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Short Communication

Separation of D-galactonic and D-gluconic acids by capillary zone electrophoresis

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ABSTRACT

Capillary zone electrophoresis was used for the analysis of the aldonic acids, D-galactonic and D-gluconic acids. The dependence of the resolution on the pH of the running electrolyte was examined. Separation with the resolution 1.2 was achieved at both pH 4.1 and 5.0. The chiral mobile phase additive, β-cyclodextrin, showed no additional optimizing effect on the separation at the optimum pH.

INTRODUCTION

Capillary zone electrophoresis (CZE) is an efficient method for the separation of charged components. Since the aldonic acids are partly ionized in aqueous solution, they are suitable candidates for CZE investigations. In the study described here we successfully applied the CZE technique to separate the free D-galactonic and D-gluconic acids.

Previously, D-galactonic and D-gluconic acids have been analysed using liquid chromatographic (LC) systems based on strong basic anion-exchange resin [1–3]. Recently, however, separation of the aldonic acids by high-performance liquid chromatography (HPLC) was examined. The analysis was carried out on an anion-exchange column, but no separation was achieved [4]. In the same reference separation was achieved by applying pre-column derivatization followed by capillary gas chromatography (GC). The above-mentioned methods can only be applied to aldonic acids that have been altered by derivatization or complexation.

When the CZE technique is utilized to analyse the aldonic acids, detection is a challenging problem owing to the very low UV absorbance of the aldonic acids. A similar problem is encountered when investigating carbohydrates. This problem can be solved by derivatization [5,6], complexation [7] or by combination of both [8–

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10]. Alternatively, indirect photometric detection can be applied using sorbic acid as both carrier electrolyte anion and chromophore. Using this method various aldonic acids were examined at pH 12.1, but separation of D-galactonic and D-gluconic acids was not achieved [10,11].

By investigating the effect of pH on the resolution we separated and detected the free D-galactonic and D-gluconic acids applying the CZE technique and indirect photometric detection.

EXPERIMENTAL

Materials

Standard solutions of D-galactonic acid (Sigma, St. Louis, MO, USA) and D-gluconic acid (Merck, Darmstadt, Germany) were prepared in purified water obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA) at concentrations of 0.4 mg/ml (pK_a values of the aldonic acids are approximately 3.6). The buffers employed were prepared by dissolving 0.67 g of sorbic acid (Merck) in 1000 ml of Milli-Q water to yield a final concentration of 6 m*M* (pK_a of value of sorbic acid is 4.8). When working with β-cyclodextrin (Sigma) 1.7 g of β-cyclodextrin were added to 100 ml of the buffer, resulting in concentrations of 15 m*M*. The pH was adjusted with 1 *M* sodium hydroxide. The final electrolytes were filtered through HV 0.45-μm filters (Millipore) and degassed in a vacuum for 30 min.

Equipment

All the experiments were performed on a Quanta 4000 capillary electrophoresis system from Waters (Waters Chromatography Division, Millipore, Milford, MA, USA). The Quanta 4000 was equipped with an untreated fused-silica capillary (Waters) of total length 100 cm and internal diameter 50 μm. The capillary was ventilated to ensure a uniform temperature. Hydrostatic injection was performed by raising the injection end 9.8 cm relative to the detector end for 10 s. Detection was carried out by on-column measurement of UV absorption at 254 nm at 7.6 cm from the detection end using a time constant of 0.1 s.

The electrophoretic data system was a Millennium 2010 chromatography manager (Millipore) operated on a PowerMate 386/33i computer (NEC Technologies, Boxborough, MA, USA). The data were collected at a rate of five data points per second.

Capillary conditioning

Every new fused-silica capillary was washed with 1 *M* sodium hydroxide for 1 h followed by 0.001 *M* sodium hydroxide for 20 min. In between runs the capillary was treated with 1 *M* sodium hydroxide for 2 min, and then with 0.001 *M* sodium hydroxide for 2 min, and finally it was equilibrated with the buffer for 8 min. When the capillary was stored for more than 24 h it was flushed with Milli-Q water.

All experiments was carried out at room temperature.

