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Significance of Redox Studies on Branched Polysaccharides during Metal Complex Formation

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ABSTRACT

Detailed studies on oxidation and reduction of complex polysaccharides were studied. Low molecular weight glucan like dextran has been oxidized using different quantities of sodium hypochlorite and the number of glucose units oxidized has been analyzed using potentiometric titrations. Different factors such as the quantity of sodium hypochlorite, reaction duration, isolation have been comprehensively studied. The oxidized product has been analyzed by Proton Nuclear Magnetic Resonance (^{1}HNMR), Carbon-13 nuclear magnetic resonance (^{13}C NMR), Infra-Red (IR) and Gel Permeation Chromatography (GPC). Similarly low molecular weight dextran has been hydrogenated using different quantities sodium borohydride and percentage reduction was calculated using reducing sugar content.

Keywords: Polysaccharides, Dextran, Oxidation, Reduction, Reducing sugars

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INTRODUCTION

Glucan with low molecular weight like dextran and Maltodextrin (MD) are polysaccharide generally synthesized by various strains of microbes. Dextran is synthesized by *Leuconostoc mesenteroides* (strain B-512), when grown on sucrose-containing media while MD are enzymatic and/or acid hydrolysis products of starch. Alpha-amylases from *Bacillus licheniformis* or *Bacillus stearothermophilus* split the 1, 4 bonds and form maltose and limit dextrins. Structure of these polysaccharides is characterized by having varying degree of α-1, 6 linkages and some low degree of branching (Table 1) [1-7].

Table 1: Dextran linkage variation and how it differs with the use of different microbial strains

Microbe	Strain	α- 1,6 linkage	α- 1,3 linkage	α- 1,3 linkage branch	α- 1,4 linkage branch	α- 1,2 branch
Leuconostoc mesenteroides	B 512 F (Class 1)	95	5	-	-	-
	B-742	87	-	-	13	-
	B-1355	95	-	5	-	-
	Class 2	-	-	-	-	-
	B-1299	66	-	7	-	27
	B-1498	50	50	-	-	-
	B-1501	50	50	-	-	-
Streptococcus downei	Mfe28	12	88			
Streptococcus mutans	GS5	13	87			
	6715	64		36		

Adapted from International Dairy Journal, 2001, 11, 680

Various by-products formation during fermentative biosynthesis of polysaccharides is a general phenomenon. Common byproducts in dextran biosynthesis are Panose, Leucrose, Isomaltulose and Levan. Dextrose equivalent (DE) is a measure of the amount of reducing sugars present in a sugar product, relative to dextrose, expressed as a percentage on a dry basis.

MD and Dextran's have attracted attention world over due to their biotechnological applications. Dextran is accepted as a plasma volume expander, due to its linear structure, high water solubility, and especially owing to its α-1,6 linkages, more relevant to biological applications, in virtue of the slower hydrolysis by body enzymes, compared to the α-1,4 linkage. There are several reports for oxidation of dextran [7-13]. Several reagents such as periodic acid and sodium hypochlorite have been used to study oxidation of dextran which generally has 9-11% reducing sugar content.

On the oxidation of maltodextrin with mercuric oxide and then final precipitation with organic solvents majorly gives two fractions with specific rotatory power of 176-179ºC and 189-192ºC respectively. Second main fraction with the higher rotatory power in a pure state, is uncrystallisable, with specific rotatory power of 193. It forms salt with calcium.

Acid derivative of maltodextrin is hydrolysable both by diastase and by acids, giving, with the enzyme, a definite amount of maltose and an acid of lower molecular weight, whilst by acid hydrolysis it yields a definite amount of dextrose and a final acid of still less complexity [14]. MD has wide applications as bulking agent, in viscosity management, stabilizer & carrier, in coating, as sweetness reducer. Maltodextrin with DE above 20, is known as glucose syrup (Table 2).

