

# Determination of carboxyl groups in the starch oxidized by nitrogen dioxide

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In the studies on starches oxidized by nitrogen dioxide, a comparison of some methods of carboxyl group determination was made with low- and middle-oxidized samples. Iodometric estimation was found to be a suitable method. In a direct alkalimetric estimation, the consumption of titrating agent is not adequate and difficult to reproduce, because of the presence of lactones and other alkali labile functional groups formed in the side reactions of oxidative sample preparation. Lactones can be cleaved in the products by prolonged standing in water at room temperature or by short heating in water. Direct alkalimetric estimation affords then results comparable with other methods, but the carboxyl groups lactonize relatively quickly again.

В рамках изучения крахмалов, модифицированных двуокисью азота, в низко и средне окисленных образцах было проведено сравнительное изучение некоторых методов определения карбоксильных групп. Самым подходящим методом оказалось иодометрическое определение. При прямом алкаиметрическом определении бывает расход титранта неадекватным и плохо воспроизводимым вследствие присутствия лактонов и других алкалилабильных функциональных групп, возникающих в результате побочных реакций при окислительной модификации образцов. Лактоны в продуктах можно разрушить длительным стоянием в холодной воде или кратковременным нагревом в водной среде. Прямое алкаиметрическое определение после этого дает результаты, сравнимые с полученными другими методами, однако карбоксильные группы опять довольно быстро лактонизируются.

Polysaccharides oxidized by various oxidizing agents, and among them the oxidized starch, find an ever increasing importance in industry. In nonselective oxidations (using hypochlorites, hydrogen peroxide, etc.), it is possible to prove the presence of aldehyde, carbonyl, and other functional groups in the reaction products. In a relatively selective oxidation with nitrogen dioxide, the carboxyl groups are mainly formed and, with the substituted derivatives, the carboxyl groups are present almost exclusively. In all cases of the oxidized starches (with an exception of polysaccharide dialdehydes), it is necessary to determine the carboxyl

groups and to know their reactivity and, therefore, much attention is devoted to them in the analysis.

The products obtained on oxidation of polysaccharides of cellulose and starch types with nitrogen dioxide form, with respect to the functional groups present, a relatively heterogeneous material. In this oxidation, the main reaction is the conversion of primary hydroxyl group at the carbon C-6 of the glucose unit to a carboxyl group, which was confirmed earlier with cellulose [1] and starch [2]. So far, more attention has been devoted to cellulose, where it was also found that a part of carboxyl groups formed was present as lactones.

It is known that in carboxyl group estimation in oxidized polysaccharides by various methods, different results are obtained. It was shown that mainly in a direct alkalimetric estimation, but also in other methods, several factors play an important role. The most important one is the pH of reaction medium. In their works, authors do not give usually the pH value of final titration point. This might be, however, a source of discrepancies in the individual determinations. It concerns basically a very complex system of consecutive acid-base equilibria of variously acidic groups (with different but close  $pK$ ), the solution of which is not an easy task.

Beside the main reaction, a side reaction takes always place in oxidation of glucans with nitrogen dioxide, *i.e.* partial oxidation of the secondary hydroxyl groups of the saccharide unit giving rise to an  $\alpha$ -hydroxyketo grouping, which is further converted to an endiol in alkaline medium or an opening of the pyranose ring of glucose moiety takes place between the carbon atoms C-2—C-3 [1]. In the oxidized polysaccharides of cellulose or starch types it can be presumed a similar  $\beta$ -carbonyl ether degradation in alkaline medium, caused by an inductive effect of electronegative group as it is reported, *e.g.* in alkaline cooking and other processes [3].

*Samuelson* and *Wennerblom* attempted to saponify lactones in a deionized sample of cellulose in 0.01 M-NaOH [4], but they were criticized as for the way they carried out the estimation [5].

Several authors [6, 7] use for the determination of carboxyl groups in oxidized starches or cellulose a method based on reaction of the carboxyl group with the  $Ca^{2+}$  or  $Ba^{2+}$  ions (the sample is allowed to stand with calcium or barium acetate and the free acetic acid is liberated and determined). The method is very simple and is performed in mild acidic medium and gives well reproducible results. We used it as a reference method in our work.

Opening of lactones at  $pH > 8$  leads to a higher alkali consumption as expected. A comparison of alkaline delactonization with the method of carboxyl group estimation by Methylene Blue was made by *Philipp et al.* [8, 9], who also found a higher alkali consumption indicating the appearance of new acidic groups. The method of determination of carboxyl groups with Methylene Blue is more suitable for the products having a low carboxyl content; we have not used it in our work.

With respect to the problems connected with the both lactone opening and reactivity of the  $\alpha$ -hydroxyketo grouping in a saccharide unit, it is recommended to estimate the carboxyls in oxidized saccharides at  $\text{pH} < 7$ . With cellulose it must be considered that a part of lactones is cleaved at  $\text{pH} > 9.5$  [10], where the destruction of pyranose ring may occur, too.

