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Analysis of cellulose degradation products by capillary electrophoresis

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Abstract

The characterization of a degraded cellulose solution was performed using capillary electrophoresis with indirect UV absorption. Chromogenic probes of various mobilities were tested to evaluate electromigration dispersion for all analytes. The best compromise was obtained with nicotinate, which exhibited a mobility intermediate between that of the slowest analyte (isosaccharinate) and that of the fastest (formate). The optimization of the separation was obtained by modification of the electrolyte pH: decreasing the pH selectively modified the effective electrophoretic mobility of the degradation compounds and thereby increased their resolution. It was found that the optimum pH range was very narrow ($5.0<pH<5.4$), and needed careful control with an appropriate buffer. © 1998 Elsevier Science B.V.

Keywords: Buffer composition; Cellulose; Organic acids

1. Introduction

A number of waste streams contain organic material like cellulose. In a nuclear waste repository, this material will be packaged in concrete containers and will eventually undergo alkaline degradation. The produced compounds are of major concern for nuclear waste management because of their suspected property to form complexes with radionuclides which would, in turn, increase the solubility of these toxic cations. The dissolution of cellulose takes place via a so-called 'peeling' process: the terminal reducing glucose units of the cellulose chain undergo rearrangements and yield intermediates (fructose derivatives and lactones) that subsequently give rise to various aliphatic hydroxycarboxylic acids, ranging from C_1 (formic) to C_6 (saccharinic) [1–5].

As many as 40 different aliphatic acids resulting from the alkaline processing of wood have been identified by gas–liquid chromatography [6] and major degradation compounds, such as the *erythro* and *threo* isomers of isosaccharinic acid have been separated. However, this technique involves sample pretreatment to per(trimethylsilylate) the analytes prior to their injection. More recently, the use of capillary electrophoresis for the analysis of small organic anions has been described by several authors [7–16]. Although some applications involve conductivity detection [8,9], most separations are detected by means of direct or indirect UV detection. For non-absorbing or slightly-absorbing analytes (like small-chain carboxylic acids), the indirect mode is preferred. The theory of indirect detection in capillary electrophoresis brings out the role of the electrolyte composition on the optimization of the efficiency [17–20] and sensitivity [20–23]. It was shown that electromigration dispersion, resulting in fronting or tailing of the peaks is limited for solutes having effective mobilities close to that of the electrolyte co-ion. Similar conclusions were drawn

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concerning sensitivity: based on the Kohlrausch regulation function, the absorbance drop of the sample zone can be derived [23] and the highest sensitivity is achieved when the effective mobility of the analyte matches that of the electrolyte co-ion. Besides, the absorptivity coefficient of the electrolyte chromophore must be high enough to provide a high background signal.

Various chromophores have been investigated for indirect detection applied to capillary electrophoresis. Naphthalene sulfonates [12,24] and benzene carboxylates [25] gave promising results for both inorganic and organic acids. They contain aromatic rings that provide for their intrinsic absorptivity, and one or several acid groups that determine their electrophoretic mobility. In this work, various derivatives of benzene and naphthalene have been evaluated as chromophores. They were selected according to their wide range of electrophoretic mobilities.

2. Experimental

2.1. Chemicals, sample and BGE preparation

1,2,4,5-Benzenetetracarboxylic acid (pyromellitic acid, PM), salicylic acid (SAL), 1,5-naphthalene disulfonic acid (NDS), tris(hydroxymethyl) aminomethane (Tris), glycolic acid and glutaric acid were obtained from Aldrich. Phthalic acid (PHT), naphthoic acid (NAPH), diphenylacetic acid (DPAC), nicotinic acid (NICO), 5-sulfosalicylic acid (SULSAL), 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)1,3-propanediol (Bis-Tris), creatinine (CREA), L-histidine (HIST), β-alanine (β-ALA), lactic acid, acetic acid, formic acid and methanol (MeOH) were from Merck. 3-Deoxy-2-C-hydroxymethyl-*erythro*-pentonic acid and 3-deoxy-2-C-hydroxymethyl-*threo*-pentonic (α-D-isosaccharinic acid, ISA) were synthesized by the Institut de Chimie Organique et Analytique of Orléans and recrystallized twice to obtain satisfactory purity (98%). In all instances, the *threo* configuration of α-isosaccharinate was detected at the same migration time as the *erythro* isomer so that the latter was used as the reference component for ISA. The water used throughout this study was purified with a Milli-Q system (Millipore).

