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Characterization of Acidic Groups in Oxycelluloses. I. Identification of Various Functional Groups

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Synopsis

A large number of oxycelluloses were prepared and modified further by treatment with sodium borohydride as well as chlorous acid, and qualitative color tests were carried out to detect the presence of various functional groups. Uronic acid groups were found to be present in most of the samples. Enediol groups were not detected in any of the samples, although the samples after sodium carbonate treatment gave distinct color tests for enediols, indicating the presence of α-hydroxymonoketo groups. Characteristic color tests for lactones were observed for most of the samples. The intensity of color developed in the lactone test was, however, found to decrease progressively on contact with a KI–KIO_3 mixture for various periods. The difference in the values of carboxyl content between the methylene blue method and the iodometric method is thus mainly caused by the opening of lactones in the latter method.

INTRODUCTION

The action of various oxidizing agents on cellulosic materials results in the formation of acidic groups as well as reducing groups, depending upon the conditions of reaction. Estimation of carboxyl groups is usually carried out by a number of methods based on the ability of acidic groups to react with organic cations or metal salts.[1–5] Thus, they can be estimated in terms of their silver binding capacity,[1] calcium binding capacity,[2] and the capacity to absorb methylene blue cations.[3] An iodometric method for the estimation of carboxyl groups was suggested by Nabar and Padmanabhan[4] and subsequently modified by Achwal, Nabar, and Padmanabhan.[5]

The presence of other types of functional groups has, however, an effect on carboxyl values as determined by various methods, and differences are always observed between carboxyl values for the same sample by different methods. Reducing groups are known to affect the carboxyl content values by methods involving treatment with alkalies.[6,7] The interference of reducing groups in oxycelluloses can be minimized by sodium borohydride and chlorous acid treatments. The presence of enediols and α-hydroxymonoketo groups has also been reported by some research workers, and these groups may interfere during determination of acid content.[8,9]

Lactone groups which may be formed in the cellulose macromolecule either during oxidation or subsequent decationization[10] can affect the acid

content values by various methods.[7,11,12] Different types of lactone groups are known to react at different rates with KI–KIO_3 solution; and on this basis Slavik, Pasteka, and Kucerova have suggested a method for the characterization of carboxylic and various lactone groups in pulps.[11,12]

As information about the presence in oxycelluloses of functional groups which are likely to interfere in the determination of acidic groups is quite limited, in the present work, a number of oxycelluloses were prepared and modified further by sodium borohydride treatment as well as chlorous acid treatment, and the presence of various types of functional groups was initially qualitatively ascertained. The possible effect of the presence of these groups on the determination of acid content of oxycelluloses by the methylene blue absorption method as well as the iodometric method was studied by special experiments and by studying typical model compounds.

EXPERIMENTAL

Standard Cellulose. 20's Single yarn made from Indian cotton was kier boiled, carefully bleached, and used as a starting material.

Preparation of Oxycelluloses. Oxidation was carried out in the dark by treating 100 g of sample with respective oxidizing agent at 30°C at a liquor ratio of 50:1 (Table I). In the case of phosphoric acid–sodium nitrite oxycellulose, however, the reaction was carried out at 10°C at a liquor ratio of

TABLE I
Preparation of Oxycelluloses

Sample	Oxidizing agent	Concentration of reagent	pH	Time of reaction, hr	Reference
S	unoxidized standard cellulose	—	—	—	—
O_1	potassium dichromate + sulfuric acid	0.033*N* 0.134*N*	strongly acidic	15.0	14
O_2	potassium dichromate + sulfuric acid	0.10*N* 0.20*N*	strongly acidic	15.0	14
O_3	potassium dichromate + oxalic acid	0.20*N* 1.00*N*	strongly acidic	4.0	4
O_4	sodium periodate + sulfuric acid	0.01*N* 0.01*N*	acidic	5.5	14
O_5	sodium periodate + sulfuric acid	0.01*N* 0.01*N*	acidic	15.5	14
O_6	sodium periodate + sulfuric acid	0.01*N* 0.01*N*	acidic	25.5	14
O_7	sodium hypochlorite	2.8 g/l. available chlorine	6.70	4.0	15, 16
O_8	phosphoric acid + sodium nitrite	87% 200 g	highly acidic	1.0 at 10°C	20
O_9	potassium permanganate	0.1*N* (buffered)	7.90	4.0	17
O_{10}	sodium hypobromite	0.04*N* (buffered)	8.85	4.0	18, 19

30:1 Part of each oxycellulose sample was further treated with sodium borohydride[20] and chlorous acid.[21] All the samples were rendered cation free and conditioned before analysis.

Determination of Acid Content of Oxycelluloses. The acid content was determined by the iodometric method[4] as well as the methylene blue absorption method.[22]

Estimation of Uronic Acid Content. The uronic acid content was measured by Anderson's method,[23] except that 12% hydrochloric acid was used as a decarboxylating agent for 6 hr.

