CHAPTER IV
MISCELLANEOUS EXPERIMENTS
(A) SEVERE OXIDATION OF STARCH

Sodium hypochlorite finds extensive application in the manufacture of oxidized starches \(^{106,107}\). The oxidation reaction is considerably complex, erratically pH dependent and highly non-specific. At higher concentrations of the oxidant, the reaction primarily proceeds to give only low molecular weight, water-soluble products, formed by the degradative breakdown of the polymer length. Therefore, the manufacture is restricted to mildly oxidized starches only. Conversion with more than ten per cent available chlorine on weight of starch is hardly feasible commercially. Attempts to recover highly oxidized starch using solvents as precipitating agents also fail, because the yields are poor. We have attempted to oxidize starch using various severe oxidative conditions and recovered the oxy-starches. The experimental procedures and results are given below:

**Experimental**

**Blank**: Dry starch (5.0 g) was placed in a three-necked 100 ml round bottom flask, 43 ml hypochlorite (77 gpl), diluted with water (7.0 ml) was added and the contents were stirred for 24 hours maintaining the temperature to \(37^\circ \pm 0.1^\circ\). At the end of the reaction, the contents were acidified with dilute acetic acid and treated with sodium thiosulphate to neutralize residual
oxidant which was less than five per cent of the original. The contents were then transferred quantitatively and precipitated with ethyl alcohol (200 ml). The precipitates were centrifuged and washed several times with acidified ethyl alcohol to remove inorganic impurities. Finally, the precipitates were washed with ethanol, methanol, ethylether and dried under vacuum. The dry weight of the oxidized starch was 1.92 g.

**Sodium chloride:** The reaction was carried out exactly as above but additionally sodium chloride (12.5 g) was added to the reaction mixture in order to ascertain the effect of ionic concentration on the yield. The oxidized starch, free from any salt, weighed 2.71 g.

**Magnesium chloride:** To dry starch (5.0 g) was added magnesium chloride (25.0 g). Then sodium hypochlorite (43.0 ml) diluted with water (7.0 ml) was added, the reaction was carried out and the product recovered as described above in the case of Blank experiment. Yield was 4.25 g.

**Cobalt chloride:** Additional reaction was also carried out using cobalt chloride (1.5 mg), as the catalyst. The rest of the conditions were the same as mentioned for the Blank experiment. The yield was 2.32 g.

**Results**

Table VII shows data on yield and other functional properties of the highly oxidized starches. Properties of the unmodified starch are also included. The yield is as high as 85 per cent.
for the reaction carried out in the presence of magnesium chloride whereas it is only about 50 per cent or less with cobalt and sodium ion or without any salts.

<table>
<thead>
<tr>
<th>No.</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Carboxyl Content m.equ./AGU</th>
<th>8-Amylolysis</th>
<th>Ferricyanide Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Uncatalyzed</td>
<td>38.0</td>
<td>91.85</td>
<td>20.4</td>
<td>2.85</td>
</tr>
<tr>
<td>2.</td>
<td>Sodium chloride</td>
<td>54.0</td>
<td>68.70</td>
<td>15.6</td>
<td>4.41</td>
</tr>
<tr>
<td>3.</td>
<td>Magnesium chloride</td>
<td>85.0</td>
<td>262.30</td>
<td>5.5</td>
<td>2.85</td>
</tr>
<tr>
<td>4.</td>
<td>Cobalt chloride</td>
<td>45.0</td>
<td>51.10</td>
<td>25.3</td>
<td>14.67</td>
</tr>
<tr>
<td>5.</td>
<td>Unmodified</td>
<td>-</td>
<td>1.09</td>
<td>61.6</td>
<td>1.21</td>
</tr>
</tbody>
</table>

The carboxyl contents are also three to four times higher for the reaction product obtained from the presence of magnesium chloride medium than other products, suggesting very effective utilization of the oxidant. 8-Amylolysis of the magnesium chloride catalyzed product is the least. 8-Amylolysis can be considered as an index of unmodified starch. The higher the value, the higher is the amount of unmodified starch. Therefore, the product obtained in magnesium chloride medium shows the least amount of the unmodified polymer as compared to others. Since the oxidation reactions are carried out in alkaline conditions, all the products show lower ferricyanide number, except the cobalt catalyzed one, which shows significantly high reducing values. Therefore, in the cobalt catalyzed reaction the rate of oxidation or depolymerization seems to be faster than the
rate of conversion of aldehydic groups to carboxyls through alkaline catalysis.

Discussion
Lower yields in uncatalyzed, sodium chloride and cobalt chloride systems suggest that certain starch granules are attacked by the oxidant preferentially. These granules which are oxidized first are further reacting with the oxidant, till most of their starch portion is converted into smaller molecules. Therefore, in such oxidative conditions relatively large proportions of unoxidized starch remain. In the presence of magnesium chloride, two advantages have been noticed. Thus, the product is not only recovered in very high yields but, most of it is oxidized as suggested by increased carboxyl contents. It is well known that magnesium and tin salts stabilize the oxidant and therefore this may lead to higher functional groups in the modified product by way of utilization of higher oxidant. However, it is surprising to note that apart from stabilizing the oxidant, this reaction medium changes drastically the course of reaction because unlike uncatalyzed oxidation, a large proportion of highly modified polymer fraction is recovered. This suggests that the magnesium chloride medium not only catalytically reduces depolymerization but also reduces formation and reaction of the smaller fragments. That this effect is not due to increased electrolyte concentration is confirmed by oxidizing starch in the presence of large amounts of sodium chloride. The latter system does not show any advantage over uncatalyzed system.
The carboxyl groups can be formed by oxidation with hypochlorite at three different sites of AGU, viz., (i) anomeric hydroxyl can be oxidized to give carboxyl, (ii) hydroxymethyl group can be oxidized to aldehyde or carboxyl, and (iii) C-2, C-3 bond can be broken to yield dialdehyde starch, which on subsequent oxidation will give dicarboxylic acid starch. As the yield of the product obtained in magnesium chloride medium is 85 per cent, the depolymerization is very less and hence the production of carboxylic groups at anomeric position would be very less.

