Effects of Salts on the Reaction of Normal Corn Starch with Propylene Oxide

The objective of this research was to develop a greater understanding of the mechanism by which salts inhibit gelatinization of starch granules. Using hydroxypropylation of corn starch in the presence of sodium sulfate under conditions employed industrially as the control reaction, reactions with propylene oxide were done with different salts at levels that matched the control reaction in anion molal concentration, cation molal concentration, and total ion molal concentration. Salts used were selected based upon their position in the lyotropic series. Reactions were characterized by determining reaction efficiency and ion concentrations. Sodium and potassium citrates, salts exhibiting a strong lyotropic effect, were extremely effective as gelatinization inhibitors, but resulted in low reaction efficiency. Salts without a strong lyotropic effect (sodium and potassium chlorides) exhibited a weak protective effect, probably at least in part based upon generation of a Donnan potential. In the hydroxypropylation reaction, sodium citrate greatly reduced swelling; sodium chloride was ineffective as a swelling inhibitor, resulting in pasting of granules, and sodium sulfate allowed restricted swelling, most likely as a result of a moderate lyotropic effect and some generation of a Donnan potential. That reaction efficiency parallels granule swelling was confirmed.

Keywords: Corn starch; Starch, derivatization of; Hydroxypropyl starch; Starch, modification of; Starch, swelling inhibitors of

1 Introduction

In the hydroxypropylation process, a salt is used to protect modified starch granules from chemical gelatinization. At the conclusion of the reaction, the mixture is neutralized; the modified granules are recovered by filtration or centrifugation, and the salt is removed by washing. Salt in the effluent waste has been the subject of concern in some municipalities. Sulfate, in particular, may be converted to hydrogen sulfide by bacteria in the waste water under anaerobic conditions. Under aerobic conditions, in which acids are produced, sulfate is converted into sulfuric acid. The purpose of this research was to understand the role of salt in starch modification reactions and possibly to identify a suitable replacement for sodium sulfate. The environmental problem of sulfate in waste water has also been recognized in the industrial purification of proteins [1]. In that industry, citrate salts were suggested as a biodegradable alternative [2], an approach investigated in this work.

1.1 Salt/starch interactions

The swelling or gelatinizing abilities of alkaline, acidic, and neutral salts on starch have long been known [3]. Sandstedt et al. [4] found that sulfate and phosphate salts increased the initial gelatinization temperature \( T_{gel} \) of wheat starch, whereas iodide and thiocyanate ions lowered it. They concluded from their data that salt solutions not only change the temperature at which gelatinization begins, but also the entire process of gelatinization. Ooster [5] examined the effect of sodium chloride on the initial gelatinization temperature of corn starch granules, with and without the addition of sodium hydroxide. His results showed that the initial gelatinization temperature increased in the presence of low concentrations of sodium hydroxide, but then the trend reversed, with sodium hydroxide destabilizing the granules as its concentration increased. Wootton and Bamunuarachchi [6] and Lii and Lee [7] recognized that salt effects were not only due to salt-water interactions, but to salt-starch interactions as well. Earlier, Evans and Haisman [8] had found that sodium sulfate increased \( T_{gel} \), whereas sodium and calcium chlorides increased \( T_{gel} \) at low concentrations, but decreased it at higher concentrations. Takahashi and Wada [9] concluded that the destabilizing effect (lowering of \( T_{gel} \)) of solutions of sodium bromide was due to a disordering of granule structure and that the granule-disordering effect of sodium bromide was completely
reversible. Chinachoti and Steinberg [10] determined that there was a strong interaction between starch and sodium chloride. Chinachoti et al. [11] found that, when water was present in amounts greater than the weight of starch, increasing concentrations of sodium chloride decreased the enthalpy of gelatinization.

Complexation of salts with glucans was investigated by Braudo [12], who concluded that glucans were not able to displace water molecules from the first coordination sphere of the cation. Oosten [13] proposed that the underlying mechanism behind electrolyte interactions with starch was based on the Donnan potential created by the weak dissociation of starch hydroxyl groups. Oosten [14] then proposed that the Donnan potential repels anions from the granule, effectively protecting the granule from gelatinization, since anions are hypothesized to induce gelatinization by rupturing hydrogen bonds.

Leach et al. [15] observed that granular starches sorb alkali from aqueous media. Oosten [14] showed that starch granules have weak ion-exchange properties and will partially neutralize and buffer an alkaline solution. Oosten [13] also found that isolated starch, in the sodium salt form, had a higher gelatinization temperature and increased water-holding capacity as compared to the corresponding native starch. This suggests that conversion of starch to its sodium salt increases granule stability. The sodium salt of starch dissociates to a greater degree than the starch hydroxyl groups, which suggests that an even greater Donnan potential is produced. Sulfate ions were proposed to penetrate the granule less than chloride ions due to increased repulsion forces on sulfate ions, which are more electronegative. Oosten [13] proposed that, at a critical concentration of alkali, the concentration gradient of the alkali dominates over the Donnan potential, allowing anions to enter the granule and effect chemical gelatinization.

