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ORIGINAL PAPER

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Determination of reducing carbonyl groups in cellulose in the solvent system LiCl/*N,N*-dimethylacetamide

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Abstract The 2,3,5-triphenyltetrazolium chloride (TTC) method for the determination of reducing carbonyl groups in cellulose in water suspensions has been thoroughly evaluated. By reduction of TTC in alkaline medium, triphenylformazane (formazane) is produced. It is thermally unstable and an unavoidable loss results in a relatively high detection limit for the determination of reducing carbonyl groups in most celluloses with a low content of such groups. Besides, oxidized cellulose is unstable in the alkaline media in which the reaction is performed. The determined content of reducing groups has also been shown to be affected by the amount of sample, indicating that absorption of triphenylformazane takes place. A new method for determination of reducing carbonyl groups in cellulose in homogeneous medium has been developed. Cellulose is dissolved in the solvent system LiCl/*N*,*N*-dimethylacetamide and reacts with TTC in the presence of *tert*-butylamine at 75 °C for 10 min and formazane is subsequently determined spectrophotometrically at 524 nm. None of the systematic errors inherent in the "aqueous" suspension method can be defined. The new procedure is also more reproducible and has a lower detection limit of 7 nmol reducing groups in a given mass of sample. The two procedures have been tested on five differently oxidised cellulose samples and the difference in the determinations are discussed.

1 Introduction

Cellulose is a relatively stable natural polymer, but may be oxidized if exposed to UV-light and atmospheric oxygen or during pulping processes. During oxidation the polymer does not necessarily decompose; it may retain the polymer structure, yet containing monomers with aldehyde, ketone or carboxyl groups. The physical and chemical properties of oxidized cellulose may be severely altered. In the polymer degradation process, introduction of oxidized functional groups is a step leading to depolymerization.

A few classical methods for the determination of functional groups exist and were recently briefly reviewed [1]. Common to the majority of methods is that they are time consuming and use alkaline media that have a destructive effect on the oxidized polymer [2]. In this paper we evaluate an existing spectrophotometric method for the determination of reducing carbonyl groups in cellulose [3] and point out some systematic errors, which may well be common to all methods in which alkaline aqueous suspensions of cellulose fibres are used:

1. Cellulose degradation under alkaline reaction conditions [4]. During the process new carbonyl groups are formed by β-alkoxyl elimination and subsequently an α-hydroxyl acid forms by the benzilic acid rearrangement mechanism. Some authors have tried to overcome this problem by time extrapolation methods [5].
2. Accessibility of functional groups. Functional groups mostly form in the readily accessible regions of cellulose fibres, *i.e.* the amorphous regions, unless an oxidizing agent is used to which the crystalline parts of cellulose are also accessible. Such an agent is iodate (VII) and possibly other low molecular weight oxidants. In such a case, the determined and the actual contents of functional groups differ, especially if the cellulose is of a high degree of polymerization [2, 6].
3. Adsorption of reaction products on suspended cellulose particles also leads to erroneous determinations.

To overcome these common problems we describe a new analytical procedure for the determination of reducing carbonyl groups in cellulose in solution. The

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solvent used should preferably be non-degrading and complex-forming in order to avoid possible side reactions of oxidized groups in cellulose. According to the literature [7] the solvent system LiCl/*N*,*N*-dimethylacetamide meets both demands.

2 Experimental

Reagents and solutions. The model compounds D(+)-glucose, D(−)-fructose, gluconic acid lactone, potassium gluconate (for biochemistry, Merck, Darmstadt), methylethylketone pur., acetophenone pur., and benzophenone pur. (Fluka, Buchs) were used to test the selectivity of the method. For the colour reaction 2,3,5-triphenyltetrazolium chloride (TTC) (for biochemistry, Merck, Darmstadt), KOH p.a. and *tert*-butylamine (*t*-BuA) p.a. (Merck, Darmstadt) were used.

All aqueous solutions were prepared with Milli-Q water. Solutions in DMA were prepared with *N*,*N*-dimethylacetamide p.s. (Merck, Darmstadt) and LiCl p.a. As a solvent for triphenylformazane and as a solvent exchanger, methanol p.a. (Merck, Darmstadt) was used.

