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Composition analysis of sulfoethylcelluloses by high-pH anion-exchange chromatography with pulsed amperometric detection

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ABSTRACT

A method for the determination of the distribution of the substituents in sulfoethylcellulose, synthesised from cellulose and ethylenesulfonic acid by a Michael reaction, involves hydrolysis with 1.2 M perchloric acid, and analysis of the resulting mixture of glucose and its sulfoethyl derivatives by high-pH anion-exchange chromatography with pulsed amperometric detection. The reactivity of the hydroxyl groups in cellulose towards sulfoethylation was found to decrease in the order HO-6 > HO-2 ≫ HO-3.

INTRODUCTION

Sulfoethylcellulose is a water-soluble cellulose derivative which can be obtained by a Michael reaction of alkali cellulose with ethylenesulfonic acid added as such[1] or formed in situ from chloroethylsulfonate[2,3]. The degree of substitution (ds) is usually < 3.0 and although reaction can yield mono- (2-, 3-, and 6-), di- (2,3-, 2,6-, and 3,6-), and tri-substituted (2,3,6-) D-glucose residues, no method has been reported for the determination of the distribution of the substituents. Recent advances[4] in the application of high-pH anion-exchange chromatography (HPAEC–PAD) to underivatised carbohydrates prompted the use of this technique to devise a method for the determination of the distribution of substituents in sulfoethylcelluloses.

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EXPERIMENTAL

Materials.—Sulfoethylcellulose samples A–C were obtained from AKZO Research (Arnhem) and methyl β-cellobioside from Koch–Light. Sulfur determinations were carried out by the Analytical Section of the Institute for Applied Chemistry TNO, Zeist (Netherlands).

Hydrolysis procedure.—Hydrolysis was carried out by a modification of the method of Reuben et al[5]. A solution of sulfoethylcellulose (5 mg) in aq 70% $HClO_4$ (0.1 mL) was kept for 10 min at room temperature, then diluted with bidistilled water (0.9 mL), heated for 16 h at 100°, and neutralised with 2 M KOH. The precipitated $KClO_4$ was collected by centrifugation (2500*g*, 5 min) and washed with bidistilled water (2 × 0.5 mL), and the supernatant solutions were combined.

High-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD).—The mixture of D-glucose and its sulfoethylated derivatives was analysed on a Dionex LC system consisting of a Dionex Bio-LC quaternary gradient module, a model PAD-2 detector, a CarboPac PA-1 pellicular anion-exchange column (250 × 9 mm), and a Shimadzu C-R3A recorder/integrator. An aliquot (25 μL) of the combined supernatant solutions was applied to the column, and eluted with 95:5 *A* (0.1 M NaOH)–*B* (0.1 M NaOH containing 2 M NaOAc) for 0.3 min, then with a linear gradient to *B* during 30 min, at 4.0 mL/min and ambient temperature. Detection was performed by PAD with a gold working-electrode and triple-pulse amperometry comprising the following pulse potentials and durations: E_1 0.05 V and 300 ms, E_2 0.65 V and 60 ms, E_3 −0.95 V and 180 ms. The response time of the PAD was set to 1 s. For identification of structures, fractions were isolated, decationised on a column (200 × 20 mm) of Dowex 50W-X8 (H^+) resin (100–200 mesh, Bio-Rad), and lyophilised.

^{13}C NMR and ^1H NMR spectroscopy.—Samples were treated repeatedly with D_2O (99.9 atom% D) and finally dissolved in 99.96 atom% D at pD ≥ 7. Resolution-enhanced 75-MHz ^{13}C NMR spectra (internal acetone, δ 31.55) were recorded at 27° using a Bruker AC-300 spectrometer, and 300-MHz ^1H NMR spectra (internal acetone, δ 2.225) were recorded at 20° using the same spectrometer. In order to obtain quantitatively reliable ^1H NMR results, no resolution enhancement was applied, the HOD signal was not suppressed, and a repetition delay of 5 s was used.

RESULTS AND DISCUSSION

For a reliable analysis of the distribution of substituents in sulfoethylcelluloses, solvolysis must be complete and side reactions avoided. A short incubation with aq 70% $HClO_4$ at room temperature improved the solubility of the substrate and made it accessible for complete hydrolysis in 1.2 M $HClO_4$ (16 h, 100°) without browning. Most of the $HClO_4$ was removed by precipitation of the potassium salt to give samples amenable to HPAEC–PAD.