Chinachoti et al. [16] concluded that creation of a Donnan potential is a plausible explanation for the protecting action of salts, but that it is probably limited and dependent upon salt concentration. Chinachoti et al. [17] mentioned that salt effects may be caused by several mechanisms, including both Donnan potential and lyotropic effects.1

Jane [19] found that sodium sulfate solutions effected an increased up-field 13C-NMR shift when compared to sodium chloride solutions matched at equivalent cation concentrations, a result attributed to the hydration of sulfate ions which enhanced dipole interactions as the amount of free water was reduced.

1 The lyotropic (Hofmeister) series is a relative ranking of ions based on their ability to precipitate colloids [18].

Mangels and Bailey [3] provided the first quantitative results on gelatinization of starch by salts at various concentrations. Wootton and Bamunuarachchi [6] described the effect of solutes on the gelatinization temperature of wheat starch as being caused by the ionic nature of the salt and its position in the lyotropic series. Sandstedt et al. [4] also related gelatinization patterns of wheat starch in the presence of salts to their lyotropic values. Gough and Pybus [20] studied effects of chlorides of 24 metal ions on swelling and gelatinization of A granules of wheat starch. For most, as salt concentration increased, \( T_{gel} \) first increased, then decreased, as was found for sodium chloride [6–8].

Takahashi et al. [21] were able to evaluate the magnitude of salt effects by determining the minimum concentration required to cause a change in \( T_{gel} \). Magnitudes were found to be related to lyotropic values and hydration number in a linear manner. When electrolytes were added to moistened potato starch, a heat-evolving adsorption occurred [22]. The heat generated by various treatments with potassium salts was measured and was found to follow the lyotropic series, with sulfate producing little evolved heat. Jane [23] found that salts affected the onset temperature and the enthalpy of gelatinization of starch granules and concluded that the effects of the anions seemed to parallel their water structure-making and -breaking effects [24].

In summary, there is evidence in the literature that both Donnan equilibria and lyotropic effects are plausible explanations for salt/starch interactions [6, 17] and that the effect(s) of salts appear to be governed, not by a single property, but by multiple properties of the solution [4].

1.2 Water sorption by starch

Water sorption and swelling of starch is a widely studied topic. Oosten [25] concluded that a reversible increase in volume of about 4–7% occurred when potato starch granules were suspended in 0.05 M sodium sulfate solution. The explanation for this phenomenon was substitution of the dissociating hydrogen ions of starch hydroxyl groups by larger volume cations. Evans and Haisman [8] found that starch granules slurred in sodium chloride solutions of <20% concentration sorbed more water as the salt concentration was increased; when using sodium sulfate, the opposite trend was observed.

1.3 Objectives

The objectives of this research were (1) to characterize interactions of salts with normal corn starch granules throughout hydroxypropylation to gain additional evi-
dence of the mechanism(s) which govern the ability of salts to inhibit gelatinization from among the several proposed and (2) to determine if a more biodegradable salt, viz., sodium citrate, could be used in the reaction. Normal corn starch was chosen for this study because of its large volume of use in hydroxyalkylated forms. Hydroxypropylation was chosen as the reaction to be studied because of ease of working with propylene oxide as compared with ethylene oxide and because it is the reaction used to make modified food starch. Determination of water and ion uptake by starch granules was used to characterize interactions. Reaction efficiency was used to determine how different salt systems affect hydroxypropylation. Correlations were made between granule swelling and reaction efficiency to determine if salts affect chemical modification by controlling the degree of granular swelling or by a more direct mechanism. Ion concentration and swelling results were evaluated in terms of known starch/salt interactions, viz., Donnan potential and lyotropic effects, to determine the presence of these interactions.

2 Experimental

2.1 Materials

Normal corn starch was obtained from Tate & Lyle North America Co., Decatur, IL. Propylene oxide was purchased from EM Science (Gibbstown, NJ). Sodium chloride, potassium chloride, potassium sulfate, potassium citrate monohydrate, and hydrochloric acid were purchased from Mallinckrodt, Paris, KY. Sodium sulfate was purchased from J. T. Baker, Phillipsburg, NJ. Sodium citrate dihydrate was purchased from Aldrich Chemical Co., Milwaukee, WI. Sodium hydroxide was purchased from Fisher Scientific, Fair Lawn, NJ. Blue dextran was purchased from Sigma Chemical Co., St. Louis, MO. Syringe filters (25-mm diam.) of supported acrylic copolymer were purchased from Gelman, Ann Arbor, MI.

2.2 Hydroxypropylation

The following general procedure was used for all hydroxypropylation reactions. Moisture content of the corn starch was determined using a vacuum oven (Standard Analytical Methods of the Member Companies of the Corn Refiners Association, Inc.). Reactions were conducted in 125-mL Erlenmeyer flasks equipped with Teflon stir bars and septums. Normal corn starch (36.0 g db; 0.222 mol glucosyl units) was dispersed into 70.2 g of distilled water. The appropriate quantity of each salt was then added.