The solution of cellulose degradation products was obtained by heating (60°C) a synthetic cement interstitial water equilibrated with 10% cellulose (Sigma). After 60 days, the sample was filtered and stored at 4°C. It was diluted by a factor of 200 immediately prior to analysis. The electrolytes were prepared daily (see text and figures for composition), degassed and filtered through 0.45-μm filters (Millipore). Methanol (2%) was added to NAPH- and DPAC-based electrolytes to increase the solubility of these two acids.

2.2. Apparatus, analytical conditions

The CZE apparatus was a Quanta 4000 (Waters) equipped with a zinc lamp and a 214-nm filter. An Accusep 60 cm×75 μm I.D. fused-silica capillary was used in this study; its effective length to the detector window was 52.4 cm.

All sample injections were hydrostatically driven (Δh_o=10 cm for 30 s), which corresponds to an injection volume of ca. 43 nl.

The run voltage was 20 or 25 kV (see figure legends).

All probe mobilities were determined by injecting the probe in a transparent electrolyte (3 m*M* H_3PO_4, 10 m*M* Tris, pH 8).

Absorptivity determinations were performed with a Lambda5 UV–visible spectrophotometer (Perkin-Elmer).

3. Results and discussion

3.1. Choice of the chromophore

The relevant characteristics of various chromogenic probes are collected in Table 1. The pK_a values of the buffers used in this study are also reported. This table is a guideline for the choice of the best probe, provided that one knows the corresponding characteristics of the compounds to be analysed, particularly their mobility.

For this particular method development, no electroosmotic flow (EOF) modifier was added to the electrolyte. The injection was at the anodic side and

Table 1
Characterization of the selected chromogenic probes and cationic buffers

	z_E (pH 8)	m_E (10^{-5} cm^2 V^{-1} s^{-1})	pK_a	$\epsilon_{E,214nm}$ (l mol^{-1} cm^{-1})
Chromogenic probe				
SULSAL	−2	−57.1	≤0; 2.49; 12.00 [31]	28 100
PM	−4	−55.1	1.92; 2.87; 4.49; 5.63 [31]	26 200
NDS	−2	−51.1	≤0; ≤0	31 000
PHT	−2	−48.1	2.95; 5.41 [31]	9950
SAL	−1	−35.3	2.97; 13.74 [32]	7950
NICO	−1	−32.7	2.05 (NH^+); 4.81 [33]	7420
NAPH	−1	−27.9	3.70 [32]	44 200
DPAC	−1	−24.0	3.94 [31]	15 220
Buffer				
β-ALA			3.55 [31]	
CREA			4.83 [34]	
HIST			1.82; 6.00; 9.16 [31]	
Bis-Tris			6.46 [31]	
Tris			8.08 [31]	

z_E, ionic charge at pH 8; m_E, effective mobility measured at pH 8; ϵ_E, absorptivity coefficient at 214 nm.

ISA, which was the slowest anion, was detected first, immediately after the peak of electroosmosis. This configuration was chosen: (i) to avoid ion-pairing with the solutes and possible precipitation of surfactant salts at acidic pH values [26], (ii) because the major degradation products are only slightly mobile and may be too slow to be detected from the capillary in a reasonable time with reversed EOF; and (iii) because the mobility of the solutes is suspected to decrease at low pH values and, in this case, alteration of the electrolyte pH in view of resolution optimization may end up in very large migration times again.

Fig. 1 illustrates the importance of the mobility match between the analyte and the probe. Three electropherograms were recorded with similar run conditions, except for the chromogenic probe of the electrolyte that was chosen according to its mobility. In electropherogram (a), DPAC, which is a slow-moving anion, is best suited for the analysis of the slowest anions like ISA, the mobility of which was determined to be -26.0×10^{-5} cm^2 V^{-1} s^{-1} from this run. Similarly, SULSAL, a fast moving anion, gives better peak shape for the last eluting solute that was identified as formate (electropherogram c). Its mobility, -52.7×10^{-5} cm^2 V^{-1} s^{-1}, was deduced from this run. Some other peaks were identified by spiking the sample with the corresponding acid (see legend of Fig. 1). 'A' is an unknown compound that

