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Note

Estimation of dialdehyde groups in 2,3-dialdehyde bead-cellulose

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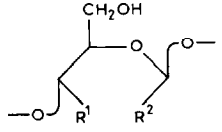
Cellulose in the form of macroporous beads is an important support material for chromatography[1–5]. Periodate oxidation is used widely to activate polysaccharides prior to the covalent binding of bioaffinic groups and spacers, and for the immobilisation of biomacromolecules[1–5]. For these applications, the degree of oxidation has to be controlled and oxidative degradation of the polysaccharides must be minimised[4,6].

Several methods have been described[7–9] for the estimation of aldehyde groups in polysaccharides. The method[7,8] based on the consumption of hydroxyl ions in the Cannizzaro reaction of dialdehyde groups is simple, but the necessary short reaction time is a disadvantage. We now report conditions which allow the Cannizzaro reaction to be applied conveniently to swollen, water-insoluble 2,3-dialdehyde bead-cellulose in the heterogeneous phase.

Treatment of bead-cellulose with periodate cleaves the C-2–C-3 bond in the glucose residues (**1**). In general, periodate-oxidised polysaccharides are unstable in mildly alkaline solutions, as demonstrated by the formation of acidic groups and cleavage of the polysaccharide chain[7,10], which can lead to solubilisation[11]. Thus, limited oxidation is necessary for the formation of a reactive bead-cellulose suitable for immobilisation purposes.



1 $R^1 = R^2 = CHO$
2 $R^1 = COOH, R^2 = CH_2OH$
3 $R^1 = CH_2OH, R^2 = COOH$

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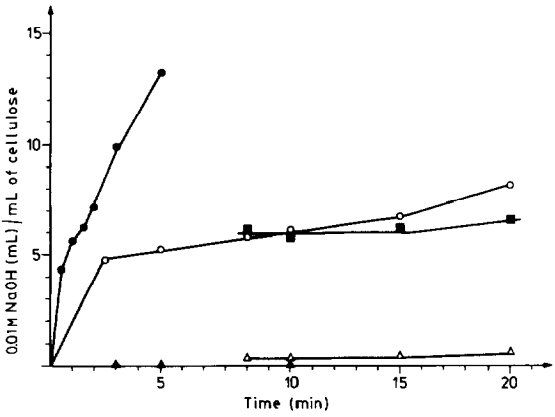

Fig. 1. Consumption of alkali in the Cannizzaro reaction: bead-cellulose (2 mL) in 0.05 M NaOH (4 mL) at 70° (▲) and 100° (△); 2,3-dialdehyde bead-cellulose (2 mL) in 0.25 M NaOH (2 mL) at 100° (●) and 70° (○), and in 0.05 M NaOH (4 mL) at 70° (■).

Hofreiter et al.[7] found that the Cannizzaro reaction of periodate-oxidised starch occurs rapidly with stoichiometric consumption of hydroxyl ions per dialdehyde group. Two possible reaction products (**2** or **3**) are expected[10] by the Cannizzaro reaction of 2,3-dialdehyde bead-cellulose. Bead-cellulose does not react with hydroxyl ions (Fig. 1). The reaction of 2,3-dialdehyde bead-cellulose with hydroxyl ions at 100° (0.25 M NaOH) is not terminated after disproportionation of the aldehyde groups but, at 70° and with 0.05 M NaOH, it reached a plateau after ~10 min (Fig. 1) and, as in the work with periodate-oxidised starch, the hydroxyl ions consumed corresponded stoichiometrically to the concentration of the dialdehyde groups.

With a sufficiently long reaction time, periodate reacts quantitatively with bead-cellulose (Table I). Furthermore, the concentration of dialdehyde groups calculated from the periodate consumed corresponds with that estimated on the basis of the Cannizzaro reaction (Table II).

The effects of temperature and time on the Cannizzaro reaction are illustrated in Fig. 2. The consumption of hydroxyl ions from 0.05 M NaOH was complete within 8–15 min at 70° and after 10 h at room temperature. Table III shows that

TABLE I

Oxidation of bead-cellulose with periodate

Periodate		Reaction time (h)	Dialdehyde groups (μmol/mL beads)
(mg/mL of beads)	(μmol/mL of beads)		
2.6	12.2	2	13.7
3.9	18.2	2	17.6
6.5	30.4	6	31.8
8.4	39.2	4	38.8

TABLE II

Estimation of the dialdehyde groups in periodate-oxidised bead-cellulose

Oxidation time (h)	Dialdehyde groups [a] (μmol/mL of beads)	
	A	B
2	21.5	22.6
6	42.7	44.1

[a] A, Cannizzaro reaction; B, consumption of $NaIO_4$; 13 mg of $NaIO_4$/mL of beads at room temperature.