The salt conditions of the reactions were done in three series termed “anion concentration constant”, “cation concentration constant”, and “total ion concentration constant”. The amounts of anion, cation, and total ions added were based on a control formulation which used 5.25 g of sodium sulfate. The anion constant series contained a molal quantity of anion equivalent to that of the control (0.527 m); the cation constant series matched molal cation concentrations, and the total ion concentration series matched the molal ion concentration of the control. Reactions with each salt system were done in duplicate. After the salt was dissolved, 5 mL of 7% sodium hydroxide solution was added dropwise and slowly with good agitation. Based on 70.2 g of water, the final concentrations were 51%, w/w starch, 0.5%, w/w (0.126 m) sodium hydroxide, and 0.527 m sodium sulfate (7.5% db, 17% Na₂SO₄·10 H₂O).

Propylene oxide (4.22 mL, 0.0604 mol) was then injected into the slurry in each flask, and the flasks were placed on a submersible multiple stir plate in a preheated 49°C (120°F) water bath. Aliquots of slurry (6 mL) were removed at 2 h, 16 h, and 24 h and centrifuged at 405 x g for 5 min. The supernatant was passed through a 5-μm syringe filter to remove any granular starch. The starch pellet was neutralized by resuspending it and adding 1 mL of 2.5% HCl. The mixture was again centrifuged. The pellet was frozen and stored at −20°C for later use.

2.3 Water sorption

All reactions were done in sealed Erlenmeyer flasks as described above with addition of an additional 1.0 mL of water. This procedure includes an 8-h equilibration step where samples were stirred at room temperature before injecting propylene oxide and immersing the flasks in a 49°C water bath. Only sodium salts (sodium sulfate, sodium chloride, and sodium citrate) were used, and in all cases were added in quantities so that the anion molal concentrations were equal, which means that equimolal concentrations of salts (0.527 m) were used. Each parameter examined was done in duplicate reaction flasks. A separate sealed flask was used for each data point and was opened only at the appropriate time.

At the time of sampling, the slurry (~ 25 mL) in each duplicate flask was poured into a 50-mL graduated centrifuge tube. The tube was immediately sealed tightly with a double layer of Parafilm M and placed upright within the 49°C water bath. After 2 h of sedimentation by gravity, the volume of starch bed and total volume were recorded. Sedimentation values are reported as (bed volume/total volume) × 100.
Immediately after sampling, ~5 mL of slurry was filtered using a Büchner funnel containing glass fiber filter paper, and the cake was washed thoroughly with dd water. Washed starch filter cakes were transferred to Eppendorf tubes and stored at −20°C until subjected to molar substitution (MS) analysis.

2.4 Ion concentration

Sodium and potassium ion concentrations were determined by inductively coupled plasma (ICP) spectroscopy using a Perkin Elmer Plasma 400 ICP/AES instrument (Norwalk, CT) with a model AS 90 autosampler. Samples of the filtered supernatant were diluted to the appropriate sensitivity range and processed without digestion. Citrate concentration was determined using a citric acid analysis kit obtained from Boehringer Mannheim (Indianapolis, IN). Chloride was determined according to AOAC method 973.51 for chloride determination in water (AOAC Official Methods of Analysis, 1995). Appropriate dilutions were made as necessary. Sulfate was determined by the turbidometric method for sulfate determination in water (973.57, AOAC Official Methods of Analysis, 1995). A spectrophotometer (Beckman, Spectronic 20) was used in place of a nephelometer, and appropriate dilutions were made. Each sample was assayed in duplicate.

2.5 Molar substitution

Two procedures were used to determine MS values. To determine the data in Tab. 2, a frozen starch pellet was thawed at room temperature. The starch was collected in a Büchner funnel equipped with a glass-fiber filter disc and washed thoroughly with distilled water. The filter cake (about 0.3 g) was placed in a 1000-mL round-bottom flask. Trifluoroacetic acid (TFA; 1.5 M, 50 mL) was added to the flask, which was then heated 4 h at 80°C with stirring. (Wootton et al. [26] reported complete hydrolysis of starch with 1.5 M TFA at 100°C in 4 h, but complete hydrolysis is not necessary; only viscosity reduction is.) TFA was removed by evaporation at 80°C under reduced pressure until a thick syrup was obtained. Residual TFA was removed by placing the flask in an evacuated desiccator over sodium hydroxide pellets overnight at room temperature.

The hydrolyzate in the round-bottom flask was dissolved in distilled water, and the solution was transferred to vials and freeze-dried. Samples (40 mg) were then exchanged with D2O twice and again freeze-dried. Just prior to NMR analysis, the deuterated sample was dissolved in D2O (0.0169 mL) was added to ensure complete solubility. The samples were analyzed by 1H NMR at 300 MHz (QE 300 spectrometer, General Electric Co.).

To determine the data in Tab. 3, an improved method was used. Frozen starch samples (50–60 mg) were weighed into Teflon-lined screw-capped vials. Acetate buffer (5.0 mL, 150 mmol, pH 6.9) was added to each vial, which was then heated 4 h at 80°C to gelatinize the starch. After cooling the starch solutions to room temperature, 1.0 mL of the acetate buffer containing α-amyrase (final conc. 0.5 unit/mL; EC 3.2.2.1, from Bacillus sp. (A-6814), Sigma Chemical Co., St. Louis, MO) was added to each vial. (One unit liberates reducing power equivalent to 1.0 mg of maltose from starch in 3 min at pH 6.9 and 20°C.) After digesting samples overnight, they were freeze dried. Freeze-dried samples were dissolved in d4-DMSO:D2O (1.0:0.3 mL/mL), placed in NMR tubes, and subjected to NMR analysis.