Table 2: Saccharide content of various syrup

S. No.	**High maltotetraose syrup**		**Acid-conversion syrup**	**Enzyme-conversion syrup**
	Type I	Type II		
DE	30.8	36.2	32.6	47
Glucose	1	2	10.3	3
Maltose	7.8	8.5	8.3	53.5
Maltotriose	10.2	11	8.4	15.8
Maltotetraose	50.5	72	8.1	3.2
Maltopentaose	2.5	1	6.9	2

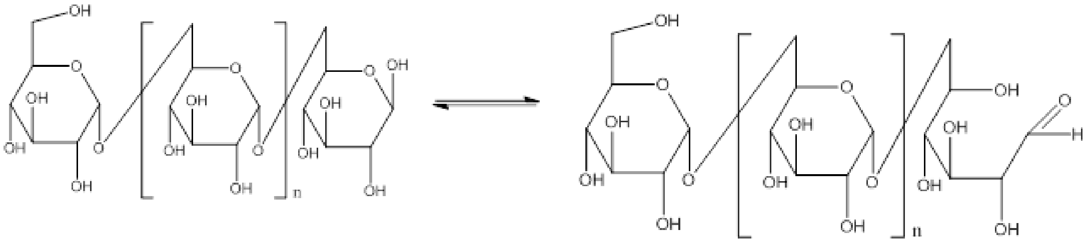
Common by-products during redox reactions of polysaccharides are illustrated in Table 3.

Table 3: Common byproducts during polysaccharides synthesis and there identification test

S. No.	**Benedict (Red ppt)**	**Barfoed (Red ppt)**	**Molisch (Red/Violet ppt)**	**Resorcinol (red ppt)**
Sucrose	-	-	+	+
Lactose	+	-	+	-
Maltose	+	-	+	-
Fructose	+	+	+	+
Glucose	+	+	+	-
Galactose	+	+	+	-
D-Gluconic acid	-	-	-	-
D-glucuronic acid	-	-	-	-
D-Glucaric acid	-	-	-	-
D-Gluconic acid lactone	-	-	-	-
Sorbitol	-	-	-	-
Mannitol	-	-	-	-
Galacitol	-	-	-	-

RESULT AND DISCUSSION

Oxidation studies were conducted for low molecular weight dextran with a wide variety of oxidizing agents such as sodium periodates, sodium chlorite and sodium hypochlorite. Effect of addition of sodium hydroxide, reaction duration and quantity of sodium hypochlorite has also been analyzed. Degree of oxidation was analyzed by titration with 2 M sodium hydroxide (NaOH) solution. Reaction mixture having dextran dissolved in milli-Q water along with NaOH and Sodium hypochlorite was stirred at room temperature with pH maintained at 12.5 using 2M NaOH by potentiometric titrator. It was observed that the number of glucose units oxidized varied with the quantity of sodium hypochlorite used. However it was observed that there was no variation in the number of glucose units oxidized on increasing the duration of reaction beyond 6 h. The oxidized polysaccharide could further be used for preparation of complexes with various metals. Upon oxidation with sodium periodate (Table 4) no change in IR spectra was observed. However on oxidation with sodium chlorite (Table 5) or sodium perchlorate some variation was observed by the way of absence of peak at 1648 cm^{-1} and by presence of peak at 1605 cm^{-1} indicating formation of carboxylic acid in the process of oxidation. It was observed that a total of 6-7 glucose units were cleaved when 1.5 times sodium hypochlorite was used while this number increased to 9-10 when the quantity of sodium hypochlorite was raised to 2 times the quantity of dextran. The oxidized polysaccharide in which 6-7 glucose units were oxidized was used for preparation of metal-polysaccharide complex and it was found that a complex with required molecular weight was obtained (Scheme 1).



Polysaccharide (n ~ 30)

Scheme 1: Formation of desired polysaccharide by oxidation

(a) On oxidation with sodium periodate: On reaction with sodium periodate no major variation in structure is observed as indicated by ^{13}C NMR and IR.

