohol concentrations.

Mansikkamäki et al. (2007) also found that the degree of mercerization increased with increasing NaOH concentrations. In this study too, the average degree of crystallinity increased slightly with increasing amounts of NaOH from 30g NaOH (0.32) to 40g NaOH (0.35) and 50g NaOH (0.36).

3.3. Degree of Substitution
The degrees of substitutions of carboxymethyl cellulose are summarized in Table 3. Statistical analysis of the degree of substitution revealed a significant interaction between NaOH and H₂O (P=0.0308), suggesting that the effect of one experimental variable was
dependent on the level of the other. The effect of the amount of H$_2$O used was significant at all levels of NaOH ($P<0.0001$). The effect of NaOH was not significant when 70% H$_2$O was used ($P=0.4725$) but was significant for all other levels of H$_2$O.

When the amount of NaOH was increased from 30g to 40g there was a significant decrease in the DS at 100% and 30% H$_2$O, and when the NaOH was further increased from 40g to 50g there was a significant decrease in DS at 50% and 10% H$_2$O. At all other levels of H$_2$O there were no significant differences. The decrease in DS with an increase in NaOH concentration, above a critical NaOH concentration, was reported by Barai et al. (1997) and Heinze and Pfeiffer (1999). The effect of NaOH concentration on the DS may have been due to the predomination of the side reaction which results in the formation of sodium glycolate, over the carboxymethylation reaction, at high NaOH concentrations (Barai et al., 1997).

The H$_2$O/ethanol compositions also affected the DS. When the H$_2$O content decreased from 100% to 70%, the increase in DS was found to be significant when 40g ($P=0.0014$) and 50g ($P=0.0484$) of NaOH were used but not when 30g NaOH were used ($P=0.3276$). With a further decrease in H$_2$O content from 70% to 50%, the DS again increased which was found to be significant when 30g ($P=0.0484$) and 40g ($P=0.0097$) of NaOH were used but not when 50g of NaOH were used ($P=0.7780$). With further decrease in H$_2$O content from 50% H$_2$O to 30% H$_2$O, the DS decreased at 40g NaOH ($P=0.0008$) but no difference was found at 30g ($P=0.7780$) and 50g ($P=0.0748$) NaOH. Finally, when H$_2$O content was decreased from 30% H$_2$O to 10% H$_2$O, the DS decreased for all values of NaOH ($P<0.0001$). The DS at 30% H$_2$O was higher than that at 100% H$_2$O and was
significant at 30g (0.0024) and 40g (0.0161) NaOH. The DS at 10% H\textsubscript{2}O was lower than that at 100% H\textsubscript{2}O at all levels of NaOH (\textit{P}<0.0001).

The DS at 70%, 50% and 30% H\textsubscript{2}O, in general, was found to be higher than that at 100% H\textsubscript{2}O. This may have been a result of the lower degrees of crystallinities and fractions of cellulose-II crystalline forms at 70% (0.36 and 0.18), 50% (0.25 and 0.19) and 30% (0.26 and 0.21) H\textsubscript{2}O as compared to 100% H\textsubscript{2}O (0.47 and 0.31). Cellulose-II crystalline form, especially, is compact and difficult for the reactants to penetrate, resulting in a lower rate of carboxymethylation (Zhang et al., 1993). However, despite the lower degree of crystallinity and cellulose-II crystalline fraction at 10% H\textsubscript{2}O (0.38 and 0.26), its DS was not higher than that at 100% H\textsubscript{2}O. The presence of ethanol also may have contributed to the higher DS by reducing the water binding tendency of carboxymethyl cellulose and hence facilitating better reactant penetration. The highest DS (0.73) was obtained at a 50% H\textsubscript{2}O concentration when 40g of NaOH was used, at reaction efficiency (RE) of 26.3%.

3.4. Liquid Uptake

The rates of liquid uptake for carboxymethyl cellulose, AQUASORB A500 and sodium polyacrylate are illustrated in Figures 4 and 5. The liquid uptake for carboxymethyl cellulose prepared with 100% H\textsubscript{2}O seemed to be higher in rate and extent than those prepared with 70, 50, 30 and 10% H\textsubscript{2}O and AQUASORB A500. However, statistical analysis revealed that there were no significant difference between the extents of liquid uptake for carboxymethyl cellulose prepared with 30g of NaOH + 100%H\textsubscript{2}O and 50g of NaOH + 10% H\textsubscript{2}O and 30g of NaOH + 70%H\textsubscript{2}O at intervals of 1 min (\textit{P}=0.9358,
\( P = 0.4696 \) and 5 min \( (P = 0.1599, P = 0.1378) \) and also between 30g of NaOH +100%H\(_2\)O and 40g of NaOH +70%H\(_2\)O, 50g of NaOH + 70%H\(_2\)O and AQUASORB A500 at a time period of 1 min \( (P = 0.2621, P = 0.2621, P = 0.1083) \). However, the extents of liquid uptake of carboxymethyl cellulose prepared with 40g and 50g NaOH +100% H\(_2\)O was found to be statistically higher than those prepared with 70, 50, 30 and 10% H\(_2\)O and AQUASORB A500 at all time periods.