Molar substitution was calculated by the following formula which accounts for the fact that each hydroxypropyl group has three methyl protons (1.2 ppm) and three non-methyl (2 methylene, 1 methyne) protons which fall within the range of the four glucosyl ring protons (3.1 – 4.1 ppm).

\[
MS = \frac{\text{integration of 1 ppm peak}}{3} - \frac{\text{integration of peaks at 3.1 – 4.1 ppm} - \text{integration of 1 ppm peak}}{7}
\]

If the reaction was 100% efficient, 0.27 MS would be expected based upon the amount of starch and propylene oxide present. Reaction efficiency was determined by dividing the molar substitution by 0.27.

3 Results

3.1 Water sorption

First, the blue dextran method [27], with corrections for light scattering by leached amylase [28], was used. The blue dextran concentration of the supernatant decreased in all samples from 0 h to 2 h and tended to decrease further in most salt systems, most likely as a result of adsorption of the blue dextran onto the starch [29]. Potassium sulfate, sodium citrate and potassium citrate-treated samples showed the most dramatic losses of blue dextran concentration. With these salts, the loss in blue dextran concentration was more pronounced as the concentration of salt increased. Therefore, the blue dextran method could not be applied in this work due to salting out of blue dextran and/or its adsorption onto starch granules. Precipitation of blue dextran by citrate ions was confirmed in a separate experiment (data not given).
As a result, sediment volume, an only semi-quantitative measure, was used to determine relative degrees of swelling. The method used was based on those used by others, especially those of Leach et al. [30] and Crosbie [31]. Results (Tab. 3) indicate that there is little change in the volume of starch in the absence of hydroxypropylation and that swelling in the absence of hydroxypropylation is independent of anion type. During and after hydroxypropylation, citrate ions inhibited swelling to the point that it was only slightly greater than that of the unmodified starch. At the other extreme, granules reacted in the presence of sodium chloride were gelatinized by 20 h of reaction. Sodium sulfate appeared to allow some swelling without gelatinization.

### 3.2 Ion concentration and molar substitution/reaction efficiency

The concentration of specific ions present in the supernatants was determined and subtracted from the concentration expected if solution entering granules was unchanged. Results are presented in Tab. 1. Negative values indicate a flux of the ion into the granule, whereas positive values indicate that the ion was excluded. All values are molar concentrations.

#### Tab. 1. Molar concentration differences due to the presence of starch.

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>K</th>
<th>Anion</th>
<th>Na</th>
<th>K</th>
<th>Anion</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
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<td>0.022</td>
<td>b</td>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.13</td>
<td>0.098</td>
<td>0.19</td>
<td>0.082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na&lt;sub&gt;3&lt;/sub&gt;Cit</td>
<td>0.02</td>
<td>0.040</td>
<td>0.08</td>
<td>0.044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
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<td>−0.081</td>
<td>0.025</td>
<td>−0.001</td>
<td>−0.105</td>
<td>0.025</td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.021</td>
<td>0.02</td>
<td>0.080</td>
<td>−0.001</td>
<td>−0.105</td>
<td>0.032</td>
</tr>
<tr>
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<td>−0.05</td>
<td>0.025</td>
<td>0.012</td>
<td>−0.06</td>
<td>0.017</td>
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<td>0.13</td>
<td>0.098</td>
<td>0.19</td>
<td>0.082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na&lt;sub&gt;3&lt;/sub&gt;Cit</td>
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<td>0.024</td>
<td>0.03</td>
<td>0.020</td>
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<tr>
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<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>0.02</td>
<td>0.080</td>
<td>0.025</td>
<td>−0.03</td>
<td>0.032</td>
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<td>−0.10</td>
<td>0.020</td>
<td>0.010</td>
<td>−0.09</td>
<td>0.011</td>
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<td><strong>Cation constant series</strong></td>
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<td>0.098</td>
<td>0.19</td>
<td>0.082</td>
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<tr>
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<td>0.04</td>
<td>0.046</td>
<td></td>
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</tr>
<tr>
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<td>K&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>0.02</td>
<td>0.080</td>
<td>0.025</td>
<td>−0.03</td>
<td>0.032</td>
</tr>
<tr>
<td>K&lt;sub&gt;3&lt;/sub&gt;Cit</td>
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<td>−0.04</td>
<td>0.015</td>
<td>0.017</td>
<td>0.00</td>
<td>0.009</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Section 2.2.

<sup>b</sup> Starch swelled excessively or pasted.