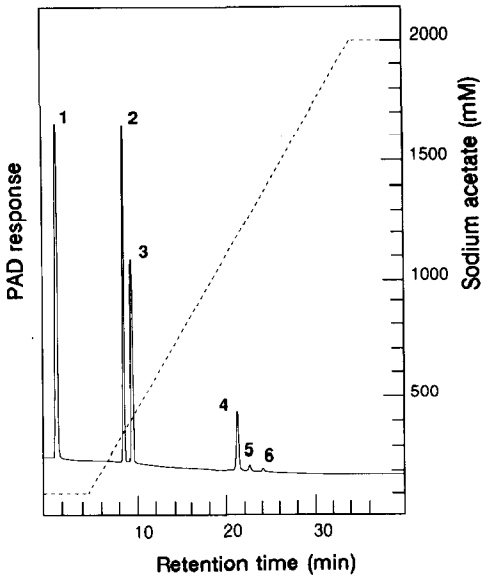

Fig. 1. HPAEC–PAD chromatogram of a sulfoethylcellulose hydrolysate separated on CarboPac PA-1, using a gradient of NaOAc (dotted line) in 0.1 M NaOH at 4 mL/min: **1** D-glucose, **2** 6-*O*-sulfoethyl-D-glucose, **3** 2-*O*-sulfoethyl-D-glucose, **4** 2,6-di-*O*-sulfoethyl-D-glucose, **5** 3,6-di-*O*-sulfoethyl-D-glucose, **6** 2,3-di-*O*-sulfoethyl-D-glucose.

A typical chromatogram of such a hydrolysate is shown in Fig. 1. Fractions **1–6** were collected, desalted, and analysed by ^{13}C NMR spectroscopy (Table I). The spectra of the monosubstituted derivatives were assigned on the basis of the

TABLE I

^{13}C NMR chemical shift data (δ) [a] for glucose and its sulfoethylated derivatives

Carbon	Compound											
	Glc [b]		Position of $CH_2CH_2SO_3$ group									
			2		6		2,3		2,6		3,6	
	α	β	α	β	α	β	α	β	α	β	α	β
C-1	93.24	97.06	90.90	96.79	93.41	97.27	91.38	97.14	91.13	97.07	93.21	96.99
C-2	72.64	75.30	81.01	84.26	72.73	75.37	81.00	84.09	81.17	84.38	72.08	74.64
C-3	73.94	76.92	72.94	76.04	73.98	76.96	82.86	85.73	73.08	76.16	83.56	86.27
C-4	70.83	70.77	70.52	70.38	70.97	70.97	70.14	70.29	70.70	70.70	70.39	70.39
C-5	72.58	77.06	72.35	76.99	71.49	75.95	72.49	76.81	71.30	75.91	71.36	75.62
C-6	61.82	61.96	61.62	61.78	70.74	70.97	61.82	61.97	70.70	70.82	70.60	70.74
2-$CH_2CH_2SO_3$			66.62	68.56			67.01	68.89	66.86	68.78		
2-$CH_2CH_2SO_3$			52.02	51.94			52.33	51.99	52.16	52.25		
3-$CH_2CH_2SO_3$							69.21	69.34			68.96	68.88
3-$CH_2CH_2SO_3$							52.33	52.33			52.19	52.19
6-$CH_2CH_2SO_3$					67.38	67.49			67.39	67.49	67.36	67.46
6-$CH_2CH_2SO_3$					51.71	51.71			51.69	51.69	51.70	51.63

[a] Relative to the signal for internal acetone (δ 31.55) in D_2O. [b] Assignments made according to ref. 6.

expected substituent shift effects[7], namely, signals of α-carbons shifted downfield by 7–11 ppm and signals of β-carbons shifted upfield by 1–2 ppm. The effects of monosubstitution, as determined for the 2- and 6-substituted derivatives, were used to assign the spectrum of the 2,6-disubstituted derivative, taking into account additivity rules[8]. A ^{13}C NMR spectrum of the 3-substituted derivative was not available, so that the spectra of the 2,3- and 3,6-disubstituted derivatives were assigned on the basis of those of 2,3- and 3,6-di-*O*-carboxymethyl-D-glucose, making use of similar substitution effects[5]. Thus, it was found that fraction **1** contained D-glucose, fractions **2** and **3** contained 6- and 2-*O*-sulfoethyl-D-glucose, respectively, and fractions **4**, **5**, and **6** contained 2,6-, 3,6-, and 2,3-di-*O*-sulfoethyl-D-glucose, respectively. The remaining two monomers that could be present theoretically, namely, 3- and 2,3,6-tri-*O*-sulfoethyl-D-glucose, were not observed. The order of elution of the sulfoethylated glucoses on CarboPac PA-1 was identical to that of the corresponding carboxymethyl derivatives[9], which suggests that the nature of the anionic substituent is not important, although the sulfoethyl derivatives required a higher concentration of NaOAc for elution, reflecting the more acidic sulfoethyl group.

