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Note

Analytical control of arabonic acid production by isotachophoresis

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Arabonic acid is an intermediate compound in the industrial synthesis of riboflavin. It is produced by the oxidation of glucose in aqueous alkaline solutions[1]. Analytical control of its industrial production is a serious problem because classical analytical methods fail due to numerous side products.

Isotachophoresis has already successfully been used for such types of analyses, *e.g.*, of anionic products formed by alkaline oxidation of sorbose and fructose[2] and of products formed by periodate oxidation of some carbohydrates[3].

The present paper describes the use of isotachophoresis for the control of arabonic acid production, and shows that isotachophoresis can serve as a fast and reliable routine method for this purpose.

EXPERIMENTAL

Potassium arabonate used as the analytical standard was prepared by glucose oxidation (see below) and purified by multiple crystallization. All other chemicals were supplied by Lachema (Brno,Czechoslovakia).

Arabonic acid was prepared by oxidation of a 10–30% alkaline solution of glucose at 30–50°C with oxygen at a pressure of 0–2 MPa for 3–18 h[4].

The isotachophoretic analyses were carried out at room temperature in equipment for capillary isotachophoresis using a potential gradient detector. A detailed description of the instrumentation can be found elsewhere[5,6].

The leading electrolyte was prepared by adding β-alanine to 10 mmole/l HCl to give a final pH of 3.3. In order to improve separation, 0.3% polyacrylamide was applied: 0.3% Cyanogum 41 [mixture of acrylamide and N,N'-methylenebis(acrylamide); American Cyanamid] and 0.001% riboflavin were added to the leading electrolyte and the solution was left to polymerize under UV light for several hours. Insoluble particles were removed by filtration. Propionic acid (10 mmole/l) was used as terminating electrolyte.

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RESULTS AND DISCUSSION

For the analyses, the leading electrolyte was HCl–β-alanine, pH 3.3, since this had proved suitable for the separation of simple hydroxy and keto acids[7].

Fig. 1 shows analyses of samples obtained after potassium arabonate crystallization. In both samples, mother-liquor and crystalloid, arabonic acid yielded good isotachophoretic zones and most side products of oxidation were separated. The zones were identified by comparison of the step-heights of the sample components with those of the standard substances. Thus, the following acids were identified in the analyzed samples: oxalic acid, oxalacetic acid, formic acid, glyoxalic acid, glycolic acid, lactic acid, arabonic acid, gluconic acid and 3-hydroxypropionic acid. The unidentified step behind glycolic acid is probably due to erythronic acid.

Fig. 1. Mother-liquor after potassium arabonate crystallization. Sample injected: 1 μl of sample diluted 1:100. (b) Potassium arabonate crystalloid. Sample injected: 5 μl of 0.2% solution. h = Detector signal. Leading (L) and terminating (T) anions were chloride and propionate, respectively.

For the quantitation of arabonic acid, a calibration curve (step-length *vs.* amount injected) was constructed in the range 10–60 nmole (Fig. 2). It was linear with a correlation coefficient of 0.9999 and standard deviation of the regression line of 0.29 nmole. The relative standard deviation was 3.5% at the 50-nmole level for eight analyses.

We have analyzed several batches of mother-liquors and crystalloids. The ar-

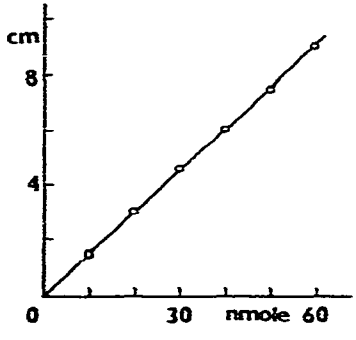

Fig. 2. Dependence of step length on the analyzed amount. Sample injected: 1–6 μl of 10 mmole/l potassium arabonate. Chart speed: 8 cm/min. Driving current: 200 μA.

abonic acid contents fluctuated in mother-liquors in the range of 0.12–0.19 mole/l, whereas those in the solid products were higher than 88.6% (w/w).

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