RESULTS AND DISCUSSION

The D-galactonic and D-gluconic acids shown in Fig. 1 are chiral 4-epimers, and therefore their electrophoretic mobilities differ only slightly. Since the components are acids, the difference between the electrophoretic mobilities depends on pH. Therefore, we examined how the resolution depends on pH from pH 3.8 to pH 11.0 using the conditions described in the Experimen-

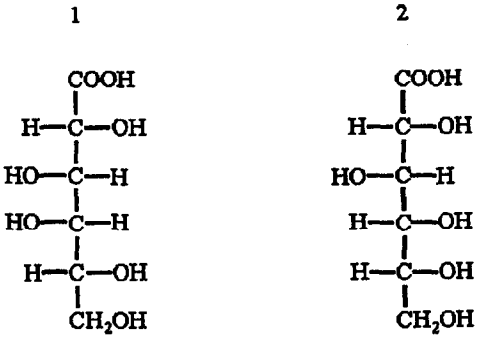

Fig. 1. Constitutional formulas of the 4-epimer aldonic acids. 1 = D-Galactonic acid; 2 = D-gluconic acid.

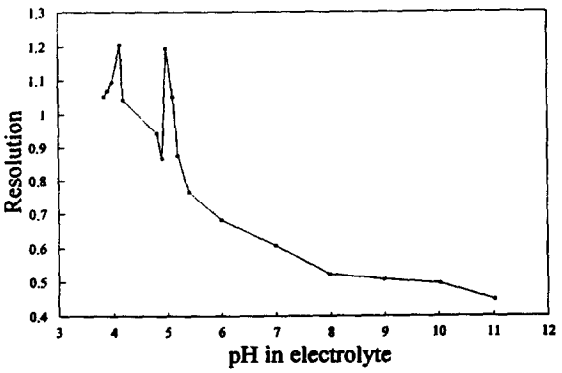

Fig. 2. Effect of pH on the resolution of D-galactonic and D-gluconic acids. Electrolyte: 6 m*M* sorbate at pH 3.8, 3.9, 4.0, 4.1, 4.2, 4.8, 4.9, 5.0, 5.1, 5.2, 5.4, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0. Current: 0.4–4.2 μA. Temperature: room temperature. Capillary: fused silica of length 92.4 cm and internal diameter 50 μm. Voltage: 20 kV. UV detection: indirect at 254 nm. Injection: hydrostatic in 10 s.

tal section. The results are depicted in Fig. 2 in terms of resolution *versus* pH of the buffer solution. The resolution, R_s, is calculated from the equation, $R_s = 2(t_{m2} - t_{m1})/(W_2 + W_1)$, where t_m is the migration time and W is the peak width at baseline using tangent lines drawn through 50th percentile points until they intercept the baseline.

It was noted that the resolution is very sensitive to changes in pH around 4 and 5. Therefore the resolution in the pH range 3.8–5.2 was further investigated. The best resolutions were obtained at the pH values 4.1 and 5.0, and the results at these pH values are reported in Table I.

In Fig. 3a we show the baseline separation of the 4-epimer aldonic acids at pH 4.1. The tailing is due to a higher mobility of the electrolyte compared with the mobility of the sample at this low pH. Fig. 3a and Table I show separation with resolution 1.2 was achieved at pH 4.1. The number of theoretical plates per metre is around 10^4, and the migration times are more than 30 min. Correspondingly, Fig. 3b shows the separation of the aldonic acids at pH 5.0. By altering the pH from 4.1 to 5.0 it is noted from Fig. 3b and Table I that the number of theoretical plates is enhanced by an order of magnitude, and the separation of the aldonic acids is achieved with a migration time of approximately 25 min. The shorter migration time is due to an increase in the electro-osmotic flow, because the higher electrolyte pH enhances the ionization of the silanol groups on the surface of fused-silica capillaries.

Equal concentrations were analysed during all runs. At a signal-to-noise ratio of 3 the detection limit is 18 fmol at pH 4.1. Correspondingly, the detection limit is 18 fmol at pH 5.0 with a signal-to-noise ratio of 5.