The source of cellulose was 2040A chromatographic paper of 100% cotton pulp (Schleicher-Schüll, Dassel). Torn in pieces, the paper was put into deionized water and stirred well to produce a pulp suspension. The cellulose was filtered through a glass filter (porosity grade: 4), dried and weighed outside the oven (ASTM Standard Test D 1348-89).

Potassium phenoxide was synthesised according to a known procedure [8].

All solutions are stable for at least a month, provided that light is excluded. TTC solutions are light-sensitive and become coloured within a few hours if not protected. KOH, *t*-BuA and TTC solutions were thus freshly prepared on a daily basis. Cellulose was dissolved under room conditions in a 2 mol L^{-1} solution of LiCl in DMA by a solvent exchange procedure in order to facilitate the swelling of fibres in the following manner: MilliQ water/methanol/DMA/ 2 mol L^{-1} LiCl in DMA.

Instrumentation. All measurements were made at $22 \pm 2\,^{\circ}C$ on a Milton Roy Spectronic 1201 spectrophotometer using a 1 cm quartz cuvette.

Cellulose samples. To acquire different samples, cellulose was oxidized under room conditions ($22 \pm 2\,^{\circ}C$) in four ways: with a 0.051 mol L^{-1} solution of NaClO for 210 min at pH 12, with a 0.046 mol L^{-1} aqueous solution of H_2O_2 (made up from 4% and 30% commercial solutions) for 150 min at pH 12, and with a 0.02 mol L^{-1} aqueous solution of KIO_4 p.a. for 210 min at pH 12, every batch containing 1 g of pulp per 100 mL of solution. The fourth sample was irradiated with an Osram-Vitalux 300 W lamp at a distance of 60 cm from the source of UV-light, firstly for 5 h and subsequently six times for 10 h, with pauses of 14 h, during which the sample was stored without access of light.

DMA purification. Commercially available DMA is not sufficiently pure to be used for analytical purposes. We therefore designed the following procedure to achieve a minimal blank value of the absorbance determined according to the new analytical procedure:

- addition of BaO (1 g per L), followed by distillation under reduced pressure;
- refluxing with CaH_2 (1 g per L) for 1 h, followed by distillation under reduced pressure;
- addition of H_3BO_3 (1 g per L), followed by mixing for 1 h;
- addition of BaO (1 g per L), followed by distillation under reduced pressure;
- repeated distillation under reduced pressure.

3 Results and discussion

3.1 Evaluation of the "aqueous" method

Optimization of the original procedure. The original procedure [3] is as follows: to 0.5 mL of 0.2 mol L^{-1} KOH solution and 0.5 mL of 0.0059 mol L^{-1} TTC solution, a sample of cellulose (ca. 0.01 g) is added and heated in a water bath at $100\,^{\circ}C$ for 10 min in an open 10 mL tube. After immediate cooling to room temperature, the reaction suspension is quantitatively given into a vacuum funnel and filtered through a glass filter (porosity grade: 4) into a 10 mL tube. The pulp is rinsed with p.a. methanol unit a 10 mL mark is reached. Formazane is determined spectrophotometrically at 546 nm.

By recording the absorbance spectrum, we initially showed that the absorbance maximum is at 482 nm and not at 546 nm as reported. The absorbance of a formazane solution in a 9 : 1 methanol : water mixture does not change within 60 min at room temperatures if exposed to daylight and its stability enabled us to make measurements without precautions regarding the exclusion of light.

Optimization of the procedure was performed using glucose as a model compound. Selectivity was proven by the use of fructose, gluconic acid lactone, potassium gluconate, methylethylketone, acetophenone and benzophenone. The reaction took place with none of the above compounds except fructose. Although a ketose, it has reducing properties in alkaline media due to isomerization.