The liquid uptakes for 50g of NaOH + 100%H\(_2\)O, at intervals of 1, 5 and 10 min, were statistically higher than those for 40g of NaOH + 100%H\(_2\)O \( (P = 0.0224, p < 0.0001, P = 0.0002) \), which in turn were higher than those for 30g of NaOH + 100%H\(_2\)O \( (P = 0.0004, p < 0.0001, p < 0.0001) \). The highest rate and extent of liquid uptake, for carboxymethyl cellulose, was obtained at 50g NaOH + 100%H\(_2\)O with absorption of 4.2 ml (21 ml/g) 1% NaCl solution after 15 min. However, that was found to be lower than the extent of water uptake of sodium polyacrylate, a synthetic superabsorbent, at 5 \( (P < 0.0001) \), 10 \( (P < 0.0001) \) and 15 min \( (P < 0.0001) \), while no differences were found at 1 min \( (P = 0.3494) \). Sodium polyacrylate had an impressive rate and extent of water absorption with an absorption capacity of 5.7 ml (28.5 ml/g) of 1% NaCl after 15 min.

During the early stages of liquid uptake, carboxymethyl cellulose, prepared with 70%, 50% and 30% H\(_2\)O, began to form a viscous mass after absorbing water which acted as a barrier between the filter plate and some of the still dry, powder mass. Even after large time intervals (~10 min), a significant amount of carboxymethyl cellulose powder remained dry because of this viscous barrier. This phenomenon, called gel blocking, significantly limited the liquid uptake for carboxymethyl cellulose prepared at these
conditions. Carboxymethyl cellulose prepared using 10% H₂O did not exhibit gel blocking, but had low rates and extents of water absorptions due to low absorption power, likely to have resulted from the lower DS. The higher rate and extent of water uptake by carboxymethyl cellulose, prepared with 100% H₂O, may have been because of its higher degrees of crystallinity. Cellulose crystallites are rendered insoluble by the presence of hydrogen bonds. This may have contributed in preventing the formation of a viscous barrier, which permitted a rapid rate of water uptake.

3.5. Water Absorption
The water absorption capacities for carboxymethyl cellulose, AQUASORB A500 and sodium polyacrylate are presented in Table 3. Statistical analyses revealed significant effects of the H₂O/ethanol ratio on the water absorption before and after centrifugation \((P<0.0001)\). No difference were found in the absorption using 100% H₂O and 50% H₂O \((P=0.0702)\) and between 50% H₂O and 30% H₂O \((P=0.3740)\) before centrifugation and between the use of 100% H₂O and 70% H₂O \((P=0.0904)\) and again between 50% H₂O and 30% H₂O \((P=0.3416)\) after centrifugation. The highest absorption before centrifugation was obtained by carboxymethyl cellulose prepared with 30g of NaOH + 70% H₂O (15.5 ml), however, after centrifugation, carboxymethyl cellulose prepared with 50g of NaOH + 70% H₂O had the highest absorption (12.3 ml). The highest retention was obtained at 50g NaOH + 50%H₂O (98%). AQUASORB and sodium polyacrylate had water absorption capacities of 8 ml and 9.8 ml after centrifugation with water retention capacities of 61.5% and 97.5%, respectively.
3.6. Carboxymethyl cellulose microstructures

Figures 6 and 7 illustrate the microstructures of carboxymethyl cellulose, at magnifications of 120X and 900X, prepared with 10% H₂O and 100% H₂O respectively, at all levels of NaOH. From the microstructures of carboxymethyl cellulose prepared using 10% H₂O, at 120X magnification, fibers 100µm to >800µm in length and 0.8-2µm in breadth are observed for 50g NaOH (Figure 6C), ≤375µm in length and 1.7-3.3µm in breadth for 40g NaOH (Figure 6B) and ≤440µm in length and 1.75-3.75µm in breadth for 30g NaOH (Figure 6A) were observed. For 30g and 40g of NaOH, after purification, extrudates were ground to pass through a 70 mesh screen, whereas for 50g NaOH, because of the difficulty in size reduction, the fraction retained on 70 mesh also was used which explains the longer fiber length. On the other hand, the lower thickness of fibers at 50g NaOH may have been because of its lower DS (0.1). The microstructure images at 900X magnification reveal some peeling and pitting of the carboxymethyl cellulose surfaces which may have occurred due to mechanical damage during extrusion or during grinding after product purification. No differences were observed found between the microstructures of carboxymethyl cellulosics prepared using 30g, 40g and 50g of NaOH.

Microstructure images of carboxymethyl cellulose, prepared with 100% H₂O (Figure 7), at 120X magnification exhibited fibers with dimensions of ≤330µm and 1.25–2.5µm (Figure 7C), ≤430µm and 1.6-3.3µm (Figure 7B), and 670µm and 1.6-3.3µm (Figure 7A) at 50g, 40g and 30g of NaOH, respectively. These images also showed some non fibrous particles, 0.8-6.7µm in dimensions, likely to have formed during extrudate grinding, after purification. Images at 900X magnification revealed fractured and rough fiber surfaces, similar to those observed in Figure 6. No differences were observed between the
microstructures of carboxymethyl cellulose prepared with 30, 40 and 50g of NaOH at 100% H₂O.

