Starch dialdehyde. V.* Indirect determination of the degree of oxidation by a hydrazone method

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A method for indirect determination of the degree of oxidation of starch dialdehyde has been elaborated. It is based on the reaction of 2,4-dinitrophenyl- or 4-nitrophenylhydrazine with aldehyde groups in starch dialdehyde followed by the iodometric determination of the excess of the reagent using biamperometric indication. The formed hydrazones present in the analyzed mixture do not interfere with the determination.

The advantage of the newly developed method lies in its simplicity, and its results are virtually identical with those obtained by means of the standard hydroxylamine method.

Разработан метод косвенного определения степени окисления диальдегида крахмала, который основан на реакции с 2,4-динитрофенилгидразином или 4-нитрофенилгидразином и последующем определении не прореагированного реагента иодометрически с биамперометрической индикацией. Присутствие образовавшихся гидразонов не мешает определению и их удаление перед анализом не требуется.

Метод приводит к результатам практически тождественным с результатами стандартного гидроксиламинового метода и его преимущество состоит в относительной несложности.

The determination of the degree of oxidation of starch, *i.e.* the aldehyde group assay in starch dialdehyde, can be accomplished by a number of methods but the obtained results vary somewhat since each method is carried out under different working conditions [1]. For routine analysis of starch dialdehyde we have found most preferable, although relatively time-consuming, hydroxylamine method based on the original procedure of *Gladding* and *Purves* [2].

Another method, based on the reaction of the aldehyde groups in starch dialdehyde with 4-nitrophenylhydrazine [3] and applicable mainly for samples with low oxidation degrees, requires isolation of the formed hydrazones to be subse-

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* For Part IV see Ref. [1].

quently determined colorimetrically. Since the key reaction involved here is performed at elevated temperature the products may undergo degradation which may, in turn, distort the results.

In older applications of the hydrazone method the products were determined gravimetrically [4]. More advantageous are procedures involving extraction of the formed hydrazones with a suitable solvent and their determination by spectrophotometry at 345 nm or, alternatively, in an alkaline medium at 480 nm. The method [5], however, is rather tedious.

In the method of Japanese workers [6] according to which the oxidation by periodate is immediately followed by deionization all products resulting from the reaction of aldehydes are converted to hydrazones and the individual products, isolated by chromatography, are determined spectrophotometrically. The method is not applicable to starch dialdehyde owing to its insolubility.

Attempts have also been made to determine aldehydes or their hydrazones indirectly, by the assay of 2,4-dinitrophenylhydrazine, added in excess. *Berka* and *Zýka* [7] have determined the unused reagent with chloramine T in the presence of potassium bromide. According to *Buděšínský* [8] the assay of the same reagent can be performed by its oxidation with copper(II) acetate directly in the reaction mixture in an inert atmosphere.

Attempts to determine the excess of phenylhydrazine acidimetrically or oxidimetrically with Fehling's solution in pyridine—benzene mixture were unsuccessful since the procedure was found to be very laborious and gave inaccurate results [5].

Iodometrical phenylhydrazine assay, using iodine as the oxidizing reagent, has been described [9] but the results were inaccurate and reproducible only with difficulty, mainly when starch was used as the indicator.

It has been pointed out by Japanese authors [10] that simple aldehydes or their corresponding 2,4-dinitrophenylhydrazones can be determined indirectly by oxition with iodine of the unused reagent and subsequent assay of iodine, added in excess, by titration with sodium thiosulfate and bipotentiometric indication. In the present work the reaction of starch dialdehyde with 2,4-dinitro- and 4-nitrophenylhydrazine has been studied from a similar point of view.

Experimental

Samples of starch dialdehyde used throughout this work were prepared [11] from maize starch produced at Slovak Starch Works, Boleráz.

The content of dialdehyde groups (DAG), based on dry samples, determined using the standard oximation method [2], was as follows: Sample No. *1:* 99.2% DAU, and Sample No. *2:* 72.9% DAU.