The yield of formazane served as a measure of the extent of the reaction. A plot of the absorbance of formazane solutions *vs.* time was prepared at different reaction temperatures (Fig. 1). The extent of the reaction reaches its maximum only at temperatures higher than $80\,^{\circ}C$; yet at the same time formazane decomposes at temperatures higher than $75\,^{\circ}C$. The decomposition products cannot be dissolved in methanol. The originally proposed conditions of $100\,^{\circ}C$ for 10 min were therefore changed to $80\,^{\circ}C$ for 6 min, conditions under which the extent of formazane decomposition is minimal and at the same time the extent of reaction is maximal.

The concentration of the two reagents TTC and KOH was also optimized. By using slightly higher concentrations than proposed, a more complete reaction was obtained. We changed the original concentrations from 0.0059 mol L^{-1} TTC and 0.02 mol L^{-1} KOH to 0.01 mol L^{-1} and 0.03 mol L^{-1}, respectively.

Measurements on cellulose samples. To prepare a calibration graph, the established changes in the procedure were followed and adequate amounts (0–100 μl) of a 0.0055 mol L^{-1} glucose solution were added to the reaction solution. The statistical parameters are listed in Table 1. The detection limit was defined as the

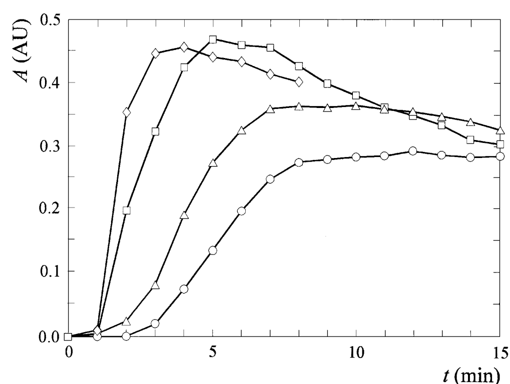

Fig. 1 Dependence of the extent of reaction on the time at different reaction temperatures. ○ = 70 °C; △ = 75 °C; □ = 80 °C; ◇ = 90 °C for the aqueous method

analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank. Formazane solutions follow Beer's law in the calibration region. It is convenient to construct the calibration graph by plotting A *vs.* n (μmol) and not *vs.* c (mol L^{-1}), thus making the calculation of the content of reducing carbonyl groups (μmol g^{-1}) in cellulose samples easier.

The inherent loss of formazane is clearly shown by the shift of the interception point of the calibration graph with the horizontal axis from the origin to 0.11 μmol.

To determine the content of reducing carbonyl groups in five cellulose samples, seven individual measurements were made. In Table 2 the mean and relative standard deviations at 6 degrees of freedom are listed for all samples.

For all samples except iodate (VII) oxidized cellulose, the reducing carbonyl group content is low; so we cannot achieve an absorbance higher than 0.1 AU, even with higher amounts of sample (up to 0.07 g in the case of untreated cellulose). The uncertainty of values is thus too high.

Identification of systematic errors. By heating the cellulose sample in alkaline solution we actually perform an alkaline extraction. The fluctuation in the content of reducing carbonyl groups under such conditions is illustrated in Fig. 2. The reactions taking place have been studied in detail by numerous authors [4, 9–12] especially in the case of iodate (VII) treated celluloses. One of the basic processes is β-alkoxycarbonyl elimination. More reducing groups form in the first step, but after the benzilic acid rearrangement of a vicinal dicarbonyl compound an α-hydroxy-carboxylic acid forms, which is no longer a reducing compound. In order to follow the process, we altered the determination procedure and first added only KOH solution to the cellulose sample, heated the mixture and only after a specified time added the TTC solution to allow reduction to take place.

By plotting the content of the reducing carbonyl groups *vs.* mass of sample (Fig. 3), we can observe a dependence that can be attributed to adsorption of formazane on cellulose. In a homogeneous solution, this phenomenon should not be present.