4. Conclusion

Reactive extrusion presents a continuous, reduced solvent, convenient and fast process for carboxymethylation of cellulose. The reaction time for the reactive extrusion process was less than 2 min. The water:ethanol ratios and NaOH concentrations were found to have significant effects on the DS and crystallinity which effected the liquid uptake properties of carboxymethyl cellulose. Carboxymethyl cellulose prepared using 100% H₂O, as revealed by x-ray diffraction analysis, had higher degree of crystallinity and fraction of cellulose-II crystalline content than those at lower H₂O contents. The higher crystalline content may have been responsible for the lower DS when 100% H₂O was used as compared to those prepared with lower water contents. The higher rate of liquid-uptake, also, may have resulted from the higher degree of crystallinity. The rate of liquid uptake of carboxymethyl cellulose prepared using 100% H₂O was significantly higher than that of AQUASORB A500. The rapid liquid uptake properties of such carboxymethyl cellulose render them ideal for applications as a super absorbent.
References


Table 1. Elaboration of screw profile

<table>
<thead>
<tr>
<th>No. of screw elements (from die end)</th>
<th>Screw Type</th>
<th>Length of each element (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Compression</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Conveying</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>Conveying</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>Kneading</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Conveying</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>Kneading</td>
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<tr>
<td>1</td>
<td>Conveying</td>
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<tr>
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<td>Kneading</td>
<td>20</td>
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<tr>
<td>6</td>
<td>Conveying</td>
<td>30</td>
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</table>
Table 2. Degree of substitutions, reaction efficiencies, degree of crystallinity and cellulose-II crystalline fraction for carboxymethyl cellulose, AQUASORB A500 and cotton linter pulp.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DS</th>
<th>RE (%)</th>
<th>X</th>
<th>X_{II}</th>
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<tr>
<td>30g NaOH and 100%H₂O</td>
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<td>20.12</td>
<td>0.43</td>
<td>0.27</td>
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<td>40g NaOH and 100%H₂O</td>
<td>0.43</td>
<td>15.61</td>
<td>0.48</td>
<td>0.36</td>
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<tr>
<td>50g NaOH and 100%H₂O</td>
<td>0.46</td>
<td>16.37</td>
<td>0.51</td>
<td>0.30</td>
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<tr>
<td>30g NaOH and 70%H₂O</td>
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<td>21.69</td>
<td>0.36</td>
<td>0.15</td>
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<tr>
<td>40g NaOH and 70%H₂O</td>
<td>0.60</td>
<td>21.57</td>
<td>0.36</td>
<td>0.18</td>
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<tr>
<td>50g NaOH and 70%H₂O</td>
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<td>19.94</td>
<td>0.35</td>
<td>0.20</td>
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<td>30g NaOH and 50%H₂O</td>
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<td>25.29</td>
<td>0.20</td>
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<tr>
<td>40g NaOH and 50%H₂O</td>
<td>0.73</td>
<td>26.27</td>
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<td>0.23</td>
</tr>
<tr>
<td>50g NaOH and 50%H₂O</td>
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<td>20.31</td>
<td>0.27</td>
<td>0.19</td>
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<tr>
<td>30g NaOH and 30%H₂O</td>
<td>0.72</td>
<td>25.77</td>
<td>0.18</td>
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<td>40g NaOH and 30%H₂O</td>
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<td>19.93</td>
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<td>50g NaOH and 30%H₂O</td>
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<td>17.29</td>
<td>0.33</td>
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<td>30g NaOH and 10%H₂O</td>
<td>0.30</td>
<td>10.64</td>
<td>0.43</td>
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<td>40g NaOH and 10%H₂O</td>
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<td>7.84</td>
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<td>50g NaOH and 10%H₂O</td>
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<td>3.46</td>
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<tr>
<td>AQUASORB A500</td>
<td>0.70</td>
<td>-</td>
<td>0.37</td>
<td>0.12</td>
</tr>
<tr>
<td>Cotton Linter Pulp</td>
<td>-</td>
<td>-</td>
<td>0.70</td>
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Reaction conditions: 30g cellulose, 60g SMCA and 130g aqueous ethanol/H₂O
Table 3. Water Absorption using flood and centrifuge methods for carboxymethyl cellulose, AQUASORB A500 and sodium polyacrylate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorption (ml)</th>
<th>Absorption after centrifugation (ml)</th>
<th>Retention (%)</th>
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<td>8.3</td>
<td>89.3</td>
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<td>40g NAOH and 100%H₂O</td>
<td>9.8</td>
<td>8.7</td>
<td>88.1</td>
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<tr>
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<td>89.0</td>
</tr>
<tr>
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<td>9.0</td>
<td>58.1</td>
</tr>
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<td>13.8</td>
<td>8.5</td>
<td>61.8</td>
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<td>12.3</td>
<td>90.2</td>
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<td>6.2</td>
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<td>92.9</td>
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<td>1.0</td>
<td>37.5</td>
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<td>AQUASORB A500</td>
<td>13.0</td>
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<td>61.5</td>
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<td>Sodium polyacrylate</td>
<td>10.0</td>
<td>9.8</td>
<td>97.5</td>
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Figure 1. FTIR spectra of carboxymethyl celluloses.
Figure 2. X-ray diffraction pattern for cotton linter pulp
Figure 3. X-ray diffraction patterns of carboxymethyl cellulose and AQUASORB A500.
Figure 4. Water uptake measurements for carboxymethyl celluloses prepared using 100% H2O, AQUASORB A500 and sodium polyacrylate.
Figure 5. Water uptake measurements for carboxymethyl celluloses prepared using 10%, 30%, 50% and 70% H2O.
Figure 6. Scanning electron micrograph images (120X and 900X magnification) of carboxymethyl cellulosates prepared using 10% H2O and 30g NaOH (A & D), 40g NaOH (B & E) and 50g NaOH (C & F).
Figure 7. Scanning electron micrograph images (120X and 900X magnification) of carboxymethyl celluloses prepared using 100% H2O and 30g NaOH (A & D), 40g NaOH (B & E) and 50g NaOH (C & F)
CHAPTER VI