### Tab. 2. Molar substitution and reaction efficiency.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>Reaction efficiency [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride – total&lt;sup&gt;a&lt;/sup&gt;</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Sodium chloride – anion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Sodium chloride – cation&lt;sup&gt;a&lt;/sup&gt;</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>0.153</td>
<td>56.5</td>
</tr>
<tr>
<td>Sodium citrate – total</td>
<td>0.085</td>
<td>31.3</td>
</tr>
<tr>
<td>Sodium citrate – anion</td>
<td>0.086</td>
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<tr>
<td>Sodium citrate – cation</td>
<td>0.089</td>
<td>48.3</td>
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<tr>
<td>Potassium chloride – total</td>
<td>0.128</td>
<td>47.0</td>
</tr>
<tr>
<td>Potassium chloride – anion</td>
<td>0.123</td>
<td>45.4</td>
</tr>
<tr>
<td>Potassium chloride – cation</td>
<td>0.131</td>
<td>48.4</td>
</tr>
<tr>
<td>Potassium sulfate</td>
<td>0.135</td>
<td>49.8</td>
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<tr>
<td>Potassium citrate – total</td>
<td>0.082</td>
<td>30.3</td>
</tr>
<tr>
<td>Potassium citrate – anion</td>
<td>0.077</td>
<td>28.5</td>
</tr>
<tr>
<td>Potassium citrate – cation</td>
<td>0.100</td>
<td>37.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Total = total ion concentration constant, Anion = anion concentration constant, Cation = cation concentration constant. See Section 2.2.

<sup>b</sup> Starch pasted.

Results obtained using the first method of analysis for MS (trifluoroacetic acid-catalyzed hydrolysis) are given in Tab. 2. Several methods have been used to determine the molar substitution (MS) of hydroxypropyl starch. In 1970, Stahl and McNaught [32] introduced a simple, accurate method using proton NMR which has since been applied in several forms, all of which involve integration of the resonance of the methyl protons of the hydroxypropyl group and comparison to some internal standard. The method included an acid-catalyzed hydrolysis step for the purpose of reducing viscosity. De Graaf et al. [33] analyzed nonhydrolyzed hydroxypropyl starches by the NMR method and found that the values correlated well with those obtained with the Johnson [34] method through a MS range of 0.04 to 0.50.

Identical reactions were done with sodium citrate added at concentrations of 0.100, 0.200, 0.300, 0.400 and 0.500 M to further elucidate the effect of sodium citrate on reaction efficiency (data not given). Sodium citrate at 0 100 M did not inhibit gelatinization. At concentrations greater than 0.1 M, it was inhibitory at all times of reaction, i.e., at all MS values; so its effect seems to be all-or-nothing in terms of gelatinization inhibition.

### 3.3 Swelling and molar substitution

It appeared that the efficiency of the hydroxypropylation reaction was a function of granule swelling, i.e., in the absence of swelling (in the presence of a strong lyotropic salt, such as citrate), probably only very limited reaction
occurred and was restricted to the limited areas available to aqueous solutions in native granules [35], but this hypothesis was based on empirical observations. Experimental data based on water sorption were not available for reasons discussed above, so sediment volumes were obtained and compared to degrees of chemical modification in order to test the hypothesis. Sediment volumes are strong indicators of the swollen state of starch granules; however, they are not an accurate measure of water/reagent solution absorbed by granules because they ignore intergranular space, which is a function of granule geometry, and intragranular voids such as channels and cavities. In addition, if the granules are not permitted to settle to equilibrium, then one must also consider sedimentation rates, which are related to the g-force and viscosity. Such was the case in this study where settling to equilibrium was not an option due to the experimental design. Nevertheless, important information about relative degrees of granule swelling was obtained.

In development of the second method of analysis for MS (using α-amylase for hydrolysis), it was determined that the chemical shifts related to acetate and the enzyme did not overlap with any peaks from a hydroxypropyl starch hydrolyzate. It was also determined that purifying digests by removal of buffer salt and enzyme was not only unnecessary, it may introduce error by inadvertent fractionation of hydrolyzate components. Dissolution in neat $d_6$-DMSO was difficult, and a low, wide peak appeared in the hydroxyl group region of the NMR spectrum (4.5–5.0 ppm) that disrupted the baseline, even in the glucosyl unit ring proton region (3.0–4.0 ppm). It also caused the entire spectrum to compress upfield. Addition of $D_2O$ directly into the NMR tubes containing $d_6$-DMSO eliminated the low, wide peak.

Swelling and molar substitution data obtained using this method are presented in Tab. 3. Reaction efficiency of the control (sodium sulfate) was 56.5%, a value reasonably close to the literature-reported maximum value of ≈ 60%. In this system, increases in reaction efficiency were slight after 16 h of reaction time. Sodium citrate-containing reactions increased in efficiency slowly up to 16 h, then leveled-off at a MS of 0.08 (37% reaction efficiency). Sodium chloride-containing systems also increased in reaction efficiency up to 16 h, but continued to increase during pasting to >70% efficiency.

Sediment volumes stayed constant at about 74% for systems not containing propylene oxide throughout the 24-h period, regardless of the salt system used. (Note that the volumes were greater at 0 h because the vials had not yet been placed in the warm water bath.) The control system (sodium sulfate) increased in sediment volume up to 16 h, at which time volumes appeared to level-out, with the exception of the 24 h sample, whose sediment volume had increased about 10%. When reactions were conducted in the presence of sodium citrate, sediment volumes that were only very slightly greater than those of the unreacted controls were obtained. Sodium chloride-containing samples, conversely, were very swollen at 16 h and yielded no supernatant at all by the 20th hour of reaction because of gelatinization.