Quantitative Test for Uronic Acid Groups.[24–26] The test was performed on the hydrolyzate, using ethanolic carbazole solution, when purple color stable for about 1 hr developed owing to the formation of 5-carboxy-2-formylfuran.

Qualitative Test for α-Hydroxymonoketo Groups.[9] The test was performed after initial isomerization to enediol groups on heating with sodium carbonate solution. Blue color developed on addition of phosphotungstic reagent to the above.

Qualitative Test for Lactones.[27,28] The test involves treatment of sample with hydroxylamine hydrochloride followed by ferric chloride when brown or pink color development takes place. The intensities of colors developed during above three tests were graded as follows:

	No color	Tinge	Pale	Light	Medium	Dark	Intense
Grade	0	1	2	3	4	5	6

Determination of Bromine Number. Bromine number was determined by the standard method of Mcilhiney.[29]

RESULTS AND DISCUSSION

Acid Content of Oxycelluloses

In the present work, different types of oxycelluloses were prepared under standard conditions by using a number of oxidizing agents to get oxycelluloses differing in their functional groups (Table I). Some of these oxycelluloses were subjected to sodium borohydride treatment and chlorous acid treatment. For all these samples, total carboxyl content was determined by the iodometric method as well as the methylene blue method, and uronic acid content was determined separately (Table II).

It can be seen from Table II that in all the dichromate–sulfuric acid and periodate-oxidized celluloses, as well as in celluloses oxidized by phosphoric acid–sodium nitrite and sodium hypochlorite, the acid content values by the iodometric method are higher than the values by the methylene blue absorption method. In periodate oxycelluloses, as the degree of oxidation increases, the difference in the acid content values measured by the two methods also increases. In the case of sodium hypobromite, potassium permanganate, and potassium dichromate–oxalic acid oxycelluloses, the values by both methods are of similar order. The value of uronic acid content is found to be quite high in the case of phosphoric acid–sodium

TABLE II
Carboxyl Content Values by Various Methods

Sample[a]	Carboxyl content, meq/100 g: Iodometric method	Methylene blue method	Uronic acid content
S	1.10	1.10	0.30
SB	1.00	1.10	0.30
SC	1.40	1.40	0.80
O_1	2.60	2.40	1.90
O_1B	2.30	2.30	1.35
O_1C	10.00	10.10	8.00
O_2	4.50	4.05	3.10
O_2B	4.10	4.00	2.45
O_2C	12.00	11.90	8.00
O_3	4.10	4.15	3.95
O_3B	4.00	3.95	3.45
O_3C	20.60	21.10	18.55
O_4	1.90	1.30	0.35
O_4B	1.60	1.25	0.25
O_4C	19.40	19.75	0.90
O_5	3.00	1.30	0.40
O_5B	1.50	1.20	0.25
O_5C	25.10	24.90	1.45
O_6	3.90	1.35	0.40
O_6B	1.60	1.25	0.25
O_6C	29.10	28.95	1.45
O_7	4.40	3.65	2.40
O_7B	3.50	3.55	1.30
O_7C	10.00	9.10	6.70
O_8	105.60	86.50	79.70
O_8B	83.20	83.80	65.05
O_8C	121.00	114.80	84.60
O_9	3.40	3.50	1.60
O_9B	3.20	3.40	1.25
O_9C	6.00	6.10	3.05
O_{10}	2.85	2.80	1.70
$O_{10}B$	2.90	2.80	1.45
$O_{10}C$	5.30	4.45	3.85

[a] Suffixes B and C represent oxycelluloses after treatment with sodium borohydride (48 hr) and chlorous acid, respectively.

nitrite oxycellulose, showing that this sample contains acid groups mainly of glucuronic type, confirming the earlier observations.[19]

The values of acid content for all the oxycelluloses after sodium borohydride treatment for 48 hr are also given in Table II. Most of the reducing action of sodium borohydride was found to be taking place in the first 48 hr, and further change on prolonging the time up to 120 hr was found to be of a small order. Acid content estimated by the methylene blue absorption method shows practically no change for all the samples. Values of uronic acid content are found to be decreased in all cases. A de-

crease in uronic acid content after borohydride treatment indicates that in all the samples, some of these groups exist in the lactonized form, which is destroyed by the sodium borohydride treatment.

On comparison of the carboxyl content values after chlorous acid treatment (Table II), a considerable increase is noticed in the values of both the iodometric and the methylene blue absorption methods. Uronic acid content also shows an appreciable increase after chlorous acid treatment in most of the oxycelluloses. A slight but definite increase is observed in uronic acid content of periodate oxycellulose. This can be due to the fact that although the oxidation is confined mainly to the C 2 and C 3 positions, there is always a side reaction occurring at the C 6 position forming aldehydic groups.