ACETYLATION OF CELLULOSE USING SOLVENT-LESS REACTIVE EXTRUSION WITH IODINE CATALYST

This research paper is in review:

ACETYLATION OF CELLULOSE USING SOLVENT-LESS REACTIVE
EXTRUSION WITH IODINE CATALYST

Abstract

Cellulose acetate was prepared using a reactive extrusion process without the use of a solvent. Iodine was used as the catalyst for the acetylation reaction. The acetylation of cellulose was confirmed by Fourier-transform infrared spectroscopy. The effects of the acetic anhydride:cellulose ratio (4, 5.25 and 6 v/w) and iodine concentration (0.25, 0.5, 1, 2 and 3 weight% of acetic anhydride) on the physical, chemical and morphological properties of cellulose acetate were investigated using a 5×3 blocked factorial design. Statistical analyses failed to detect any significant interaction in the effects of acetic anhydride:cellulose ratio and iodine concentration on the degree of substitution. The effects of acetic anhydride:cellulose ratio and iodine concentration on the degree of substitution were significant (P=0.0001). The maximum degree of substitution of 1.41 was observed when an acetic anhydride:cellulose ratio of 5.25 (v/w) and 3% iodine concentration were used, at a reaction efficiency of 15.7%. X-ray diffraction analyses revealed CTA-II crystalline forms at iodine concentrations of 2 and 3%. This may have been due to homogeneous acetylation at high iodine concentrations. $^1$H-NMR spectroscopy revealed the presence of carbohydrate oligomers. These oligomers were observed even at iodine concentrations as low as, 0.25%. Scanning electron microscopy studies revealed the presence of fibers, 11-30 µm in thickness, with partially damaged
surfaces. Fiber coiling and agglomeration to form granular-like materials also were observed.

**Keywords:** Cellulose acetate, iodine catalyst, reactive extrusion, scanning electron microscopy, x-ray diffraction.
1. Introduction

Cellulose is a renewable natural polymer with the largest annual production (by volume), and is used as a starting material for various products with miscellaneous applications.¹ Cellulose acetate is an organic acid ester of cellulose which finds applications in textiles, cigarette filters, photographic, motion picture and audiotape films, sheet and molded objects, surface coatings and ink and membranes for separation processes, with a total production of 1.5 billion pounds per annum. It was first prepared by Schutzenberger in 1865 by reacting cellulose with acetic anhydride in a sealed tube at 180°C.² Cellulose acetate has been prepared traditionally, in a heterogeneous process, by reacting cellulose with acetic anhydride in the presence of acetic acid and a catalyst.

\[
R-(OH)_3 + 3(CH_3CO)_2O \rightarrow R(OOC.CH_3)_3 + 3CH_3COOH
\]

Before the reaction, cellulose is pretreated with glacial or dilute acetic acid at temperatures below 50°C, with or without a catalyst, typically using about 1:7 ratio of cellulose to acetic acid.² The pretreated product is then suspended in a mixture of acetic anhydride, acetic acid and a catalyst (sulfuric acid being the most popular catalyst). Typically, 1 part of the cellulose to about 3 parts of 85% acetic anhydride and 0.07 parts of sulfuric acid are used for acetylation for a total reaction time of 90 min.² The cellulose triacetate thus formed is then partially hydrolyzed at temperatures between 50-80 °C for several hours to get cellulose acetate with a slightly lower DS.³

In recent times, several new techniques for the acetylation of cellulose have been developed. Yan et al.⁴ prepared cellulose acetate with a ball-mill reactor in a solvent-free process using \(\text{SO}_4^{2-}/\text{ZrO}_2\) solid superacid catalysts. Researchers have used homogeneous
processes for acetylation of cellulose using various solvents, acetylation agents and catalysts, which have been listed in Table 1.

More recently, iodine has been found to be an efficient catalyst for several reactions\textsuperscript{14-16}, including the acetylation of alcohols, amines, phenols and anilines\textsuperscript{17-19} under solvent-less conditions. Biswas et al.\textsuperscript{20-22} applied the iodine catalyzed, solvent-less acetylation process to poly-alcohols such as starch and cellulose. Researchers from the same group also studied the iodine catalyzed esterification of agriculture byproducts and wastes such as cottonseed hulls and cotton burrs.\textsuperscript{23, 24} Li et al.\textsuperscript{25} studied the iodine catalyzed, solvent free acetylation of cellulose using microwave heating. For these reactions, a glass vial and block heater combination, equipped with magnetic stirrer, were used as a reactor. However, while reacting larger quantities of fibrous cellulose in a solvent-less system, mixing of the reactants and efficient heat transfer is a problem.

The objective of this study was to explore the acetylation of cellulose using reactive extrusion. The effects of the reaction stoichiometry on the physicochemical properties of the cellulose acetate prepared using reactive extrusion were studied using acetic anhydride (AA):cellulose ratio and the iodine concentration as the experimental variables. The use of iodine as a catalyst was well suited for the reactive extrusion because of the elimination of additional solvents. The elimination of solvents also resulted in higher reactant concentrations in the extruder. In a twin screw extruder very good mixing and heat transfer to the reactants can be achieved.