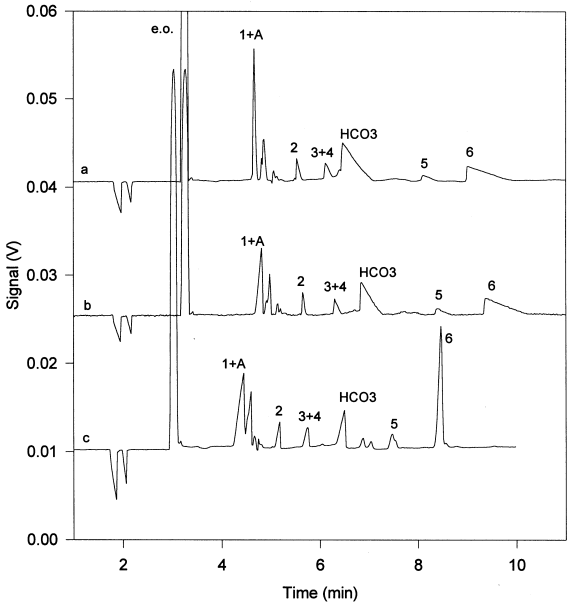

Fig. 1. Influence of the mobility of the chromophore on the peak shape of the degradation products of cellulose (dilution factor 200). (1) ISA, (2) lactate, (3) acetate, (4) glycolate, (5) glutarate, (6) formate, (A) unknown. Analytical conditions: capillary, fused-silica, 60 cm×75 μm I.D.; injection, hydrostatic, $\Delta h_0 = 10$ cm for 30 s; applied voltage, 20 kV; detection, indirect, $\lambda = 214$ nm. Electrolyte: (a) 4 m*M* DPAC, 10 m*M* Tris, 2% MeOH, pH 8.0; (b) 4 m*M* NICO, 10 m*M* Tris, pH 8.0; (c) 2 m*M* SULSAL, 10 m*M* Tris, pH 8.0.

co-migrates with ISA; its occurrence will be discussed later. Lactic, acetic, glycolic and formic acids are long known to be formed during the alkali treatment of cellulose matter [1–5]. The occurrence of glutaric acid is consistent with previous observations of small amounts of dicarboxylic acids during the sodium hydroxide treatment of cellobiose [4]. The peaks that are located between ISA and lactic acid are supposed to be C_5- and C_4-monohydroxycarboxylates, such as 3,4-dideoxypentonic acid [4], 2-deoxypentonic acid [5], 2,4-dihydroxybutanoic acid [4], 3,4-dihydroxybutanoic acid [1–5] and 2-methylglyceric [4,5]. The relative amount of their production depends on such parameters as temperature [3] and calcium [3] or carbonate [4,5] content of the solution.

The efficiency of each peak can be evaluated by the calculation of the number of theoretical plates *N*. The plot of *N* as a function of the ratio between the analyte mobility m_A and that of the probe, m_E (Fig. 2), shows that the efficiency drops quite steeply (75% for ISA) when m_E differs from m_A by more than 10%. However, the degradation products display too wide a mobility range to allow for a satisfactory efficiency for all the peaks with one single electrolyte. Fig. 1b corresponds to the compromise when all the solutes are to be quantitatively determined. Considering the mobility values of the chromophore candidates, either NICO or SAL could be used but NICO was preferred because of its higher solubility.

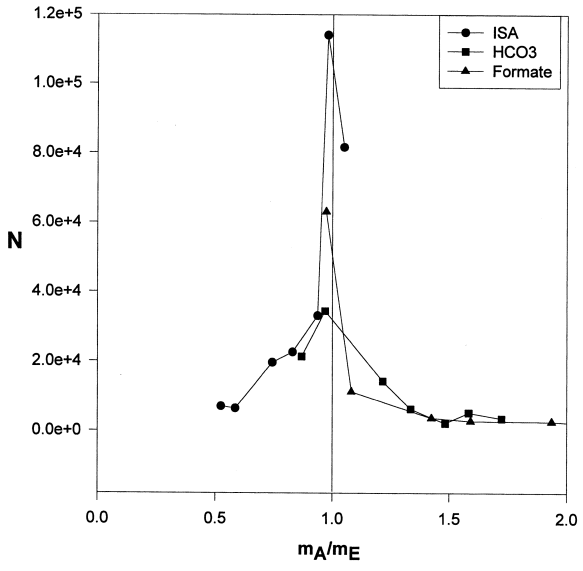

Fig. 2. Dependence of the efficiency on the mobility match between the chromophore and the analyte for ISA, carbonate, and formate. Analytical conditions: applied voltage, 20 kV; other conditions as in Fig. 1. Electrolyte: each composed of $4/z_E$ m*M* of a chromogenic probe listed in Table 1, 10 m*M* Tris, pH 8.0.