In principle, the Michael addition reaction for the sulfoethylation of cellulose should be reversible. Treatment of sulfoethylcellulose with 9:1 2-propanol–water containing 2 mol of NaOH per mol of "anhydroglucose" unit for 25 h at room temperature resulted[9] in a small decrease in ds. This result points to the instability of the sulfoethyl group under alkaline conditions and possible interference with the HPAEC. However, the comparatively mild conditions (0.1 M NaOH, 30 min, room temperature) used in the HPAEC–PAD analysis make such interference unlikely, and, in fact, Fig. 1 shows sharp, symmetrical peaks without tailing or excessive broadening, which indicates that no significant degradation occurred.

For the quantification of the distribution of substituents in sulfoethylcellulose, the molar response of each monomer in the PAD detector was determined by HPAEC of mixtures of each substituted monomer with a defined amount of methyl β-cellobioside, the composition of which had been determined by integration of the H-1 signals in the 300-MHz ^1H NMR spectra [2-*O*-sulfoethyl-D-Glc δ 5.429 (α) and 4.693 (β), 6-*O*-sulfoethyl-D-Glc δ 5.210 (α) and 4.626 (β), 2,3-di-*O*-sulfoethyl-D-Glc δ 5.419 (α) and 4.699 (β), 2,6-di-*O*-sulfoethyl-D-Glc δ 5.418 (α) and 4.658 (β), 3,6-di-*O*-sulfoethyl-D-Glc δ 5.218 (α) and 4.660 (β)]. The results are given in Table II.

The quantitative HPAEC–PAD analysis procedure was applied to sulfoethylcellulose samples A–C (Table III), and the ds values found (0.33, 0.53, and 0.81 $\pm$ 0.03, respectively) were in excellent agreement with those (0.33, 0.51, and 0.83) based on a sulfur determination. The data in Table III indicate the order of reactivity for cellulose towards sulfoethylation to be HO-6 > HO-2 $\gg$ HO-3, as also found for carbamoylethyl-[10] and methylsulfonylethyl-cellulose[11], synthesised from cellulose by Michael additions to acrylamide and methyl vinyl sulfone, respectively. The low reactivity of HO-3 is a common feature for cellulose derivatives prepared by S_N2

TABLE II

Molar PAD response factors of sulfoethylated derivatives of D-glucose (see Experimental)

Monomer	Response factor [a]
D-Glucose	1.00
2-*O*-Sulfoethyl-D-glucose	0.71
6-*O*-Sulfoethyl-D-glucose	0.77
2,3-Di-*O*-sulfoethyl-D-glucose	0.16
2,6-Di-*O*-sulfoethyl-D-glucose	0.36
3,6-Di-*O*-sulfoethyl-D-glucose	0.17

[a] Relative to that of D-glucose.

TABLE III

Distribution of substituents in sulfoethylcelluloses A–C as determined by HPAEC–PAD after solvolysis

Monomer [a]	Distribution (mol %)		
	A	B	C [b]
D-Glucose	70.1	56.6	38.2 ± 1.3 [c]
2-*O*-Sulfoethyl-D-glucose	9.4	11.2	16.3 ± 0.3
6-*O*-Sulfoethyl-D-glucose	17.5	22.0	26.7 ± 0.4
2,3-Di-*O*-sulfoethyl-D-glucose		1.3	1.5 ± 0.1
2,6-Di-*O*-sulfoethyl-D-glucose	3.0	6.6	13.9 ± 0.3
3,6-Di-*O*-sulfoethyl-D-glucose		2.2	3.4 ± 0.2

[a] The 3- and 2,3,6-tri-*O*-sulfoethyl derivatives of D-glucose were not detected. [b] Average results of four analyses with standard deviation. [c] This larger standard deviation probably results from contamination with additional glucose.

reactions with neutral components and has been attributed to the involvement of HO-3 in an intramolecular hydrogen bond[12,13]. However, when the negatively charged sodium chloroacetate was used in the synthesis of carboxymethylcellulose by an S_N2 reaction with alkali cellulose, a comparatively high relative reactivity of HO-3 was observed[13] and ascribed to disruption of hydrogen bonds by the anionic alkylating agent[13]. Analogously, with respect to cellulose derivatives prepared by Michael addition reactions, an increase in the relative reactivity of HO-3 was expected when the negatively charged sodium ethylenesulfonate reacted with alkali cellulose to give sulfoethylcellulose. However, the relative amounts of 3-substituted derivatives found here for sulfoethylcellulose are similar to those in the Michael addition products carbamoylethyl-[10] and methylsulfonylethyl-cellulose[11]. This indicates that the use of a negatively charged reagent does not increase the relative reactivity of HO-3 in the now investigated Michael reaction.

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