For a long time, reactive extrusion has been an extremely popular technique for chemical modification of starches.\textsuperscript{26} However, it also has been used in the past decades, in some
processes, for chemical modifications of cellulose such as saccharification\textsuperscript{27,28}, partial hydrolysis using mineral acids\textsuperscript{29} and hydrogen peroxide\textsuperscript{30,27}, surface esterification using maleated polyolefins\textsuperscript{31}, carboxymethylation\textsuperscript{32} and alkaline pretreatment of ligno-cellulosic materials for enzymatic hydrolysis.\textsuperscript{33}

2. Experimental

2.1. Extrusion
Cotton linter pulp sheets (Grade 1ARY, DP\textsubscript{w}=2934, Buckeye Technologies Inc., Memphis, Tennessee) were cut into 15×15 mm pieces using a sheet cutter and shredded into a fluff using a blender (model 31BL92, Waring Products Limited, New Hartford, Connecticut). Acetic anhydride (99.7\%, Fisher Scientific; Pittsburg, Pennsylvania), with iodine (99.5\%, resublimed, Acros Organics, New Jersey) dissolved in it, was added to the cellulose and then shredded in a blender. A co-rotating twin-screw extruder (model TSE-20, Brabender Technologies Inc., South Hackensack, New Jersey), having four heating zones, was used as the reactor for the acetylation of cellulose. The reaction formulation was hand fed to the second zone. The temperature profile for the extruder was set at 160-160-160°C. The extruder screw configuration consisted of two kneading blocks and nine compression screw sections is illustrated in Figure 1. Also, the fibrous nature of the cellulose necessitated that the extruder be used without a die. The extrudates were collected, ground using a grinder (model F203, Krups, USA) and washed with ethanol (Decon Labs, King of Prussia, Pennsylvania) (1:20 w/v ratio) for 5 h. and repeated five times. The purified extrudates were then dried in a vacuum oven.
2.2. Degree of Substitution
The degree of substitution of cellulose acetate was determined using the procedure described by Green et al.\textsuperscript{34} with slight modifications. Cellulose acetate was dried overnight, in a vacuum oven, at 50°C to remove any moisture present. In a 250 ml Erlenmeyer flask, 0.5 g cellulose acetate was accurately added and suspended in 20 mL of 75\% aqueous ethanol solution. The flasks were heated in a shaking water bath at 50-60°C for 30 min. Then, 20 mL of 0.5N NaOH solution was accurately added to the flask and heated again in a shaking water bath at 50-60°C for 15 min. The flask was stoppered and allowed to stand at room temperature for 48 h, after which the excess alkali was back titrated using 0.5N sulfuric acid. Reagent blanks also were prepared and a similar procedure was followed for them. Each measurement was replicated once. The \% acetyl content was then calculated as,

\[
\% \text{ Acetyl} = \frac{[(A-B) N_b - (C-D) N_a] \times 4.3}{W},
\]

where:

A = amount of NaOH solution added to the sample (mL),
B = amount of NaOH solution added to the blank (mL),
\(N_b\) = normality of NaOH solution,
C = amount of sulfuric acid solution added to the sample (mL),
D = amount of sulfuric acid solution added to the blank (mL),
\(N_a\) = normality of sulfuric acid solution, and
W = weight of sample (g).

The degree of substitution was determined as,
DS = \((3.86 \times \% \text{ acetyl}) / (102.4 - \% \text{ acetyl})\).

2.3. Fourier-transform infrared spectroscopy
Fourier-transform infrared (FTIR) spectra for cellulose acetate, in the 650 cm\(^{-1}\) to 4000 cm\(^{-1}\) region, were obtained using a Thermo Nicolet Avatar 360 FT-IR E.S.P. spectrometer. Sixteen scans were obtained per image with a resolution of 4 cm\(^{-1}\).

2.4. Scanning electron microscopy
Microstructures of cellulose acetate were determined using a variable pressure scanning electron microscope (Model S-3000N, Hitachi High Technologies America Inc., San Jose, California) as described by Bhandari et al.\(^{35}\)

2.5. X-ray Diffraction
X-ray diffractograms, used to study the crystalline structures of cellulose acetate and cotton linter pulp, were obtained using Rigaku D/Max-B diffractometer (Rigaku Americas, TheWoodlands, Texas) as described by Bhandari et al.\(^{36}\) Diffraction patterns were obtained for a diffraction angle (2\(\theta\)) range of 5-35° at a scanning rate of 4°/min. and step size of 0.02°.

2.6. H-NMR
The H-NMR spectra were acquired using a Bruker Avance 400 (Billerica, MA) spectrometer operated at a 400 MHz magnetic field. DMSO-\(d_6\) was used as a solvent for cellulose acetate. Spectra were obtained at 100°C using 512 scans for each sample.
2.7. Experimental design and data analyses
Fifteen treatment combinations (5 levels of iodine concentration × 3 levels of AA:cellulose ratio) were applied to the acetylation of cellulose using reactive extrusion. These treatment combinations were arranged using a factorial design, blocked and then replicated twice. The data on the degrees of substitution was analyzed using ‘proc mixed’ procedure of SAS version 9.1 (SAS Institute Inc., Cary, North Carolina). A significance level of α≤0.05 was used.

3. Results and Discussion

3.1. Fourier-transform infrared spectroscopy
The FTIR spectra of cellulose acetate with degrees of substitution 0.42, 0.89, 0.92 and 1.32, in the wave numbers of 650-4000 cm\(^{-1}\), are summarized in Figure 2. The region 3000-3600 cm\(^{-1}\) corresponds to stretching of hydroxyl groups, 1650-1800 cm\(^{-1}\) corresponds to C=O and 1200-1350 cm\(^{-1}\) corresponds to the methyl from the acetyl group.\(^{21}\) The peaks observed at 1250 cm\(^{-1}\) and 1750 cm\(^{-1}\) confirm the acetylation of cellulose.