3.2. Optimization of the sensitivity

The highest sensitivities can be obtained with low-concentration electrolytes containing a chromophore with a high molar absorptivity. [21]. However, the chromophore concentration should be at least two orders of magnitude higher than that of the solute to keep electromigration dispersion reasonable [27]. Consequently, the optimum concentration of the chromophore will be attained when the background signal corresponds to the dynamic range of the detector. The sample of degraded cellulose products was injected in electrolytes containing varied concentrations of NICO, keeping the electrolyte pH in the range 8.0–8.2. The evolution of the corrected areas of the different identified peaks as a function of the concentration of NICO is reported in Fig. 3. The corrected areas of all peaks decrease for NICO concentrations higher than 4 m*M*, indicating that the dynamic range of the detector has been reached. The chromophore concentration was kept at 4 m*M* in further experiments.

3.3. Optimization of the resolution

The resolution between the peaks of ISA and its neighbors can be optimized either by addition of a complexing agent into the electrolyte (typically, a cation [15,28]) or by alteration of the pH of the electrolyte.

In our case, the addition of 0.1 m*M* calcium hydroxide to the electrolyte did not improve the resolution of the first-eluted anions, possibly because these anions are monovalent carboxylates with only little complexation ability. Besides, the electroosmotic flow was reduced because of the adsorption of Ca^{2+} on the capillary wall, and the analysis time was increased. The addition of a larger amount of calcium hydroxide into the electrolyte was not tested.

The degradation products all contain at least one

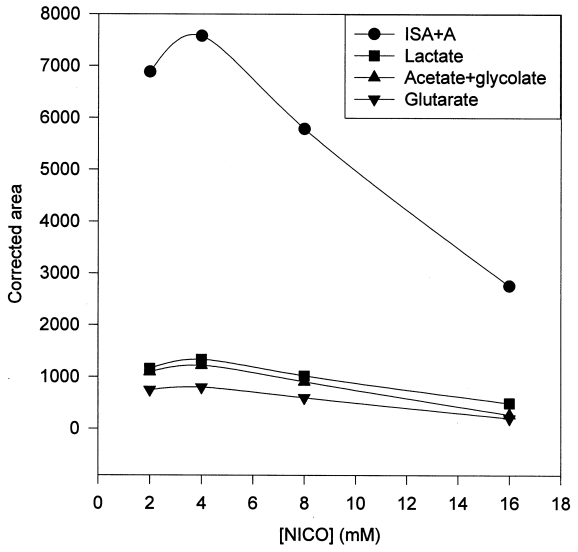

Fig. 3. Optimization of the sensitivity for major peaks by alteration of the chromophore concentration in the electrolyte. Analytical conditions: applied voltage, 20 kV; other conditions as in Fig. 1. Electrolyte: each composed of n mM NICO, 2.5n mM Tris, $8.0 \leq pH \leq 8.2$.

carboxyl group that accounts for their acid–base properties. One can reasonably assume that these weak acidic compounds have pK_a values in the range 3–7. Their effective mobility is given by:

$$m_A = \sum_{i=1}^{n} x_i m_i \quad (1)$$

where x_i and m_i are the molar fraction and the electrophoretic mobility of the n forms of species A. For a weak acid, x_i is a function of its pK_a values and the pH [26,29,30]. Eq. (1) can be written:

$$m_A = \frac{\sum_{i=0}^{n} \left(m_i [H]^{n-i} \prod_{j=1}^{i} K_j \right)}{\sum_{i=0}^{n} \left([H]^{n-i} \prod_{j=1}^{i} K_j \right)} \quad (2)$$

When operating the capillary electrophoresis separation at pH 8 (as described previously), the carboxylic acids are totally ionized and move as carboxylate anions. A decrease of the pH of the electrolyte should alter their speciation (partial protonation) and consequently selectively decrease their effective mobility.

Fig. 4 shows the evolution of the electropherogram for discrete values of the pH of the electrolyte from 8 to 3.65.

First, it can be observed that the carbonate species no longer interfere with the degradation products below pH 6. Hydrogencarbonate anions are converted to carbonic acid (pK_a = 6.37) while the other solutes, more acidic, remain ionized.

Second, a separation arose at pH 5.76 between ISA and another species (named A). This second major degradation compound was not identified but could be assigned to 3-deoxyhexonic acid (metasaccharinic acid, MSA) which chemical formula is similar to that of ISA and has already been observed as a major degradation product of cellulose matter [2,4–6].