Table 4: NMR. IR and MW data pre and post oxidation with sodium periodate

Analysis	**Result of polysaccharide before reaction**	**Analysis result of product obtained upon oxidation with sodium periodate**
^{13}C NMR δ (ppm)	63.2, 68.2, 68.4, 72.2, 72.9, 74.1, 74.5	51.6, 68.2, 72.2, 72.9, 74.1, 74.2, 74.5, 76.1, 100.4
IR (cm^{-1})	1646.0, 1460, 1420, 1345, 1270, 1154, 1108, 1077, 1040, 1012, 914, 865	1646.3, 1421.1, 1348.2, 1275.1, 1155.0, 1109.8, 1015.4
Average molecular weight	Around 5000	5173

(b) **On oxidation with sodium chlorite:** On reaction with sodium chlorite carboxylic acid group is introduced in the polysaccharide as indicated by peak of 1607 in IR spectrum.

Table 5: NMR. IR and MW data pre and post oxidation with sodium chlorite

Analysis data	**Result of polysaccharide before reaction**	**Analysis result of product obtained upon oxidation with sodium chlorite**
^{13}C NMR δ (ppm)	63.2, 68.2, 68.4, 72.2, 72.9, 74.1, 74.5	51.7, 63.2, 68.3, 70.9, 72.0, 72.0, 72.3, 72.9, 73.7, 73.9, 74.2, 74.3, 74.6, 74.8, 75.3, 75.8, 76.2
IR (cm^{-1})	1646.0, 1460, 1420, 1345, 1270, 1154, 1108, 1077, 1040, 1012, 914, 865	1607, 1419, 1345, 1272, 1154, 1107, 1015, 916, 863
Average molecular weight	Around 5000	5524

(c) On oxidation with sodium hypochlorite/ Sodium hydroxide: On reaction with sodium hypochlorite/ sodium hydroxide polysaccharide is oxidized to corresponding carboxylic acid as indicated by presence of 1607 peak in IR. Presence of peaks such as 157.2 and 189.6 (Table 6).

Table 6: NMR. IR and MW data pre and post oxidation with sodium hypochlorite/sodium hydroxide

Analysis data	**Result of polysaccharide before reaction**	**Analysis result of product obtained upon oxidation with sodium chlorite**
^{13}C NMR δ (ppm)	63.2, 68.2, 68.4, 72.2, 72.9, 74.1, 74.5	68.3, 72.3, 72.4, 72.9, 73.0, 74.2, 74.3, 76.2, 76.2, 100.5, 100.6, 100.9, 107.2, 157.2, 189.6
IR (cm^{-1})	1646.0, 1460, 1420, 1345, 1270, 1154, 1108, 1077, 1040, 1012, 914, 865	1607, 1416, 1347, 1277, 1152, 1107, 1076, 1016, 917, 844
Average molecular weight	Around 5000	4763

(c) GPC of oxidized Polysaccharide before and after the dia filtrations (Table 7).

Table 7: GPC data pre and post oxidation and dia-filtration

Material	**Average MW in Daltons**
Polysaccharide	4500 to 5500
After oxidation	3000-3500 (Because of glucose units formation)
After Dia filtrations	4000-4500 (After removal of glucose units through permeate)

(d) Degree of oxidation monitored by HPLC (Table 8).