3.2. Degree of Substitution
Table 2 summarizes the effects of the AA:cellulose ratios and iodine concentrations on the average DS and reaction efficiency (RE) of cellulose acetate. RE is defined as the mole percentage of acetic anhydride charged that reacts to form cellulose acetate. The maximum DS (1.41) was obtained, at a reaction efficiency of 15.7%, when an AA:cellulose ratio of 5.25 v/w and a 3% iodine concentration were used. The maximum reaction efficiency of 17.3% was obtained when a AA:cellulose ratio of 4 v/w and a 3%
iodine concentration were used. Statistical analysis revealed that the effects of the AA:cellulose ratio \((P<0.0001)\) and iodine concentration \((P<0.0001)\), on the DS, were significant and also that there was no significant interaction between the AA:cellulose ratio and iodine concentration \((P=0.2945)\). The DS obtained when AA:cellulose ratios of 5.25 and 6 v/w were used, were significantly higher than when a AA:cellulose ratio of 4 v/w was used \((P=0.0005\) and \(P<0.0001\) respectively). However, no statistical difference could be found between the DS obtained for AA:cellulose ratios of 5.25 and 6 v/w \((P=0.2249)\). The fact that increasing the AA:cellulose ratio from 5.25 to 6 did not result in an increase in DS, indicated that time may have been a limiting factor in the reactive extrusion process. The DS also increased with an increase in the iodine concentration from 0.25% to 0.5% \((P<0.0001)\), 0.5% to 1% \((P<0.0001)\), 1% to 2% \((P=0.0003)\) and 2% to 3% \((P<0.0001)\). Hence, the reaction rate increased significantly with increasing iodine concentration.

Statistical analysis revealed a significant interaction between the effects of AA:cellulose ratio and iodine concentration on the reaction efficiency \((P=0.007)\). The effect of iodine on the reaction efficiency was significant at all AA:cellulose ratios \((P<0.0001)\). The effect of AA:cellulose ratio was significant at iodine concentrations of 0.25% \((P=0.0454)\), 1% \((P=0.0151)\), 2% \((P=0.0084)\) and 3% \((P=0.0002)\).

Biswas et al.\(^{21}\), using a AA:cellulose ratio of 3.07 (v/w), iodine concentration of 2.18 (w/v %) and a reaction time of 10 min at 100°C, obtained cellulose triacetate (DS~3) with a yield of almost 100%. The reaction was conducted in a 10 mL glass vile reactor with magnetic stirrers and without the use of any additional solvent. In comparison, in the
reactive extrusion process, the cellulose, acetic anhydride and iodine reaction formulations (~25°C) were fed to the extruder with barrel temperature of 160°C. The residence time in the extruder was less than 2 min. For a short residence time in the extruder, during which the temperature of the formulation gradually increased from the feed temperature, a DS of 1.4 was obtained which indicates that the reaction proceeded rapidly. The time-temperature profile for the reactant formulation in the extruder is not known, since it is difficult to measure the temperature of the formulation inside the extruder, especially so, for non-thermoplastic materials like cellulose, and also because of the mixing of the formulation in the extruder which results in residence time distribution. Increasing the residence time in the extruder could result in a higher DS and RE even at milder reaction conditions. This is because, while the thermodynamic parameters of the reaction (temperature, pressure, concentration and time) determine the rate of reaction in all reactors, there is an additional parameter affecting the rate of reaction in reactive extrusion. Because of the intimate contact between the reactants as it passes through the interstices between the screws and between the screw and barrel and the resulting shear forces, there is an increase in the frequency of contact between the reactant functional groups. This increases the pre-exponential constant in the Arrhenius equation and increases the reaction rate, even while the thermodynamic parameters remain constant.37

3.3. X-ray Diffraction
X-ray diffractograms, illustrating the effects of AA:cellulose ratios and iodine concentrations and the corresponding DS, on the crystalline structures of cellulose acetate are summarized in Figure 3. The diffractogram of cotton linter pulp (Figure 3a), exhibiting peaks at 20 angles of 14.7°, 16.1°, 22.4° and 34.2°, is characteristic of
cellulose I crystalline structure.\textsuperscript{38} In the diffractograms of cellulose acetates prepared at all conditions (Figure 3b-3o), the peak at 22.4° is retained and prominently displayed, indicating the preservation of cellulose I crystalline structure. However, the intensity of this peak decreases with an increase in DS. This indicates that the amorphous regions of cellulose were preferentially acetylated, however, as the DS increased there may have been some acetylation in the crystalline regions causing a reduction in crystallinity. The diffractogram of cellulose acetate prepared with an AA:cellulose ratio of 4.5 and 1% iodine concentration (DS=1.04, Figure 3h), illustrates the first evidence of peaks in the 2θ range of 5-10°. In the diffractograms of cellulose acetate prepared with 2% and 3% iodine concentrations (Figure 3k-3o), these peaks become prominent at 2θ angles of around 8.5° and 10.5°. These diffractograms also illustrate a peak at around 13.5° and another broad peak at 17°. According to the literature, cellulose triacetate exists in two polymorphic crystalline forms CTA I and CTA II.\textsuperscript{39} These crystalline forms have been observed previously to exist even at low DS (0.4).\textsuperscript{40} The diffractograms of cellulose acetate prepared in this study resembled that of CTA II crystalline form which had characteristic peaks at 8.4°, 10.4°, 13.4°, 16.3°, 16.7°, 18.6° and 23.4°.\textsuperscript{39} According to some researchers, CTA II crystalline form was obtained by homogeneous acetylation\textsuperscript{41} or high degree of swelling of cellulose I.\textsuperscript{42} Also, there was some evidence that CTA II could be obtained at high acetylating temperatures.\textsuperscript{43}