Another systematic error inherent in all aqueous methods may also be pointed to: we can never determine all the reducing groups as they are not accessible to the reagent. This point can only be proven if we raise the accessibility of the TTC reagent to 100% by

Table 1 Statistical parameters of the calibration graphs

Parameter	Aqueous method	DMA method
Intercept a	– 0.32 AU	– 0.005 AU
Gradient b	2.9 AU μmol^{-1}	5.7 AU μmol^{-1}
Correlation coefficient r	0.9983	0.9992
Number of measurements n	15	18
Limit of detection LOD	0.11 μmol	0.007 μmol
Number of measurements of the blank n_b	28	34

Table 2 Content of reducing carbonyl groups in different cellulose samples as determined by the aqueous method and by the new DMA method

Sample	Aqueous method		DMA method	
	Content of –CHO (μmol g^{-1})	RSD (%)	Content of –CHO (μmol g^{-1})	RSD (%)
Untreated cellulose	4.2	40	7.7	6.2
Cellulose irradiated with UV-light	7.2	15	10.9	4.7
Celllulose treated with H_2O_2	5.1	23	6.1	6.9
Celllulose treated with NaClO	11.3	8.8	9.7	4.2
Celllulose treated with KIO_4	57.9	3.4	43.3	1.4

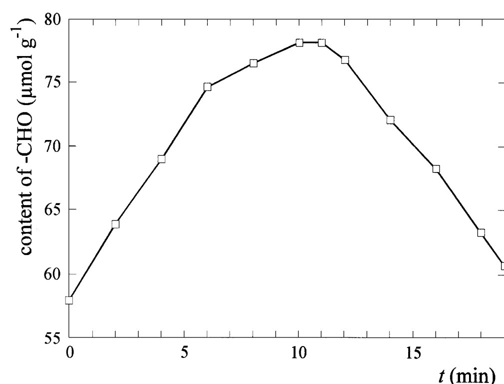

Fig. 2 Dependence of the content of reducing groups on the alkali extraction time of a KIO_4-treated sample as determined by the aqueous method

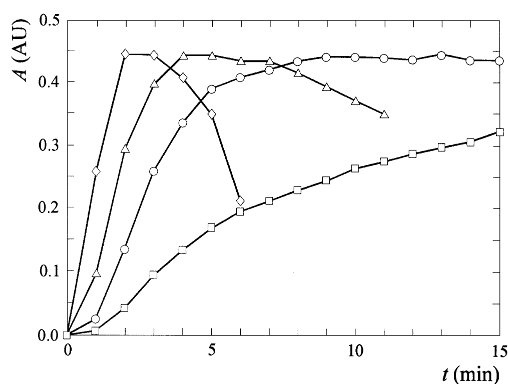

Fig. 4 Extent of reaction as a function of time at different reaction temperatures: □ = 60 °C; ○ = 70 °C; △ = 80 °C; ◇ = 90 °C for the DMA method

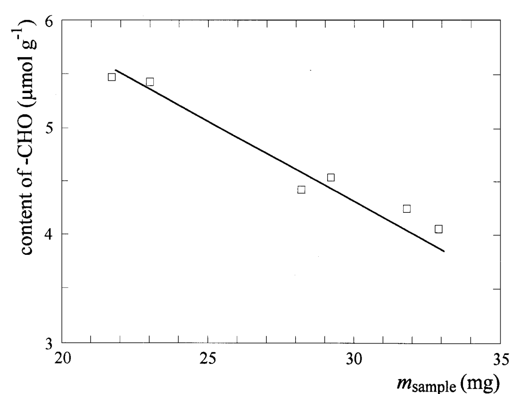

Fig. 3 Dependence of the determined content of reducing groups on the mass of H_2O_2-treated sample as determined by the aqueous method

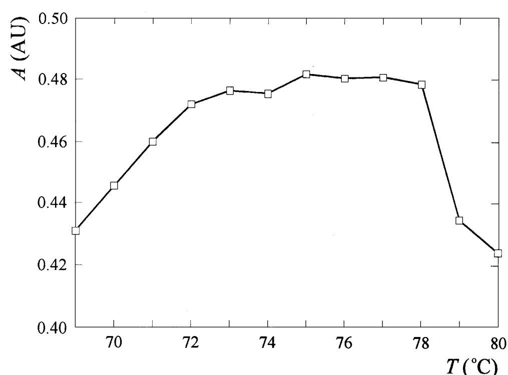

Fig. 5 Extent of reaction as a function of temperature for the DMA method. Reaction time: 10 min

conducting the reaction in a homogeneous solution. Some discussions regarding this problem were already published [13, 14].