Reagents

The following standard solutions were prepared:
0.0125 M-2,4-dinitrophenylhydrazine in 1 M hydrochloric acid (*I*),
0.0125 M-4-nitrophenylhydrazine in 1 M hydrochloric acid (*II*),
0.01 M iodine (I), *c* 1.2692 mg/ml,
0.01 M sodium thiosulfate.

The titrations were monitored using a biamperometric equipment described previously [12].

Indirect determination of aldehyde groups (degree of oxidation) in starch dialdehyde with 2,4-dinitro- or 4-nitrophenylhydrazine by the new method

The suspension of a sample of starch dialdehyde, corresponding to 16—20 mg of dry highly oxidized starch, in water (10 ml) is treated with stirring in a stoppered flask with *I* or *II* (10 ml) for 2 h at room temperature. After addition of the standard, 0.01 M solution of iodine the mixture is left in the closed flask for 20 min and 0.01 M sodium thiosulfate (15 ml) is added. Its excess is then titrated with 0.01 M iodine solution using biamperometric indication.

When using 2,4-dinitrophenylhydrazine the calculation of the per cent amount of oxidized (dialdehyde) groups in starch is as follows

$$\%\ \mathrm{DAU} = \frac{80.822 \times \text{used-up reagent [mg]}}{\text{dry sample [mg]}}$$

The amount of the used-up reagent in mg is equal to the reagent added [mg] minus 0.495 $(\mathrm{A}+\mathrm{X}-\mathrm{B})$.

When 4-nitrophenylhydrazine is the reagent used,

$$\%\ \mathrm{DAU} = \frac{104.571 \times \text{used-up reagent [mg]}}{\text{dry sample [mg]}}$$

where the amount of the used-up reagent in mg is equal to the reagent added [mg] minus 0.382 $(\mathrm{A}+\mathrm{X}-\mathrm{B})$, and here:

A = ml of the 0.01 M iodine solution added to the mixture after the hydrazone had been formed,

X = ml of the 0.01 M iodine solution used-up in the back-titration,

B = ml of the 0.01 M sodium thiosulfate solution added prior to the back-titration.

Results and discussion

In continuation of our previous work [1] we have studied the reaction of two highly oxidized samples of starch with 2,4-dinitro- and 4-nitrophenylhydrazine. The aim of the present study was to elaborate an indirect method of determining the degree of oxidation of starch by way of iodometric determination of the excess of the used reagent using biamperometric indication. The method was to eliminate the tediousness of the oximation method [2] and, at the same time, was hoped to be less laborious than the hydrazone method [3] requiring the isolation of the formed hydrazone. We have modified the conditions commonly applied when simple aldehydes are determined [10] in a way to suit the determination of dialdehyde groups in starch without removing the hydrazone formed. Hydrazones of starch dialdehyde are stable under the given conditions in the presence of iodine. Fig. 1 shows the time dependence of the reaction of starch dialdehyde with both reagents.

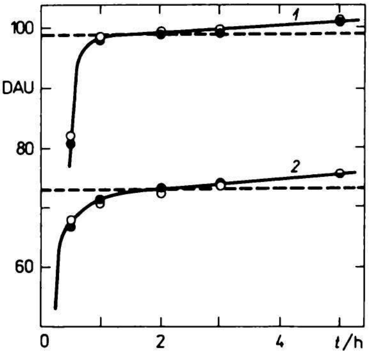

Fig. 1. Reaction of starch dialdehyde with the individual reagents.
1. Sample No. *1*; *2.* Sample No. *2.*
● 4-Nitrophenylhydrazine;
○ 2,4-dinitrophenylhydrazine.

The data shown are mean values of three determinations; the relative error of the determination was for all experiments less than ± 1%. The degree of oxidation of the studied samples (~ 100 and 70%) lies within the range of the oxidation degrees of oxidized starches having practical applicability.

The principle of the reaction of aldehydes with substituted phenylhydrazines is the nucleophilic attack of the reagent and the reaction rate depends upon its nucleophilicity and bulkiness, as well as upon the reactivity of the aldehyde, pH, and the solubility of the products. The high reactivity of phenylhydrazine is further enhanced by an electrophilic substituent (such as a nitro group) as it breaks the symmetry of the electron distribution in the molecule. The lower basicity of such compounds facilitates the shift of the reaction equilibrium towards the formation of the products making it possible for the aldehydes to react also under acidic conditions. Depending upon the type of the aldehyde the reaction time ranges from few minutes to several hours.