According to Biswas et al.\textsuperscript{22}, the acetylation reaction catalyzed by iodine is homogeneous when the iodine (I\textsubscript{2}) used approaches 5 mole\% of AGU. In this study, the molar amounts of I\textsubscript{2} exceeded 5% of that of AGU when 2% and 3% iodine concentrations in AA (w/v) were used. This explains that the prominence of the peaks at 2θ angles of 8.4°, 10.4°,
13.4° and the broad peak at 17° in the diffractogram, which correspond to the CTA II crystalline form, when iodine concentrations of 2% and 3% were used (Figures 3k-3o).

3.4. $^1H$-NMR Spectroscopy

$^1H$-NMR spectra for cellulose acetate prepared using AA:cellulose ratio of 6 and 3% iodine concentration (DS=1.49), AA:cellulose ratio of 5.25 and 1% iodine concentration (DS=1.14) and AA:cellulose ratio of 6 and 0.25% iodine concentration (DS=0.61) are illustrated in Figures 4, 5 and 6 respectively. All spectra had broad peaks, which may have been because of the poor solubility of cellulose acetate prepared using reactive extrusion in DMSO. However, the spectra of cellulose acetate prepared with AA:cellulose ratio of 6 and 3% iodine concentration had the least amount of noise. This may have been because of its slightly better solubility.

$^1H$-NMR spectra indicate the presence of carbohydrate oligomers which implies the reduction of cellulose molecular weight during the reaction. The peaks indicating the presence of carbohydrate oligomers have been indicated in the spectra and can observed around 4, 4.2, 4.75, 4.8-4.9, 5.25, 5.8 and 6.1 ppm. It is interesting to note that cellulose oligomers were observed even when a low iodine concentration (0.25%) was used. The multiple peaks in the $^1H$-NMR spectra in the range of 3.4-5.3 ppm were due to protons on the AGU.

3.5. Cellulose acetate microstructure

The microstructures of cellulose acetate prepared at various conditions are illustrated in Figure 7. Figures 7a&b illustrate the microstructure of cellulose acetate prepared using an AA:cellulose ratio of 4.5 (v/w) and a 0.25% iodine concentration at magnifications of
100x and 1000x respectively. The microstructure of cellulose acetate, at 100x magnification (Figure 7A), illustrates the presence of fibers 17-23 µm in thickness. However, the presence of fines, probably resulting from the grinding of cellulose acetate, also was observed. The microstructure at 1000x magnification (Figure 7B) indicates some damage to the fiber surface. Figures 7C&D illustrate the microstructure of cellulose acetate prepared using an AA:cellulose ratio of 6 (v/w) and a 1% iodine concentration at magnifications of 100x and 1000x respectively. The microstructures of cellulose acetate, at 100x magnification (Figure 7C), illustrates the presence of fibers 12-30 µm in thickness. The microstructure at 1000x magnification (Figure 7D) indicates that cellulose acetate had some damaged surfaces, especially the fiber ends, but also relatively smooth surfaces in other places. Figures 7E&F illustrate the microstructure of cellulose acetate prepared using an AA:cellulose ratio of 5.25 (v/w) and a 3% iodine concentration at magnifications of 200x and 1000x respectively. The microstructure images indicate the presence of fibers, 11-29 µm in thickness and relatively smooth surfaces. The microstructure in this case, however, also indicates agglomeration and coiling of some fibers to form a granular-like material in some cases, which was not observed in other microstructures.

4. Conclusion

Cellulose acetate was successfully prepared using a reactive extrusion process with an iodine catalyst. The maximum degree of substitution was 1.41. The effects of iodine catalyst and acetic anhydride:cellulose ratio on the degree of substitution were found to be significant without any significant interaction. A higher RE could be obtained by
increasing the residence time in the extruder. The presence of CTA-II crystalline forms were observed when high catalyst concentrations were used. Presence of carbohydrate oligomers also were observed, even at low catalyst concentration. Reactive extrusion presents a convenient, continuous and solvent-less process for preparing cellulose acetate.
References


33. C. Karunanithi, Muthukumarappan, K. Industrial Crops and Products 2011, 33, 188.


Table 1. Homogeneous acetylation: Solvents, Acetylating agents and catalysts.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Acetylating agent</th>
<th>Catalyst</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-allyl-3-methylimidazolium chloride</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>N,N-dimethylacetamide/LiCl</td>
<td>Acetyl chloride</td>
<td>Pyridine and cross-linked polyvinyl pyridine†</td>
<td>6, 7</td>
</tr>
<tr>
<td>Dimethyl sulfoxide / tetrabutylammonium fluoride trihydrate</td>
<td>Acetic anhydride and vinyl acetate</td>
<td>6, 8, 9</td>
<td></td>
</tr>
<tr>
<td>N-methyl-2-pyrrolidinone</td>
<td>Acetyl chloride</td>
<td>Crosslinked polyvinylpyridine</td>
<td>10</td>
</tr>
<tr>
<td>N-ethyl-pyridinium chloride</td>
<td>Acetic anhydride</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>N-methyl-morpholine-N-oxide</td>
<td>Vinyl acetate</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>1-3 dimethylimidazolidinone/LiCl</td>
<td>Acetic anhydride</td>
<td></td>
<td>13</td>
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</table>

†Reaction possible without catalyst
Table 2. Degree of substitution of cellulose acetate.