3.2 The "DMA" method

Optimization of reaction conditions. The extent of the reaction was determined by measuring the absorbance of formazane solutions in DMA at 524 nm. A considerable red shift of 42 nm compared with the absorbance maximum of a formazane solution in 9:1 methanol: water mixture can be observed.

Figures 4 and 5 indicate that the decomposition takes place at temperatures higher than 78 °C and that the optimal reaction conditions are 75 ± 2 °C and 10 min, the gain of formazane being maximal and the decomposition not determinable spectrophotometrically.

In the reaction, nucleophiles containing hydroxyl or phenoxide anions cannot be used on account of precipitation of LiOH or $LiOC_6H_5$. If *t*-BuA is used, the reaction only takes place in the presence of LiCl. Thus, the concentration of LiCl has also to be optimized, and at the same time must not drop under 1 mol L^{-1} to prevent precipitation of cellulose. Therefore, LiCl is added to all solutions of reactants. The optimization of the reagent concentration is presented in Fig. 6. To achieve maximal yield, we recommend that the minimum concentration of reactants in the reaction mixture must be 0.4 mol L^{-1} *t*-BuA and 0.4 mmol L^{-1} TTC.

In order to examine the selectivity of the method, we used the model compounds. Apart from glucose and fructose, TTC reacts with none of them.

Analytical procedure and calibration. The following stock solutions in purified DMA were prepared:
– Solution A: 0.01 mol L^{-1} TTC and 1 mol L^{-1} LiCl;
– Solution B: 2 mol L^{-1} *t*-BuA and 1 mol L^{-1} LiCl;
– Solution C: 2 mol L^{-1} LiCl.

To 0.5–1 mL of 0.1% cellulose solution (it is advisable that the cellulose solution is weighed due to its high viscosity), 2 mL of solution A are added and the

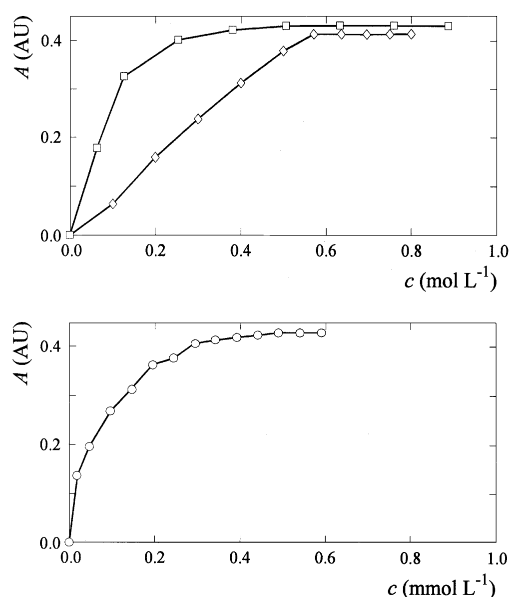

Fig. 6 Extent of reaction as a function of the concentration of the reactants. □ = *t*-BuA; ◇ = LiCl; ○ = TTC for the DMA method

mixture is stirred well in a 10 mL graduated test tube. Subsequently, 1 mL of solution B is added and the mixture is diluted by solution C to the 4 mL mark. The tube is heated for 10 min at 75 °C in a water bath, followed by immediate cooling to room temperature and diluted with purified DMA to the 5 mL mark. The absorbance of the formazane solutions is then measured.

To prepare a calibration graph, different aliquots (0–200 µl) of 1 mmol L^{-1} glucose solution in purified DMA are added to the reaction mixture instead of cellulose solutions. The graph is constructed by plotting the absorbance *vs.* amount of reducing carbonyl groups. The statistical parameters of the calibration graph are given in Table 1. Compared with the aqueous method, there is no formazane decomposition as indicated by the absence of a shift of the intercept. The limit of detection was defined as under 3.1. The new DMA method has a limit of detection 15 times lower than the aqueous method. The reproducibility is also significantly improved.