### Table 3.

<table>
<thead>
<tr>
<th>Reaction Time [h]</th>
<th>Sodium chloride</th>
<th>Sodium sulfate</th>
<th>Sodium citrate</th>
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<tr>
<td>4</td>
<td>0.032</td>
<td>0.060</td>
<td>0.067</td>
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<tr>
<td>8</td>
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<td>0.106</td>
<td>0.084</td>
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<tr>
<td>20</td>
<td>0.170</td>
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<td>0.091</td>
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<tr>
<td>24</td>
<td>0.190</td>
<td>0.149</td>
<td>0.094</td>
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<table>
<thead>
<tr>
<th>Sediment volumes [%]</th>
<th>Reaction Time [h]</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
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<td>Sodium chloride</td>
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<td>75</td>
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<tr>
<td>Sodium sulfate</td>
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<td>82</td>
<td>81</td>
<td>81</td>
<td>81</td>
<td>81</td>
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<tr>
<td>Sodium citrate</td>
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<td>78</td>
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</table>

<table>
<thead>
<tr>
<th>Sediment volumes without addition of propylene oxide [%]</th>
<th>Reaction Time [h]</th>
<th>0</th>
<th>1</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
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<td>75</td>
<td>73</td>
<td>74</td>
<td></td>
</tr>
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<td>Sodium sulfate</td>
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<td>73</td>
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<td>74</td>
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<tr>
<td>Sodium citrate</td>
<td>81</td>
<td>73</td>
<td>74</td>
<td>74</td>
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</table>

<table>
<thead>
<tr>
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<th>Reaction Time [h]</th>
<th>0</th>
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<tr>
<td>Sodium citrate</td>
<td>81</td>
<td>73</td>
<td>74</td>
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<td></td>
</tr>
</tbody>
</table>

- Averages of two measurements.
- Percent of total volume.
- No reagent was added. Otherwise, conditions were the same.
- Granules gelatinized.

4 Discussion

Ideally, various cation-anion combinations would have been used in this research, with each ion being varied as to charge and size. However, insolubility prevented use of some ion combinations. Six salts were selected. The cations used were sodium and potassium. The anions were chloride, sulfate, and citrate. Sodium and potassium ions were used because insolubility at high pH values prevented use of divalent or trivalent cations.
Chloride, sulfate, and citrate were chosen because of their position in the Hofmeister lyotropic series (from least to most effective in precipitating colloids) and because they were mono-, di-, and trivalent anions. Citrate was also chosen because of its large size and because it is biodegradable.

The control reaction mixture contained starch (51% dry solids), sodium hydroxide (0.5%, 0.126 molal), sodium sulfate (7.5% db, 0.527 molal), and enough propylene oxide (4.22 mL, 0.0604 mol) to give an MS of 0.16 (5.8% hydroxypropyl groups by weight), assuming an = 60% reaction efficiency. Three reaction conditions were used. All contained the same concentrations of all components except the salt. In one set of experiments, the total molal concentration was equated to that of the control; and in the other, the molal anion concentration was equated to that of the control. In another, the molal anion concentration was equated to that of the control; and in the other, the molal cation concentration was made equal to that of the control.

4.1 Ion concentration and molar substitution

The data in Tab. 1 indicate a greater propensity for potassium ions to penetrate corn starch granules as compared to sodium ions. Leach et al. [15] noted that KOH was taken up by starch more readily than was NaOH. The data in Tab. 1 also indicates a greater cationic charge in the extragranular solution when sulfate is the anion, suggesting that the sulfate ion restricts absorption or neutralization of hydroxide ions by corn starch granules. There is no clear relationship in this regard with chloride and citrate ions.

Because starch granules can be considered to be polyanions, a Donnan potential is created and granules attract cations and repel anions [13]. Reactions containing chloride salts had these characteristics, indicating strong evidence for Donnan potential effects. Sulfate systems exhibited similar behavior in this regard. From data in Tab. 1, it is apparent that sulfate salts were unique in that both the cation and the anion were kept out of granules in preference to water. An explanation is that the divalent sulfate anions experience greater repulsive forces from the granule than do chloride anions because they are a more concentrated source of electronegativity [14]. The law of electroneutrality must be satisfied. Therefore, because sulfate ions are divalent, two sodium or potassium ions will be held in the extragranular solution with each sulfate ion. That sulfate salts also have an effect based on their position in the lyotropic series indicated by the fact that swelling data (Tab. 3) places them in the same position that they are in the lyotropic series. Systems containing citrate ions appeared to be governed by a strong lyotropic effect which retarded water sorption, swelling, and the solubilization of starch; consequently no Donnan effects were observed.