Iodometric estimation using a solution of  $\text{KIO}_3 + \text{KI}$  is suitable for a need to work at  $\text{pH} < 7$ . *Timmel* studied by this method hydroxy and uronic acids [11]. *Nabar* and *Padmanabhan* [12, 13] applied this method for cellulose. They used  $\text{KIO}_3 + \text{KI} + \text{NaCl}$  solution in the presence of an excess of sodium thiosulfate with the subsequent estimation of remaining thiosulfate by a titration with iodine solution. Theoretical values were achieved after 24 h. In this method, an unfavourable effect of the liberated iodine is avoided, which in the original working procedure was estimated by titration with thiosulfate after terminating the reaction; this method offers a little higher values. An indirect method was modified further — the reaction time was lowered to 1 h with the simultaneous temperature increase to  $60^\circ\text{C}$ , the reaction was carried out in a closed vessel [14]. In our work we did not use the latter approach since we obtained a worse reproducibility of results.

*Slávik et al.* [10, 15, 16] undertook systematical studies on the iodometric determination of lactones in cellulose. They confirmed on the basis of kinetic measurements the presence of different type lactones in samples. The method of indirect iodometric estimation was also used in the determination of products of periodate oxidation of saccharides and mainly of ester-bound formic acid after maltose oxidation [17].

Less data can be found in literature on the nature of functional groups in starch. *Ellington* and *Purves* [18] made a systematic analysis of oxidized starches but did not consider the problems of lactones. As for the highly oxidized starches prepared by oxidation with nitrogen dioxide, a knowledge obtained with monocarboxycellulose was confirmed and complemented in the way that monocarboxystarch was even more alkali labile than the former polymer [19]. Most of analytical works is restricted to the alkalimetric estimation of samples or reports on a method using calcium acetate [7].

In this work we compared several methods of carboxyl group estimation in low- and middle-oxidized starches and attempted to find conditions where the degradation effect of alkali on this relatively reactive substrate was not yet apparent.

## Experimental

Two samples of maize starch were used, oxidized by gaseous nitrogen dioxide for 3 or 6 h according to [20]. Using the reference method I (see later), sample A contained 7.66%

COOH and sample B 14.29% COOH. Both samples gave positive reaction on lactones and the  $\alpha$ -hydroxyketo grouping.

The alkalimetric measurements were performed by potentiometric titration using  $0.1 \text{ mol dm}^{-3}$  NaOH.

### *Determination of carboxyl groups*

#### *Method I — reaction with calcium acetate*

A sample (70—100 mg) was allowed to stand with the solution of calcium acetate ( $10 \text{ ml } 0.1 \text{ mol dm}^{-3}$ ) in water (50 ml) for 24 h and the amount of acetic acid released was determined alkalimetrically being proportional to the carboxyls present [7]. The method was selected as the reference one.

#### *Method II — indirect determination of NaOH consumption*

A sample (70—100 mg) was allowed to stand with NaOH solution ( $10 \text{ ml } 0.1 \text{ mol dm}^{-3}$ ) in water (50 ml) for 24 h and the amount of remaining agent was estimated by titration with  $0.1 \text{ mol dm}^{-3}$  HCl. For better comparison the both values were expressed as COOH, like in other methods.

#### *Method III — direct alkalimetric determination of COOH*

A sample was determined after delactonization using the method VIIa.

#### *Method IV — direct alkalimetric determination of COOH*

A sample was determined after delactonization using the method VIIb.

#### *Method V — indirect iodometric determination of COOH [12]*

A sample (50 mg) is allowed to stand in a solution of  $\text{KIO}_3 + \text{KI} + \text{NaCl}$  (20 ml, solution: 50.0 g NaCl + 21.4 g  $\text{KIO}_3$  + 83 g KI + 5 g  $\text{Na}_2\text{S}_2\text{O}_3$  added to 1000 ml of distilled water) with subsequent estimation of remaining thiosulfate by titration with  $0.01 \text{ mol dm}^{-3}$  solution of iodine using the biamperometric indication in an equipment according to [21].

### *Other procedures*

#### *Method VI — determination of the carbonyl groups [22]*

Carbonyl groups were determined before and after delactonization of samples by method VIIb on a principle of the reaction of samples with hydroxylammonium chloride in ethanol followed by titration of HCl released with NaOH ( $0.1 \text{ mol dm}^{-3}$ ) to pH 3.32.

#### *Method VII — delactonization of samples*

a) A sample (50 mg) is allowed to stand in distilled water (50 ml) for 48 h (see method III).

b) A sample (50 mg) is heated in distilled water (50 ml) for 15 min to 95—100 °C and cooled (see methods IV and VI, respectively).

*Method VIII — proof of lactones [6]*

The presence of lactones was proved on a principle of the reaction of sample with hydroxylammonium chloride and a colour reaction with  $\text{FeCl}_3$ .

*Method IX — proof of  $\alpha$ -hydroxyketo grouping [23]*

The presence of  $\alpha$ -hydroxyketo grouping was proved on a principle of isomerization of this grouping to an endiol in a solution of sodium carbonate; after cooling the colour reaction was developed with a phosphotungsten agent.