Third, the sensitivity is decreased and the asymmetry of the peaks is modified when the pH drops from 5.76 to 3.65. In this pH range, the monovalent nicotinate anion is converted to the neutral form of nicotinic acid (pK_a = 4.81). The lower sensitivity at low pH values can be explained by the fact that only the ionized moiety participates in the electrophoretic process, and is to be considered when operating with indirect detection. The asymmetry of the peaks depends on the difference between the effective mobility of the probe and those of the analytes.

The evolution of the effective mobility of some degradation compounds as a function of the pH of the electrolyte is shown in Fig. 5. The plot of the effective mobility of nicotinate was calculated according to Eq. (2), given the values of its pK_a values and the mobility of nicotinate measured at pH 8 (Table 1), and assuming that the value of the cationic form below pH 2 was +32.7, that is, the opposite of that of the nicotinate anion. The explanation of the change of peak asymmetry of, e.g. ISA in Fig. 4, can be deduced from the results reported in Fig. 5. For pH values higher than 5.2, the effective mobility of ISA is lower than that of NICO: the corresponding peak shows fronting. Conversely, for pH values lower than 5.2, the effective mobility of ISA is higher than that of NICO: the peak shows tailing. Among the electropherograms presented in Fig. 4, the best efficiency for the peak of ISA is obtained for electrolytes with pH ranging between 5.0 and 5.4. According to Figs. 4 and 5, the pH range of optimum

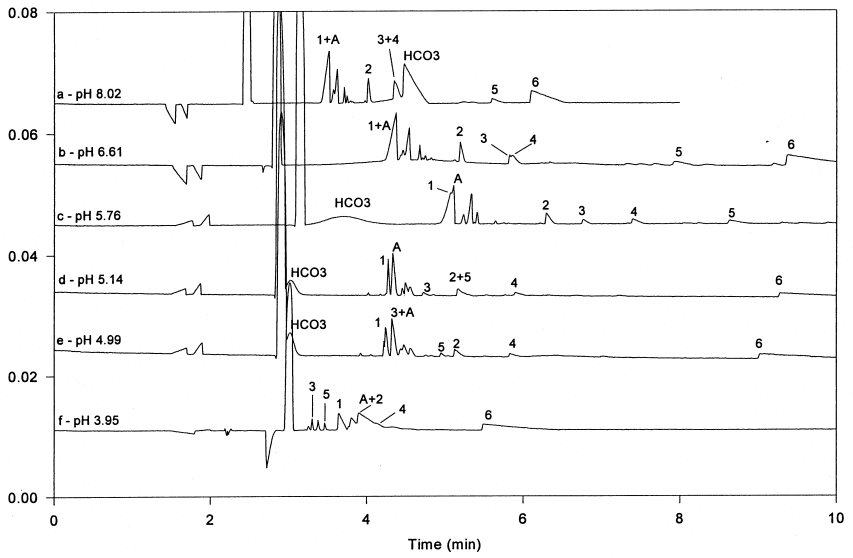

Fig. 4. Influence of the electrolyte pH on the separation of the degradation products of cellulose (dilution factor 200). Peak identification as in Fig. 1. Analytical conditions: applied voltage, 25 kV; other conditions as in Fig. 1. Electrolyte: (a) 4 m*M* NICO, 10 m*M* Tris, pH 8.02; (b) 4 m*M* NICO, 10 m*M* Bis-Tris, pH 6.61; (c) 4 m*M* NICO, 6 m*M* HIST, pH 5.76; (d) 4 m*M* NICO, 10 m*M* CREA, pH 5.14; (e) 4 m*M* NICO, 7 m*M* CREA, pH 4.99; (f) 4 m*M* NICO, 2 m*M* β-ALA, pH 3.95.

separation is very similar to that of optimum efficiency (5.0–5.4). Indeed, curve fitting from the data presented in Fig. 5 shows that most degradation products have pK_a values close to 4.0 (see Table 2). Thus, pH 5.0–5.4 corresponds to the pH range for which alteration of the effective mobility becomes significant and selective moderation of the solute velocity can take place.