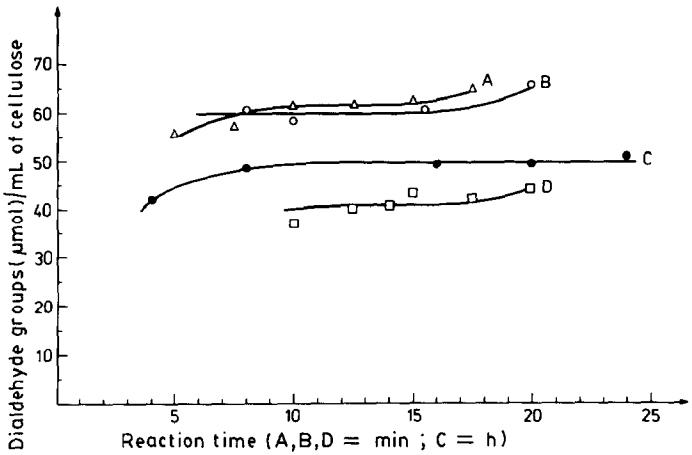

Fig. 2. Reaction of 2,3-dialdehyde bead-cellulose with alkali: A and D, 1 mL of beads in 0.05 M NaOH (4 mL) at 70°; B, 2 mL of beads in 0.25 M NaOH (2 mL) at 70°; C, 1 mL of beads in 0.05 M NaOH (4 mL) at room temperature.

TABLE III

Concentrations of dialdehyde groups in periodate-oxidised bead-cellulose

Oxidation time (h)	13 mg of $NaIO_4$/mL of beads (μmol of dialdehyde groups/mL of beads)		26 mg of $NaIO_4$/mL of beads (μmol of dialdehyde groups/mL of beads)	
	Method A [a]	Method B	Method A	Method B
0.5	7.6	8.7	17.7	16.5
1	14.1	18.4	23.8	23.3
2	21.4	21.9	39.9	37.4
3	27.2	25.8	47.2	41.8
4	31.6	32.5	61.3	62.0
8	46.3	45.4	85.3	83.2
16	55.6	53.4	105.4	110.1
24	58.6	57.0	117.3	119.2

[a] Cannizzaro reaction: A, at 70°, 10 min; B, at room temperature, 24 h.

the two analytical methods give comparable concentrations of dialdehyde groups over a wide range of degrees of oxidation (6–25% of the glucose residues).

In comparison with periodate-oxidised starch, the slower Cannizzaro reaction of periodate-oxidised bead-cellulose can be attributed to the heterogeneous conditions of the latter reaction.

EXPERIMENTAL

Bead-cellulose (particle size 80–200 μm) was obtained from Leipziger Arzneimittelwerk GmbH (Leipzig) or from the Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences (Prague, Czechoslovakia).

2,3-Dialdehyde bead-cellulose[5]. *(a) Preparation.* Bead-cellulose (100 mL) swollen in water was washed on a filter with water in order to remove the stabiliser (NaN_3), then treated with 0.05 M or 0.1 M $NaIO_4$ (120 mL). Each suspension was stirred gently at room temperature for 2, 4, or 6 h and, after the addition of ethylene glycol (30 mL), it was stirred for 1 h. The resulting 2,3-dialdehyde bead-cellulose was collected, washed with water (2 L), and stored at 4° as a suspension in water containing 0.02–0.04% of NaN_3.

(b) Estimation of the dialdehyde groups. (i) Method A (70°). 2,3-Dialdehyde bead-cellulose (1 mL), swollen in water, was freed from the excess of water, then treated with 0.05 M NaOH (4 mL), and the stirred suspension was heated at 70° for 10 min. The suspension was cooled to room temperature during 1–2 min, then added quantitatively to 0.1 M HCl (2 mL), and the excess of acid was titrated with 0.01 M NaOH, using a glass electrode and a pH meter.

(ii) Method B (room temperature). The procedure in Method A was followed, but the suspension was stirred gently for 24 h at room temperature.

The concentration of dialdehyde groups is given by [0.01 M NaOH (mL) – 0.01 M HCl (mL)] × 10 = μmol of dialdehyde groups/mL of gel.

(iii) From the consumption of periodate. Macroporous bead-cellulose (10 mL), swollen in water, was treated with 0.05 M $NaIO_4$ (10 mL) as in (*a*). After 2 and 6 h, the oxidised cellulose was collected, then washed intensively with water, and the residual $NaIO_4$ in the washings was determined iodometrically. The periodate consumed corresponds to the amount of dialdehyde groups generated.

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