Measurements on cellulose samples. The contents of reducing carbonyl groups determined in five different cellulose samples are given in Table 2. Comparing the data for both methods, we can conclude that there are no considerable differences in the determinations for samples treated with H_2O_2 and NaClO, as the main oxidation products are keto- and carboxyl groups [15, 16]. Possibly due to hindered access, determinations of reducing groups by the aqueous method are lower than those established by the new method for the untreated sample and the sample irradiated by UV light.

To prove that, unlike during the aqueous procedure, the elimination mechanism of degradation does not take place, a similar experiment was performed: To allow the elimination to occur before oxidation, the TTC solution was added to the reaction mixture of *t*-BuA and cellulose only after different time delays. No fluctuation in the determinations was observed. The difference between the determinations of reducing carbonyl groups in the cellulose treated with KIO_4 can thus be explained.

4 Conclusions

The performance of the original analytical procedure for the determination of reducing carbonyl groups in cellulose was improved by optimizing the conditions under which the colour reaction is performed, regarding time, temperature, and the concentrations of reagents. The newly recorded absorbance spectrum of triphenylformazane suggests measurements at 482 nm. The coloured substance is stable under room conditions, but not at temperatures higher than 75 °C, thermal decomposition thus leading to a higher detection limit. The method is therefore unsuitable for celluloses with a lower content of reducing groups.

Other systematic errors were identified: adsorption of formazane on cellulose and a positive error due to alkaline extraction. A possible error due to hindered access of the TTC reagent to carbonyl groups has been discussed. These three systematic errors are possibly the main drawbacks of all methods for the determination of functional groups using alkaline suspensions of cellulose.

The new DMA procedure for the determination of reducing carbonyl groups in cellulose was shown to be advantageous over the old one in many aspects: systematic errors arising from phenomena like cellulose degradation, formazane decomposition, hindered access, and adsorption are eliminated. Besides, a better reproducibility and a lower detection limit are achieved. A drawback of the new method is, however, that it is more time consuming due to the dissolution procedure. Our future task is therefore to search for a more practical procedure.

The comparison of the two methods is especially useful in the evaluation of other classical methods used in cellulose and paper chemistry.

References

1. Schleicher H, Lang H (1994) Papier 48:765–768
2. Nevell TP, Zeronian SH (1985) Cellulose chemistry and its applications. Ellis Horwood, Chichester, pp 116, 245, 262
3. Szabolcs O (1961) Papier 15:41–44
4. Lewin M (1987) Proceedings of the Fourth International Symposium on Wood and Pulping Chemistry, Paris, Vol. I, pp 345–353

5. Nevell TP (1963) In: Whistler RL (ed.) Methods in carbohydrate chemistry, Vol III. Academic Press, London, pp 43–49
6. Albertsson U, Samuelson O (1962) Anal Chim Acta 27:434–440
7. Dawsey TR, McCormick CL (1990) J Macromol Sci - Rev Macromol Chem Phys C30:405–440
8. Kornblum N, Lurie AP (1959) J Am Chem Soc 81:2705–2716
9. Albeck M, Ben-Bassat A, Epstein JA, Lewin M (1965) Text Res J 35:836–843
10. Albeck M, Ben-Bassat A, Lewin M (1965) Text Res J 35:935–942
11. Lewin M (1965) Text Res J 35:979–986
12. Fellers C, Iversen T, Lindström T, Nilsson T, Ringdahl M (1989) Ageing/Degradation of Paper - A literature survey; FoU-projektet för papperkonservering, Report No. 1E, Stockholm
13. Dautzenberg H, Phillip B (1974) Faserforsch Textiltechn 25:422–425
14. Blair HS, Cromie R (1977) J Appl Chem Biotechnol 27:205–213
15. Lewin M, Ettinger A (1969) Cell Chem Technol 3:9–20
16. Lewin M, Epstein JA (1962) J Polym Sci 58:1023–1037