The aldehyde groups in freshly prepared starch dialdehyde are very reactive. They can be reduced under mild conditions, oxidized or condensed with a variety of reagents. These reactions are quantitative or give high yields of the desired products. Older samples of starch dialdehyde show lower reactivity which manifests itself as a decrease of the number of effective aldehyde groups as a result of "aging". These phenomena were discussed in our previous work [1].

Table 1

Indirect determination with 2,4-dinitrophenylhydrazine of DAU *vs.* time in starch dialdehyde

Reaction period h	Sample No. *1*				Sample No. *2*			
	Sample size mg	Reagent used mg	DAU found mg	DAU theoretical %	Sample size mg	Reagent used mg	DAU found mg	DAU theoretical %
0.5	21.05	21.48	17.36	83.1	25.19	21.26	17.18	93.6
1.0	17.60	21.48	17.36	99.4	24.30	21.36	17.26	97.4
2.0	17.52	21.54	17.40	100.1	23.77	21.48	17.36	100.2
3.0	17.49	21.50	17.37	100.1	23.56	21.43	17.32	100.8
5.0	17.05	21.36	17.26	102.1	22.88	21.50	17.37	104.2

Table 2

Indirect determination with 4-nitrophenylhydrazine of DAU *vs.* time in starch dialdehyde

Reaction period h	Sample No. *1*				Sample No. *2*			
	Sample size mg	Reagent used mg	DAU found mg	DAU theoretical %	Sample size mg	Reagent used mg	DAU found mg	DAU theoretical %
0.5	20.91	16.20	16.94	81.7	25.35	16.25	16.99	91.9
1.0	17.24	16.25	16.99	99.3	23.79	16.27	17.01	98.1
2.0	17.12	16.27	17.01	100.1	23.21	16.23	16.97	100.3
3.0	17.02	16.30	17.04	100.9	22.96	16.30	17.04	101.8
5.0	16.89	16.35	17.09	102.0	22.43	16.20	16.94	103.6

The present method minimizes the possibilities of substrate degradation as the reaction conditions are mild (no heating is applied). In view of the reactivity and the nature of the studied material the acidic conditions of the procedure are suitable and their unfavourable effects can be noticed only after longer reaction periods.

The time-dependence curves show that the reaction is fast even in its first stages. The moderate increase of the determined values noticeable after 4—5 h is negligible compared with the methods developed previously.

In contrast to simple aldehydes a unit of starch dialdehyde having a hemialdal or a hemiacetal structure reacts merely with one molar proportion of substituted hydrazines, which is in analogy to the similar reaction with hydroxylammonium chloride.

In the newly developed method an electrometric indication is indispensable since the solutions are coloured, making the use of a colour-indicator impossible. A spectrophotometric indication cannot be used because of the presence of the interfering insoluble hydrazones. The indication by biamperometry has been chosen as this method is commonly used in starch chemistry for the determination of amylose and in the evaluation of periodate oxidation [12].

When compared with the oximation method the sample size required is reduced by a factor of 10 which is an advantage particularly when working with precious samples.

A comparison of the procedures involving 2,4-dinitro- and 4-nitrophenylhydrazine shows that the time-dependence curves are virtually identical. Thus, in view of the high reactivity of starch dialdehyde the introduction of another nitro group into the molecule of the reagent has no effect upon the reaction rate. Tables 1 and 2 show the results of experiments run with these reagents; the determined average degree of oxidation is shown in Fig. 1.

Experiments run with freshly prepared samples of starch dialdehyde showed good agreement with the widely used oximation method [2] while the time required for one determination (~2 1/2 h) is substantially shorter than that required by the standard procedure (~5 1/2 h). The method is both simple and accurate and as such it should find application in the production, processing, and use of starch dialdehyde. It should also be possible to use it for monitoring of the process of aging of starch dialdehyde and in the analysis of dialdehydes formed from other polysaccharides.

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