Table 8: HPLC monitoring data during oxidation

Volume of sodium hypo chlorite	**Reaction time**	**Oxidized polysaccharide area %**	**Polysaccharide area %**
0.5 V	2 h	4.49	90.38
1.0 V	22 h	7.56	25.51
1.5 V	6 h	16.75	62.51
1.5 V	24 h	15.02	19.77
1.5 V	144 h	21.18	36.21
2.0 V	22 h	17.04	22.93
3.0 V	6 h	31.51	34.48
3.0 V	48 h	28.01	52.18
3.0 V	96 h	31.65	31.91
4.5 V	96 h	28.37	19.32
6.0 V	20 h	35.42	ND

EXPERIMENTAL

Preparation of oxidized polysaccharide

General procedure

10 g of low molecular weight dextran was dissolved in 30 ml of Milli-Q water and stirred till the solution becomes clear. 0.45 ml of 5% NaOH solution in water was added and stirred for 30 min. Sodium hypochlorite solution was added (in different mole ratios or volumes wrt to dextran) to the reaction mixture. pH was maintained at 12.5 using 2 M NaOH by means of pH stat. Reaction mass stirred till 6 ml of NaOH was consumed. pH of the reaction mass was adjusted to 5.5 using concentrated hydrochloric acid. In a separate flask 500 ml of methanol was added. The reaction mass obtained after HCl addition was added to this methanol in 2nd flask. The mixture was stirred for 1 h. The reaction mixture was filtered and dried under vacuum to get 8.5 g of oxidized dextran (Figure 1).

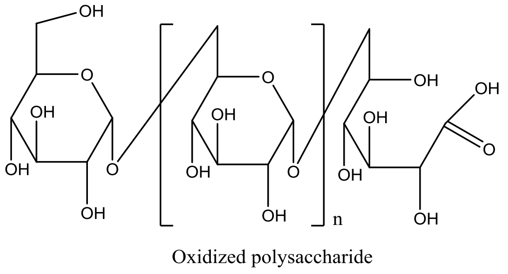


Figure 1: Oxidized polysaccharide

IR (cm^{-1}) 3391, 2926, 2075, 1606, 1418, 1347, 1272, 1207, 1154, 1106, 1077, 1014, 915, 849, 764, 1H NMR (D_2O, 300 MHz) 4.98 (1.00 H, s), 4.013-3.844 (2.196 H, m), 3.783-3.702 (2.43 H, m), 3.603-3.498 (2.05 H, m), 3.357 (0.62 H, s) ^{13}C NMR 181.5, 134.8, 100.9, 100.5, 76.2, 63.29, 51.7.

Oxidation of dextran (Average MW 5000) conducted with various amount of sodium hypo chlorite with other purification techniques

Example 1

A mixture of dextran (100 g) and sodium hydroxide (0.5 g) in water was stirred at room temperature for 30 min. Sodium hypo chlorite was added (1 volume wrt to dextran) to the mixture. The reaction mixture was stirred for 6 h at 30°C. The reaction mixture was quenched in methanol and stirred for 60 min. Solids obtained was filtered and washed with methanol. The solids was dried under vacuum afford the product as white solid. Average molecular weight (4210).

1 H NMR (D_2O, 300 MHz) δ 5.0 (1 H), 3.9 (2.39 H), 3.7 (2.47 H), 3.5(2.0 H).

Example 2

A mixture of dextran (100 g) and sodium hydroxide (0.5 g) in water was stirred at room temperature for 30 min. Sodium hypo chlorite (0.5 volumes wrt to dextran) was added mixture. The reaction mixture was stirred at room temperature for 6 h. The solution was purified using membrane process (dia filtration using 1000 Daltons molecular weight cutoff) against pure water until the specific conductivity 6 ms/cm was achieved. The solution was spray dried at 100°C to get dextran powder. The reaction mixture was the solids were dried under vacuum at 40°C to afford the product as white solid.

1 H NMR (D_2O, 300 MHz) δ 4.9 (0.99 H), 3.93 (2.27 H), 3.7 (2.59 H), 3.5(2.0 H). Average molecular weight (4320).

Example 3

A mixture of dextran (100 g) and sodium hydroxide (0.5 g) in water was stirred at room temperature for 30 min. Sodium hypo chlorite (2 volumes wrt dextran) was added mixture. The reaction mixture was stirred for 6 h at 40°C. The solution was purified using membrane process (dia filtration using 1000 Daltons molecular weight cutoff) against pure water until the specific conductivity 6 ms/cm was achieved. The reaction mixture was quenched in methanol and stirred for 60 min. Solids obtained was filtered and washed with methanol. The solids was dried under vacuum afford the product as white solid. Average molecular weight (3820).