In Chapter III we have seen that magnesium chloride does not show any catalytic effect on the rate of oxidation, when it is used in concentration of 0.5 m.moles per litre as a catalyst in 112 m.moles per litre oxidant. However, it showed significant improvement in the functional properties, as determined by arbitrary oxygen balance. This effect on the functional properties is magnified here by oxidizing starch with 1.5 moles of oxidant per AGU in 50 per cent magnesium chloride medium. The catalytic effect of magnesium can be explained by two different ways. Magnesium may form a complex with starch, and this complex may sterically hinder the hypochlorite to attack the glycosidic bond. Another possibility that magnesium chloride may swell the starch granules and leave a large and open structure of starch for the oxidation, cannot be ruled out. The reaction between the swollen granule may be more uniform and specific leading to greater oxidation over the entire length of polymer. Such a course of reaction, therefore, may reduce the formation of smaller fragments and
consumption of the oxidant in preferential reaction with such fragments.

(B) SOLVENT ISOTOPE EFFECT

Oxidation of model carbohydrate compounds with chlorine and its derivatives has been carried out by several workers. Deuterium isotope effect in the chlorine oxidation of model carbohydrate compounds has been studied by Urquiza, who oxidized the C-deuteriated glucose. He found the reaction rate ratio, $k_H/k_D$, much below 2.5. However, Urquiza suggested a hydride transfer from the carbon atom. This is contrary to known observations, because if there is a hydride transfer from the carbon atom then the ratio should be greater than 2.5. Green and Crossman studied the oxidation of C-14-labelled methyl β-D-glucopyranoside. They suggested that the main oxidative attack was on the glycosyl unit. Isbell et al. studied the oxidation of tritium labelled model carbohydrates with bromine and chlorite. They suggested that the rate determining step is C-1-H bond rupture in the case of oxidation with bromine, whereas it is not so in chlorite oxidation.

We have studied the solvent isotope effect on the oxidation of model carbohydrates and amylpectin in order to know the nature of reaction at the hydroxyl groups, because when glucose is dissolved in D$_2$O, the -H of the -OH groups exchanges with -D very rapidly. Therefore, the rates of reaction of chlorine in H$_2$O and D$_2$O are expected to be quite different, if hydroxyl groups participate. The participation of hydroxyl groups in the oxidation reaction has been shown earlier in Chapter II.
Table VLIII shows the rate constants for the oxidation reaction of methyl α-D-glucopyranoside, maltose and amylopectin in light water (H$_2$O) and in heavy water (D$_2$O) at 30°. It is observed that with all the three compounds the rate in light water is more than four times faster than that in heavy water. Whister et al. have showed that the rate and nature of oxidation of amylose and amylopectin are not only similar but both behave in same way as starch. It is further observed that the ratio $k'_H/k'_D$ is approximately the same for methyl α-D-glucopyranoside and amylopectin, whereas it is higher for maltose. This higher ratio in the case of maltose may be attributed to the free anomeric reducing groups, which are oxidized relatively rapidly. It has been predicted that the ratio $k'_0H/k'_0D$ can be as large as 10 in proton transfer reaction.

### TABLE VLIII

<table>
<thead>
<tr>
<th>No.</th>
<th>Product</th>
<th>H$_2$O Rate (k$^1_H$ min$^{-1}$)</th>
<th>D$_2$O Rate (k$^1_D$ min$^{-1}$)</th>
<th>$k'_H/k'_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl α-D-glucopyranoside</td>
<td>0.00043</td>
<td>0.00010</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>Maltose</td>
<td>0.00210</td>
<td>0.00030</td>
<td>7.0</td>
</tr>
<tr>
<td>3</td>
<td>Amylopectin</td>
<td>0.00048</td>
<td>0.00012</td>
<td>4.0</td>
</tr>
</tbody>
</table>
From the foregoing discussion it can be assumed that there is an initial attack of chlorine molecule on the hydroxyl of the substrate, which subsequently forms a hypochlorite ester, and a molecule of hydrochloric acid. This is a relatively faster reaction than the proton transfer from carbon atom which probably follows the ester formation step and can be the slow and rate determining step in acidic medium. Schematically this is shown below:

\[
\text{H-C-OH} + \text{Cl-Cl} \xrightarrow{\text{fast}} \text{H-C-O-Cl} + \text{HCl} \\
\text{(Hypochlorite ester)}
\]

\[
\text{H-C-O-Cl} \xrightarrow{\text{slow}} \text{C = O} + \text{HCl}
\]

The attack of chlorine molecule is consistent with and analogous to that proposed by Whistler et al., forming chloro-oxonium ion, followed by hypo-halite ester formation. The decomposition of the ester leads to a carbonyl containing carbon atom liberating a molecule of hydrochloric acid.