In most reaction systems, the anion concentration in the extragranular solution decreased slightly at the conclusion of the reaction. This is probably due to increased granule swelling due to hydroxypropylation and a consequent reduction in the Donnan potential, which is responsible for anion exclusion. The ability of sodium chloride to inhibit gelatinization is diminished rapidly as the reaction occurs because of this loss of Donnan potential. Sodium chloride has little, if any, lyotropic effect to help under these circumstances. This signifies that sodium chloride is not suitable for hydroxypropylation of starch to relatively high MS values, an already known fact, unless, perhaps, the reactions are performed at lower temperatures for a longer time.

Based upon the amount of propylene oxide added, a MS of 0.27 would have been expected if reactions were 100% efficient. The effects of salts on reaction efficiency (Tab. 2) suggest that use of a potassium salt results in reduced reaction efficiency when compared to an equal concentration of the corresponding sodium salt, except perhaps for potassium citrate and sodium citrate at low concentrations. This may be due to a greater stability of potassium starch alcoholate. In this case, a greater proportion of potassium starch alcoholate is in the undisassociated form, leaving less alkoxide ion to react with propylene oxide.

A rough trend observed in the results is that, for a given salt, the highest MS occurred when the salt was added at the lowest level. (Potassium chloride was an exception.) The most likely explanation for this observation is based upon lyotropic effects. At higher salt concentrations, granule swelling is more restricted by lyotropic effects, which reduces the number of sites available for reaction with propylene oxide. If this is true, the optimum salt concentration to maximize reaction efficiency would be the minimum level of salt required to inhibit gelatinization.

Wooton and Manatsathit [35] found that, for hydroxypropyl starch with MS values < 0.07, water binding and swelling were essentially independent of MS. For products with MS values > 0.07, water binding increased as MS increased; and at MS > 0.10, swelling power increased as the MS increased. Wooten and Manatsathit [36] also found that, for MS values below 0.1, the peak temperature, onset temperature, and enthalpy of gelatinization decreased as MS increased. (Above MS 0.1, DSC peaks were too broad to analyze.) One way to interpret this data is that starch granules can accommodate hydroxypropyl groups up to MS 0.07 with little change in...
their structure. Between MS 0.07 and 0.10, hydroxypropyl groups effect minor rearrangements in granules, probably within the amorphous areas. Above MS 0.10, forces created by swelling of amorphous regions strip chains from crystallites, leading to partial hydration of amylepectin and consequent even greater swelling of granules. Citrate ion seemed to keep hydroxypropylation to a minimum in the 0.08 to 0.10 MS range and the reaction efficiency to 28 – 48% (Tab. 2). This supports the view that the powerful lyotropic effect of citrate maintains crystallities, limiting hydroxypropylation to amorphous areas. Later, it was determined that the effect of sodium citrate was an all-or-none effect. Depending on the concentration, it either inhibited swelling effectively or not at all, i.e., there was no partial inhibition of swelling [37]. Sulfate produces a moderate lyotropic effect, allowing restricted swelling and enabling the production of higher MS products.

Chloride salts are known to increase $T_{gel}$ of starch up to a certain concentration of salt, after which increasing concentrations lower $T_{gel}$ [6–8]. This phenomenon must be considered when evaluating swelling and MS data when salts that exhibit the effect are used; however, the effect of a combination of salt and base has not been previously studied in the same way as it has been here. Potassium chloride appears to exhibit this effect in that reaction efficiencies increased with increasing concentrations of KCl. In this study, KCl was likely employed in the concentration range where the chloride ion had a destabilizing effect on granules, making more sites available for reaction. Sodium chloride was either not stabilizing enough or destabilizing at the concentrations employed in this investigation.

In most samples, anion concentrations in the extragranular solution decreased slightly at the conclusion of the reaction. This could be explained by expansion of the granule structure and consequent reduction of the Donnan potential, which is responsible for excluding anions. However, the exclusion of anions should be reduced less in lower-MS samples, a phenomenon not always observed.

4.2 Granule swelling and molar substitution

The results given in Tab. 3 confirm [38] that granule swelling is a prerequisite for efficient reaction to occur. Citrate, a highly lyotropic anion, appears to restrict granular swelling. Swelling did not increase significantly even when limited reaction was occurring, which suggests that the reaction was restricted to naturally occurring open regions, i.e., no new accessible regions were created via swelling. This conclusion is strongly supported by the results of Wootton and Manatsathit [35], who found that the swelling power of common corn starch did not increase until the MS value exceeded 0.10. In the presence of chloride and sulfate ions, no significant increase in granule/bed volume was observed until sometime after 12 h of reaction (MS values of 0.12 and 0.13, respectively). After this time, both granule swelling and MS values increased more rapidly for granules reacted in the presence of sodium chloride as opposed to sodium sulfate. Data for swelling (sediment volumes) in the absence of etherifying reagent seem to indicate that the effect of the alkali is realized more rapidly than the effect of the salt (0 h vs. 1 h).

Some granules reacted in the presence of sodium chloride were gelatinized by 16 h of reaction; substantial numbers were gelatinized by 20 h of reaction, confirming that sodium chloride is less effective as a swelling and gelatinization inhibitor than is sodium sulfate. The MS of granules reacted in the presence of sodium chloride increased considerably after 16 h of reaction while those reacted in the presence of sodium sulfate seemed to almost reach a plateau. This indicates that there was no shortage of reagent during the period from 16 h to 24 h (as might be indicated by the data for reactions carried out in the presence of sodium sulfate and sodium citrate) and that reaction efficiency is not a function of a competing reaction with hydroxide ions. Rather, the data for the three salts would indicate that it is a function of the degree of swelling.