Preparation of reduced polysaccharide

Example 4

A mixture of dextran (100 g) and sodium hydroxide (0.5 g) in water was stirred at room temperature for 300 min. Sodium borohydride (1 mole ratio) was added to the mixture. The reaction mixture was stirred at room temperature for 6 h. The solution was purified using membrane process (dia filtration using 1000 Daltons molecular weight cutoff) against pure water until the specific conductivity of 3 ms/cm was reached. The solution was spray dried to get reduced dextran powder. The solids was dried under vacuum afford the product as white solid. Reducing sugar: 2.4% w/w (Figure 2).

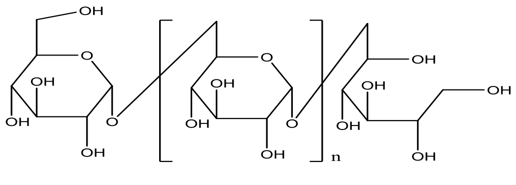


Figure 2: Reduced polysaccharide

NMR: δ 4.9 (1.0 H), 4.5 (1.02 H), 3.7 (0.97 H), 3.5(1.82 H), 2.9 (1.082 H), reducing sugar: 0.36% w/w.

Example 5

A mixture of dextran (100 g) and sodium hydroxide (0.5 g) in water was stirred at room temperature for 600 min. Sodium boro hydride (2 mole ratio) was added to the mixture. The reaction mixture was stirred for 6 h at 35°C. The solution was purified using membrane process (dia filtration using 1000 Daltons molecular weight cutoff) against pure water until the specific conductivity of 3 ms/cm was reached. The solution was spray dried to get reduced dextran powder. Reducing sugar: 1.1% w/w.

Example 6

A mixture of dextran (100 g) and sodium hydroxide (0.5 g) in water was stirred at room temperature for 30 min. Sodium borohydride (2.6 mole ratio) was added to the mixture. The reaction mixture was stirred at room temperature for 6 h. The solution was purified using membrane process (dia filtration using 1000 Daltons molecular weight cutoff) against pure water until the specific conductivity 3 ms/cm was reached. The reaction mixture was quenched in methanol and stirred for 60 min. Solids obtained was filtered and washed with methanol. The solids was dried under vacuum afford the product as white solid. Reducing sugar: 0.30% w/w (Tables 9, 9a and 9b).

Table 9: Table of reducing sugars

Material	Reducing sugars
Polysaccharide	9-10%
Oxidized polysaccharide	1-2%
Reduced polysaccharide	0.50%

Table 9a: Table of Oxidized sugars

Oxidized Polysaccharide	Reducing Sugars
0.5 volumes of sodium hypo chlorite	3.10%
1.0 volumes of sodium hypo chlorite	2.20%
1.5 volumes of sodium hypo chlorite	1.60%
2.0 volumes of sodium hypo chlorite	0.90%

Table 9b: Table of reducing sugars

Reduced Polysaccharide	Reducing Sugars
1.0 mole ratio of sodium borohydride	2.40%
1.5 mole ratio of sodium borohydride	1.60%
2.0 mole ratio of sodium borohydride	1.10%
2.6 mole ratio of sodium borohydride	0.30%

CONCLUSION

In conclusion oxidation and hydrogenation of polysaccharides has been studied comprehensively. Various conditions oxidation and hydrogenation of polysaccharide resulted in formation of the various monosaccharide, oligo- and polysaccharides and of oxidized polysaccharides. This may impact the physical and chemical properties of the product to be formed with these polysaccharides. Therefore process of preparation and consistency in preparation will determines the properties of final formulation prepared from these polysaccharides.

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