The difference in the values of carboxyl content by the methylene blue method and the iodometric method observed in the case of various samples mentioned above is likely to be caused by the interfering action of some other functional group present which probably can react only under the conditions of one of the methods. Hence, the presence of groups likely to give acidity was tested in the oxycellulose and their modifications by specific qualitative tests. The effect of typical functional groups on the value of carboxyl content by the two methods was confirmed by selecting some model compounds.

Identification of Uronic Acid Groups

The presence of the uronic type of acid groups in various oxycellulose samples was detected by means of the carbazole test after hydrolysis in sulfuric acid. The intensities of purple colors observed were in good agreement with the values of the uronic acid content in Table II. Almost all samples give fairly good tests with the exception of periodate oxycellulose as well as standard cellulose. The intensities of colors for all samples decrease to some extent after sodium borohydride reduction. Some of the uronic acid groups may be present in the lactonized form and will become reduced and hence will not contribute to the color development. Increase in color intensity after chlorous acid treatment showed the presence of some aldehyde group at C 6 in all oxycelluloses.

Identification of α-Hydroxymonoketo and Enediol Groups

A color test employing phosphotungstic reagent has been suggested by Kaverzneva and Salova[27] to detect the presence of α-hydroxymonoketo groups. In this test, these groups are first converted to enediol form by boiling in 10% sodium carbonate.

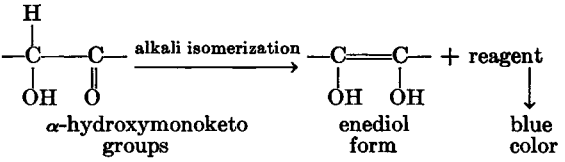


TABLE III
Qualitative Tests for α-Hydroxymonoketo and Lactone Groups in Oxycelluloses

Oxycellulose and its modifications	Intensities of colors developed due to			
	α-Hydroxy-monoketo groups	Lactone groups		
		Sample	After KI–KIO_3 treatment for	
			24 hr	120 hr
S	0	1	0	0
SB	0	0	0	0
SC	1	1	0	0
O_2	4	3	2	0
O_2B	1	3	2	0
O_2C	3	4	3	0
O_3	4	4	3	1
O_3B	3	3	2	1
O_3C	4	5	4	1
O_5	4	4	3	1
O_5B	1	3	2	0
O_5C	3	5	4	2
O_7	4	4	3	1
O_7B_2	3	3	2	1
O_7C	4	5	4	1
O_8	5	6	5	2
O_8B	4	5	4	2
O_8C	5	6	5	3
O_9	3	3	2	0
O_9B	1	3	2	0
O_9C	2	4	3	1
O_{10}	3	3	2	0
$O_{10}B$	1	3	2	0
$O_{10}C$	2	4	3	1

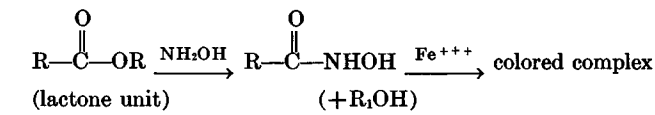
Except in the case of standard cellulose, all oxycelluloses showed some color development, indicating the formation of α-hydroxymonoketo groups during oxidation (Table III). In general, the formation of these groups seems to be relatively higher for oxidations under acidic conditions than under alkaline conditions. After borohydride treatment of all samples, only a weak test is obtained indicating practically complete reduction of these groups. Chlorous acid treatment caused only a little decrease in color intensity. However, when the isomerization pretreatment with sodium carbonate was omitted, all the oxycelluloses and their modifications gave a negative test. A significant color test was obtained under these conditions with catechol and L-ascorbic acid used as model compounds containing enediol groups. Thus, in the oxycelluloses themselves, the α-hydroxymonoketo groups are possibly present as such and not in the isomerized enediol form. During bromine number determination of ascorbic acid, a large difference was observed between the readings before and after potassium iodate addition due to oxidation reaction converting enediol groups into the diketo form. Absence of such reaction for all oxycelluloses as

well as no bromine number confirms that α-hydroxymonoketo groups are less likely to be present in oxycelluloses in the isomerized enediol form.

In order to examine the possibility of conversion of α-hydroxymonoketo groups into enediol groups under conditions of estimation of carboxyl content by the iodometric method, the following experiment was carried out. About 3–6 ml of iodometric solution (KI–KIO_3, NaCl–$Na_2S_2O_3$) was added to about 10 mg of sample and kept for 24 hr. The sample was then washed and tested with the reagent for enediol groups but without the sodium carbonate isomerization. A negative test was again obtained for all oxycelluloses and their modified samples.