<table>
<thead>
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<th>AA:Cellulose (v/w)</th>
<th>Iodine (w/v %)</th>
<th>DS</th>
<th>RE (%)</th>
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<tbody>
<tr>
<td>4.5</td>
<td>0.25</td>
<td>0.29</td>
<td>3.8</td>
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<td>5.25</td>
<td>0.25</td>
<td>0.46</td>
<td>5.1</td>
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<tr>
<td>6</td>
<td>0.25</td>
<td>0.59</td>
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<tr>
<td>4.5</td>
<td>0.5</td>
<td>0.55</td>
<td>7.2</td>
</tr>
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<td>5.25</td>
<td>0.5</td>
<td>0.70</td>
<td>7.8</td>
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<td>0.5</td>
<td>0.82</td>
<td>7.9</td>
</tr>
<tr>
<td>4.5</td>
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<td>0.98</td>
<td>12.7</td>
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<tr>
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<td>11.7</td>
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<tr>
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<tr>
<td>6</td>
<td>3</td>
<td>1.39</td>
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</tr>
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</table>
Figure 1. Elaboration of screw profile. (Extruder temperature profile was 160-160-160°C).
Figure 2. FTIR spectra for cellulose acetate with different degree of substitutions.
Figure 3. X-ray diffractogram of cotton linter pulp (a) and cellulose acetate prepared using an iodine concentration of 0.25% (v/w) and AA:cellulose ratio (v/w) of 4.5 (b), 5.25 (c) and 6 (d), iodine concentration of 0.5% (v/w) and AA:cellulose ratio (v/w) of 4.5 (e), 5.25 (f) and 6 (g), iodine concentration of 1% (v/w) and AA:cellulose ratio (v/w) of 4.5 (h), 5.25 (i) and 6 (j), iodine concentration of 2% (v/w) and AA:cellulose ratio (v/w)
of 4.5 (k) and 6 (l) and iodine concentration of 3% (v/w) and AA:cellulose ratio (v/w) of 4.5 (m), 5.25 (n) and 6 (o).
Figure 4. 1H-NMR spectra of cellulose acetate prepared using AA:cellulose ratio of 6 and 3% iodine concentration (DS=1.49).
Figure 5. $^1$H-NMR spectra of cellulose acetate prepared using AA:cellulose ratio of 5.25 and 1% iodine concentration (DS=1.14).
Figure 6. $^1$H-NMR spectra of cellulose acetate prepared using AA:cellulose ratio of 6 and 0.25% iodine concentration (DS=0.61).
Figure 7. Microstructures of cellulose acetate prepared using an AA:cellulose ratio of 4.5 (v/w) and a 0.25% iodine concentration at magnifications of 100x (A) and 1000x (B), AA:cellulose ratio of 6 (v/w) and a 1% iodine concentration at magnifications of 100x 100x (C) and 1000x (D) and AA:cellulose ratio of 5.25 (v/w) and a 3% iodine concentration at magnifications of 200x (E) and 1000x (F).
CHAPTER VII

CONCLUSIONS

Reactive extrusion was used successfully for the preparation of carboxymethyl starch, carboxymethyl cellulose and cellulose acetate. Cross-linked carboxymethyl starch was prepared by limiting the gelatinization of starch. Such carboxymethyl starch had rapid swelling properties in an aqueous medium. The physical, chemical and morphological properties of such carboxymethyl starch were then compared with VIVASTAR®P.

Reactive extrusion also was used for reacting non-thermoplastic materials like cellulose. Carboxymethyl cellulose, with a high degree of crystallinity and cellulose-II crystalline fraction, was obtained by using 100% H₂O and high concentration of NaOH. Such carboxymethyl cellulose had water-uptake properties similar to those of commercial super absorbents. Reactive extrusion presents a simple, efficient, fast and solvent-less or reduced solvent process for the preparation of these products.
CHAPTER VIII

RECOMMENDATIONS FOR FUTURE STUDY

1. In this study, liquid uptakes (water and 0.1N HCl) by sodium starch glycolate prepared using reactive extrusion were studied. These uptakes gave an indication of the functional properties of sodium starch glycolate. However, it would be more useful to study the disintegration force and disintegration time and drug dissolution for solid dosage forms prepared using this sodium starch glycolate.

2. Carboxymethyl cellulose was prepared using reactive extrusion and its water absorption properties were characterized. However, no cross-linking agent was used. Cross-linking of carboxymethyl cellulose may result in better water absorption. This could be further investigated.

3. The reaction efficiency of acetylation of cellulose using reactive extrusion could be improved by increasing the residence time in the extruder, increasing the number of kneading blocks and with the use of a post-extrusion static mixer. With this, cellulose acetate with DS~3 could be obtained. The molecular weight of this cellulose acetate (DS~3 prepared with reactive extrusion) should be characterized and compared with that of commercial cellulose acetate.

4. Reactive extrusion of cellulose with gaseous reactants like ethyl chloride, methyl chloride, ethylene oxide and propylene oxide to prepare ethyl, methyl, hydroxyethyl and hydroxypropyl cellulose, respectively, could be investigated.