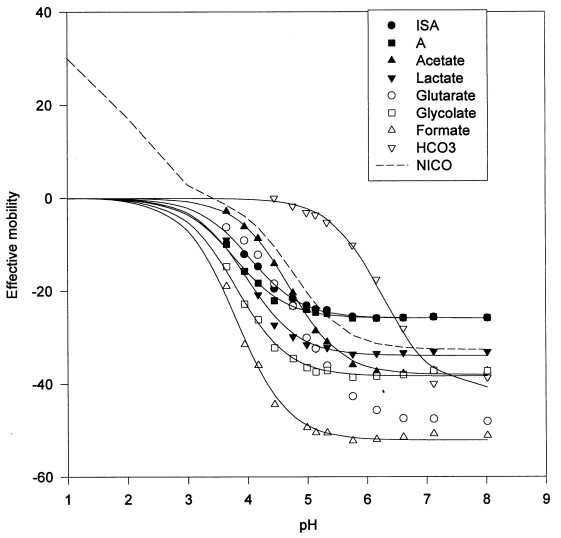

Fig. 5. Alteration of the effective mobility of the hydroxycarboxylate anions of the degraded cellulose solution as a function of the electrolyte pH. Analytical conditions as in Fig. 4.

The parameters collected in Table 2 were compared to values of pK_a values and equivalent ionic conductivities taken from the literature. Most calculated values are consistent with the tabulated ones, considering that the ionic strength is not fixed but does not exceed 2×10^{-3}. Values of 4.05 and 3.79 can be considered as good estimates of the apparent pK_a of ISA and species A, respectively.

Fig. 6 gives the optimum separation that provides complete separation of the C_6–C_4 acids with satisfactory efficiency of the first-eluted peaks. It was obtained with an electrolyte composed of 4 m*M* NICO, 15 m*M* CREA, pH 5.37. Using this buffer composition, the repeatability of the method was tested by injecting 10 times the same cellulose degradation solution. The relative standard deviations (R.S.D.s) are 0.6% on a time basis and 1.7% on a corrected area basis for the peak of ISA. They are equal to 0.6 and 1.4%, respectively, those of A. The change of migration times corresponds to a drift that

Table 2
Application and validity of the model described in Eq. (2) to the determination of pK_a and effective mobility values of degradation products

Solute	pK_a		Effective mobility (calculated) 10^{-5} cm^2 V^{-1} s^{-1}	Equivalent ionic conductivity (literature) [31] 10^{-4} m^2 S mol^{-1}
	Calculated	Literature [31]		
ISA	4.05		−25.88	
A	3.79		−25.88	
Lactate	3.98	3.86	−33.96	38.8
Acetate	4.70	4.756	−38.04	40.9
Glycolate	3.81	3.83	−38.34	
HCO_3	6.24	6.36	−41.42	44.5
Formate	3.81	3.75	−52.10	54.6

The fitted data were taken from Fig. 5.

is probably due to the lack of thermostating of the capillary. However, the separation factor α between the two peaks is 1.0122 with an R.S.D. of 0.017%, and confirms that the selectivity of the separation is not affected by this temperature effect.

4. Conclusion

The separation of cellulose degradation products was achieved with an electrolyte composed of 4 m*M* NICO, 15 m*M* CREA, pH 5.37. It is particularly suitable for the analysis of the slowest monovalent hydroxycarboxylic acids. Formic acid cannot be analysed with this electrolyte because of poor peak shape. For this particular species, 2 m*M* SULSAL, 10 m*M* Tris, pH 8.0 is preferred.

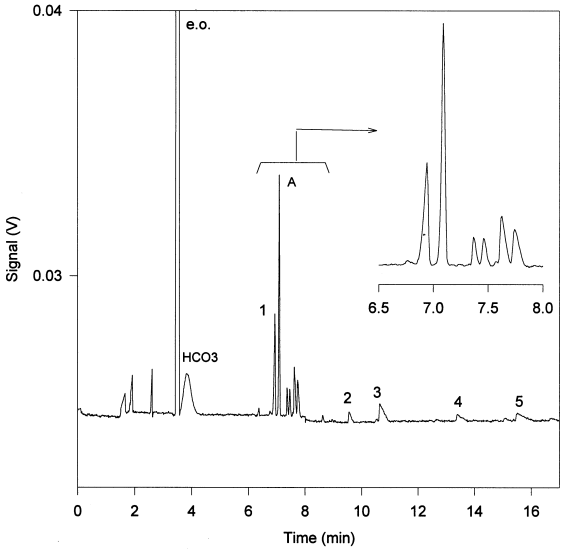

Fig. 6. Optimized separation of the degradation products of cellulose (dilution factor 200). (1) ISA, (2) acetate, (3) lactate, (4) glutarate, (5) glycolate. Analytical conditions: applied voltage, 25 kV; other conditions as in Fig. 1. Electrolyte: 4 m*M* NICO, 15 m*M* CREA, pH 5.37.

Acknowledgements

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