Although it is possible that the low reaction efficiency was due to propylene oxide reacting directly with the citrate anion [39], a follow-up study confirmed that this was not occurring to a significant extent. Citrate content was determined under reaction conditions, with and without propylene oxide present, using an enzyme test kit; no significant loss of citrate due to hydroxypropylation was found, although formation of the propylene glycol ester of citrate, followed by saponification, cannot be ruled out.

In the presence of sodium chloride, granules swelled excessively after about 16 h of reaction. Although impractical from an industrial processing standpoint, the fact that these granules were so excessively swollen allowed them to react with a reaction efficiency considerably higher than the usual 60%. The results of the control sodium sulfate reaction suggest that, even though the reaction was essentially complete at 16 hours, enough instability was introduced so that swelling continued into the 24th hour.

It cannot be concluded from these results that there are no biodegradable anions that have swelling-inhibiting effects similar to those of sodium sulfate, only that citrate is not one of them.
5 Conclusions

Results indicate that increased granule swelling results in increased hydroxypropylation efficiency. Results also indicate that the mechanisms by which salts protect granules during modification include both Donnan potential and lyotropic effects, as predicted by Chinachoti et al. [17]. Salts which exhibit a strong lyotropic effect, such as citrate, preclude Donnan potential effects. These salts are very effective at inhibiting granule swelling, but can be too effective, i.e., effective to the point of not allowing hydroxypropylation to occur efficiently. It was hypothesized that, for optimum reaction efficiency, strongly lyotropic salts should be used at the minimum level necessary to inhibit gelatinization. However, in the case of sodium citrate, the lowest level of salt which inhibited gelatinization still resulted in diminished reaction efficiency when compared to the control, and below that level, gelatinization occurred, i.e., there was an all-or-none effect.

In this study, the control reaction with sodium sulfate gave optimum reaction efficiency without gelatinization. Sulfate salts seemed to be excluded as water entered granules, suggesting a modest Donnan potential effect, preventing granule destabilization. Sulfate ions also have a more moderate lyotropic effect than do citrate ions, which allows granules to swell to a limited degree, which seems to be necessary for the reaction to occur. Thus, the role of sulfate as a protective agent is two-fold. When hydroxypropyl groups are steadily introduced into the amorphous areas of the granule, the structure experiences additional stress from steric and, perhaps, electrostatic repulsion forces. The lyotropic effect of sulfate ions provides additional protection by reducing solvation of the polymer chains in these regions. Without this protection, the amorphous regions would swell and strip chains from adjacent crystallitles, leading to gelatinization [40]. Since sulfate ions tend to be repelled/excluded from granules, it is plausible that the lyotropic effect contributed by sulfate ions is greater on the surface of granules. This may help explain the high reaction efficiency obtained using sulfate salts.

Sodium chloride does not exhibit a stabilizing lyotropic effect and, thus, fails to inhibit gelatinization as higher levels of hydroxypropyl groups are introduced. However, chloride salts sufficiently protected granules, even at 49°C, as long as the MS values were low. This is due to a Donnan potential effect, which is most likely the sole mechanism by which sodium chloride protects. Potassium chloride was probably successful only because the stability of potassium starch alcoholate retarded reaction efficiency, and consequently less stress was introduced into granules.

Each starch is unique [41]. Conclusions based on data obtained from one type of starch, in this case normal corn starch, are not necessarily applicable to other starches. Differences that could be expected to effect the generation and extent of a Donnan potential are the presence of intragranular protein, which is present in normal cereal grain starches in greater amounts than it is in waxy maize starch or potato starch [42], the presence of phosphate ester groups, of which there are more in potato starch than in cereal starches, and the presence of intragranular phospholipids.

The practical purpose of this study was to determine a suitable replacement for sodium sulfate for environmental reasons. Citrate was examined as a potential biodegradable substitute for sulfate. Although citrate was extremely effective as a gelatinization inhibitor, it did not allow achievement of the reaction efficiency obtained with sodium sulfate because of its powerful lyotropic effect.

A distinct correlation between swelling (as determined by sedimentation) and reaction efficiency was found. Hydroxypropylation in the absence of swelling, e.g., in the presence of citrate, apparently occurs only in regions that can be penetrated by reagent solution without further swelling. Without the ability to swell as a result of substituents being introduced, the remainder of the granule remains inaccessible to reagent. However, in the absence of a salt which restricts swelling, the result of derivatization is greater swelling, which opens up new regions of granules to penetration by reagent solution, which in turn allows more reaction, and so on. If unbalanced by a lyotropic swelling retardant, this situation will result in a form of chemical gelatinization. Sodium sulfate, when used at the proper concentration, seems to be ideal in controlling swelling without excessive restriction.

Epilogue

This paper is a summation of the M. S. Thesis of V. Kurtis Villwock (Purdue University 1996), which has previously been referenced in several papers [37, 43–46].

References


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