The chiral mobile phase additive, β-cyclodextrin (β-CD) has yielded good results in the separation of chiral compounds by CZE [12]. In order to optimize the separation, 15 m*M* β-CD was added at various pH values ranging from 4.1

TABLE I

EFFECT OF pH ON NUMBER OF THEORETICAL PLATES AND MIGRATION TIMES

Conditions: electrolyte: 6 m*M* sorbate. Temperature: room temperature. Capillary: fused silica of length 92.4 cm and internal diameter 50 μm. Voltage: 20 kV. Current: 0.8 μA at pH 4.1 and 1.6 μA at pH 5.0. UV detection: indirect at 254 nm. Injection: hydrostatic in 10 s.

pH in electrolyte	Standard amount (pmol)	Resolution	Number of theoretical plates per m[a]		Migration time (min)	
			Galactonic acid	Gluconic acid	Galactonic acid	Gluconic acid
4.1	3	1.2	8 000	12 000	33.1	34.8
5.0	3	1.2	75 000	100 000	24.8	25.3

[a] Number of theoretical plates: $N = 16(t/W)^2$, where t = migration time, W = peak width at baseline using tangent lines drawn through 50th percentile points until they intercept the baseline.

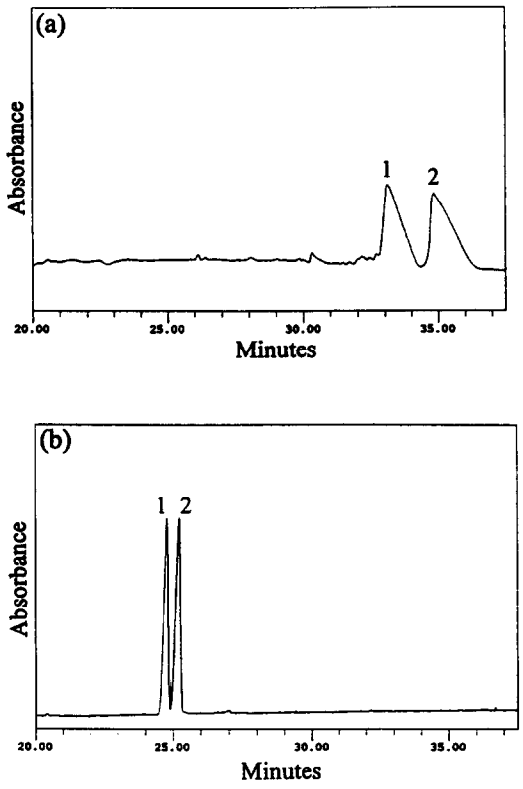

Fig. 3. CZE separation of the 4-epimer aldonic acids. (a) Electrolyte: 6 m*M* sorbate at pH 4.1. Current: 0.8 μA. Temperature: room temperature. Capillary: fused silica of length 92.4 cm and internal diameter 50 μm. Voltage: 20 kV. UV detection: indirect at 254 nm. Range: 7 mAu. Injection: hydrostatic in 10 s. (b) Electrolyte: 6 m*M* sorbate at pH 5.0. Current: 1.6 μA. All other conditions as in (a). Identification: 1 = D-galactonic acid; 2 = D-gluconic acid.

to 7.9. The results are shown in Fig. 4 in terms of resolution *versus* pH of the electrolyte solution. The results show that the effect of β-CD depends on pH, but the separation is not further improved.

CONCLUSIONS

The potential of the CZE technique for separating the free aldonic acids, D-galactonic and D-gluconic acids, has been demonstrated. It is shown that the separation of the chiral compounds by the CZE technique is very sensitive to the pH value of the buffer. Addition of β-CD to the buffer did not improve the separation at the optimum pH.

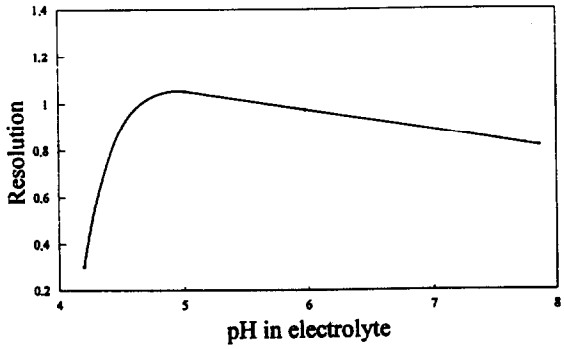

Fig. 4. The resolution of D-galactonic and D-gluconic acids as a function of pH with 15 m*M* β-cyclodextrin added to the 6 m*M* sorbate buffer. The experiment was performed at pH 4.1, 5.0, 6.0 and 7.9. Current: 0.2–2.1 μA. Temperature: room temperature. Capillary: fused silica of length 92.4 cm and internal diameter 50 μm. Voltage: 20 kV. UV detection: indirect at 254 nm. Injection: hydrostatic in 10 s.

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