Identification of Lactone Groups

All types of oxycelluloses were subjected to hydroxylamine and ferric chloride tests as follows:



A positive test (Table III) is obtained in almost all the oxycelluloses as well as their modifications, although to varying degrees. Standard cellulose(S) as well as its chlorous acid modification give just a light tinge, indicating that there are no appreciable lactone linkages. The borohydride-treated modification of standard cellulose does not give any color at all. Fairly good colors are obtained for potassium dichromate–sulfuric acid (O_1,O_2), potassium dichromate-oxalic acid (O_3), periodate (O_4,O_5,O_6), alkaline potassium permanganate (O_9), and hypobromite (O_{10}) oxycelluloses. The presence of lactone groups in pulps oxidized by periodate and sodium hypochlorite has also been reported by Kaverzneva.[9]

For all oxycelluloses, an increase in the color intensity is observed after chlorous acid treatment, indicating that some of the carboxyl groups formed by oxidation of aldehydic groups are lactonized during decationization.[10] The presence of lactone groups in periodate–chlorite oxycelluloses has been reported by various research workers.[12,30] A decrease in color intensity was observed after borohydride treatment due to the destruction of some of the existing lactone groups. However, even after borohydride treatment for 120 hr, the color, although less intense, still persists as all the lactone groups present are not destroyed. By far the most intense brown color is observed in the case of phosphoric acid–sodium nitrite oxycellulose, showing that it contains quite appreciable amount of lactone groups. This observation substantiates earlier observations that this oxycellulose contains mostly glucuronic acid lactone.[19,31] The color becomes still more intense after chlorous acid treatment, while only a little decrease in color intensity is noticed after borohydride treatment.

Cleavage of lactones with KI–KIO_3 solution has been studied earlier in sugar lactones[32] as well as in modified pulp celluloses.[11,12] Slavik et al.[11,12]

attempted the estimation of the lactone form of acidic groups in pulp celluloses by measurement of the color intensity of material in sheet form after transformation to hydroxamic acids and reaction with ferric chloride. An attempt to carry out a similar study on cotton celluloses was not successful, as it was not possible to get the fibrous samples in the form of suitable sheets for colorimetric estimation. Hence, to get qualitatively an idea of the extent of cleavage of various lactone groups, the following procedure was adopted.

Freshly decationized oxycellulose samples were treated with an iodometric solution ($KI–KIO_3$, $NaCl–Na_2S_2O_3$) for periods of 24 and 120 hr and subjected to the lactone test. From the results (Table III), it can be seen that a distinct decrease in color intensity after 24 hr of cleavage and a further decrease after 120 hr is observed for oxycelluloses as well as their borohydride-treated modifications. In contrast to this, the chlorous acid modifications of oxycelluloses show comparatively a more intense color, indicating that the cleavage of lactones by $KI–KIO_3$ is still not complete as the initial lactone content is higher.

Presence of D-glucuronic acid lactone has been confirmed in the hydrolyzates of various samples (except standard cellulose after borohydride treatment, periodate oxycellulose, and their modifications) by paper-chromatographic technique. Presence of the glucono lactone could not be definitely ascertained as the R_f value obtained was very near to that of glucose under the conditions employed for chromatographic study.

Study of Model Compounds

The possibility of the reaction of lactone groups and α-hydroxymonoketo groups during the estimation of carboxyl content by the methylene blue method as well as the iodometric method was then investigated by studying typical model compounds (Table IV). For all model compounds studied, practically no methylene blue absorption was observed (weights taken for analysis are kept low enough to avoid pH change), showing that lactones, α-hydroxymonoketo, as well as enediol groups have no effect on the acidity estimated by the methylene blue method. Under the conditions of carboxyl group estimation by the iodometric method, however, only benzoin,

TABLE IV
Studies on Model Compounds

Model compound	Methylene blue method	Iodometric method	
	acid content, meq/100 g	Acid content, meq/100 g	Extent of reaction, %
D-Glucono-δ-lactone	nil	528	94
D-Glucuronic acid lactone	nil	500	88
Benzoin	nil	nil	nil
L-Ascorbic acid	nil	1190	100

which contains α-hydroxymonoketo groups, does not show any reaction. L-Ascorbic acid, which contains enediol groups, gives quantitative evolution of iodine, while both D-glucono-δ-lactone and D-glucuronic acid lactones show a considerable extent of cleavage.

CONCLUSIONS

The presence of α-hydroxymonketo as well as lactone groups and less likelihood of the presence of enediol groups in oxycellulose has been established by qualitative tests. The extent of changes in these functional groups caused by sodium borohydride and chlorous acid treatment has also been studied. Cleavage of lactone groups by KI–KIO_3 solution has been shown to take place by qualitative tests as well as by reactions of model compounds. Hence, the differences in the carboxyl content values estimated by the methylene blue absorption method and the iodometric method for oxycelluloses as well as their modifications can be attributed to the cleavage of existing lactone groups.

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