### Results and discussion

The values obtained are always an average of three estimations with the relative error (except of method *III*)  $< 1\%$ . A method of estimation with calcium acetate, modified by Mercer, was shown to be a suitable one in the carboxyl group estimation (Table 1, method *I*). Approximately the same results were also obtained after the reaction time prolongation from 24 to 48 h. The isolated calcium salts obtained after 24 h standing of samples with the agent did not give a positive reaction on lactones (method *VIII*) thus indicating that under the given conditions of estimation the lactones present reacted, too.

Standing of the sample in an excess of  $\text{NaOH}$  led, similarly as with highly oxidized samples [18], to expected values after 4 h, but the consumption was increasing with time continuously further without an appropriate stabilization and after 24 h it represented the values inadequately high, which pointed to deeper degradation in alkaline medium (method *II*).

It was found, however, that after 48 h dialysis or standing in water (method *VIIa*) the prevailing amount of carboxyl groups was present in an opened form and it was possible to estimate them by a direct alkalimetric method (method *III*).

Table 1

Functional groups (mass %) in the starch samples partially oxidized with nitrogen dioxide estimated by various methods

Sample	COOH					CO	
	<i>I</i>	<i>II</i>	<i>III</i>	<i>IV</i>	<i>V</i>	<i>VI</i>	
						<i>a</i>	<i>b</i>
<i>A</i>	7.66	11.35	6.80	7.09	7.70	0.72	0.70
<i>B</i>	14.29	26.22	13.59	13.90	14.40	1.65	1.53

For methods *I*—*VI* see Experimental.

A similar effect was also achieved after heating of samples in water to boiling point (method IV). In both cases, the determination must be accomplished immediately and in the latter after cooling. In both cases the values were obtained, which were very close to those obtained by reference method I. No interaction of the  $\alpha$ -hydroxyketo groups with alkaline hydroxide could be observed under the conditions of estimation. The potentiometric curves had a common shape.

In the indirect iodometric carboxyl estimation (method V), the reaction course was followed for 48 h (Table 2). The constant values were achieved after 12 h. These corresponded well to the values obtained according to method I (Table 1).

In the partially oxidized starch samples the distance among individual carboxyl groups is larger than in the fully oxidized products and, consequently, their interaction is lower and acidity less suppressed, which reflected shapes of the corresponding potentiometric curves in the alkalimetric COOH estimation of samples. Interaction of COOH with the primary hydroxyl groups of unoxidized glucose units is also possible in the given situation, which is not possible with the fully oxidized samples.

The carboxyl group estimation according to method VI was accomplished with hydroxylammonium chloride in ethanol followed by the titrimetric estimation of HCl released up to pH 3.32, when lactones have not yet reacted with the agent and this was confirmed by the carbonyl determination before and after delactonization using method VIIb. Approximately the same values were obtained in both cases (Table 1).

The presence of lactones was followed only qualitatively by method VIII. As it has been stated already, lactones were not proved in calcium salts isolated from reaction medium according to method I, which indicated that lactones reacted under the conditions of estimation.

In conclusion, it can be stated that the polysaccharides of starch type possessing characteristic structural features and higher reactivity than cellulose react faster within the individual methods under study. Of course, the problems arising as a consequence of the presence of oxidized groups at carbons C-2 or C-3 of

Table 2

Time dependence of COOH estimation (mass %) in the starch samples partially oxidized with nitrogen dioxide by an indirect iodometric method V

Sample	$\tau$ /h									
	0.5	1	2	4	6	8	12	16	24	48
A	5.50	6.51	6.80	7.05	7.40	7.53	7.70	7.72	7.80	7.97
B	12.00	12.60	12.92	13.30	13.51	14.10	14.40	14.45	14.50	14.85

a saccharide unit are displayed in alkaline medium, which was used for cleavage of lactones, at first in the increased alkali consumption.

It can be concluded on the basis of results obtained that for the sample characterizations of low- and middle-oxidized starches, method *I* may be used. For a rough orientation, the alkalimetric COOH estimation is suitable after delactonization by method *VIIa* or *VIIb*. The direct titration of samples of the oxidized starch containing lactones, which are not stable in starch, does not give reliable results. Therefore, the method should also be applied in practice [24] only for other derivatives, e.g. for the determination of substitution degree of carboxymethyl derivative. Likewise, alkaline cleavage of lactones is not suitable, since it offers values considerably higher than expected (Table 1, method *II*).

The iodometric COOH determination in oxidized starches offers the most sensitive method and as far as the interference groups are not present, it can be recommended. It is more suitable to use the indirect method of estimation applied in our work, too, in which the iodine released is removed immediately from reaction medium by the reaction with sodium thiosulfate and has no possibility to react with other groups of the relatively reactive substrate. It was not possible to determine the portion of free and lactone-bound carboxyl groups because of the fast reaction of oxidized starch with the iodometric agent.

A simple method of sample delactonization was found. Lactones are little stable in the partially oxidized starch but after 48 h storage of delactonized samples in a solid state they can be estimated again.

The problems of analysis of oxidized polysaccharides and mainly of the carboxyl group estimation are very complex and the study of side reactions in interaction of samples and alkali will require further investigation.

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