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CAPILLARY ISOTACHOPHORESIS: CONTINUOUS SAMPLING TECHNIQUE IN THE ANALYSES OF LOW-CONCENTRATION SAMPLES

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SUMMARY

A method has been developed for sampling that permits an increase in the total amounts of sample components in an isotachophoretic system so that the zones which originate can be detected and evaluated. This method is based on the simultaneous pumping of the leading electrolyte and the sample in an isotachophoretic column. It is shown that the continuous sampling technique is quantitative and that it increases the operational capability of isotachophoresis at least by 1–2 orders of magnitude towards lower concentrations. As an illustration, the simultaneous analysis of chlorides and sulphates in a mineral water is reported and the results are compared with those of titrimetric, gravimetric and previous isotachophoretic analyses.

INTRODUCTION

The essential characteristic for quantitation in isotachophoresis is the fact that the length of a zone is proportional to the amount of a component injected[1–3]. In order for the detector to yield a signal that is suitable for qualitative and quantitative interpretation, the zone must be long enough, *i.e.*, the component in question must be present in an amount exceeding the minimum detectable amount. Therefore, the more the sample is diluted, the larger the volume that must be injected into the column. When classical methods of sampling are used, *e.g.*, sample taps[3–5] or syringes, the sample occupies a part of a column in which the separation path is reduced. Moreover, a long column containing a low-concentration sample suffers from considerable electric resistance, so that the use of higher electric currents and thereby the shortening of the time of analysis are limited. Therefore, the volume of sample solution is limited by the construction of the column, the constant-current source and the sampling device.

The amount of the component in question can be increased by utilizing the principle on which isotachophoresis itself is based. It would be possible to inject a low-concentration sample at the start of the analysis and, after the zones have been formed, *i.e.*, after an increase in concentration, to switch the electric current off and then to inject the sample again, and so on. After several such enrichment steps,

sufficiently long zones could be created. This procedure, however, is laborious and makes the automation of analysis difficult. Moreover, the flushing-back of the zones into the injection port by a hydrodynamic counter flow of the leading electrolyte may have deleterious effects.

In this paper, a continuous sampling technique is described that can overcome the above problems and may be helpful in the analyses of low-concentration samples. This technique is demonstrated on LKB 2127 Tachophor instrument, which at present is the only apparatus commercially available.

EXPERIMENTAL

Isotachophoretic analyses were carried out on LKB 2127 Tachophor instrument (LKB, Bromma, Sweden) equipped with a 43-cm capillary tube. The capillary was maintained at a constant temperature of 25°. A PTFE capillary tube (O.D. 0.6 mm) was inserted via a septum into the injection port. The capillary was connected by using a 200-μl micropipette with a home-made electroosmotic pump[6]. The sample doses were measured with the help of the micropipette. Similarly, another capillary connected the counter-flow input with the second electroosmotic pump. A schematic diagram of the arrangement is shown in Fig. 1.

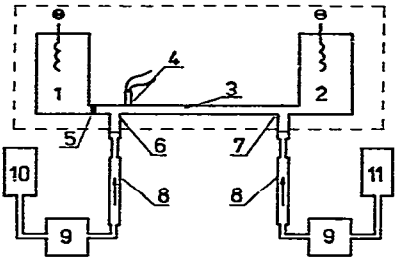

Fig. 1. System for the continuous sampling technique (anionic separations). 1 = Anode; 2 = cathode compartments; 3 = capillary; 4 = detectors; 5 = membrane; 6 = counter-flow input; 7 = injection port; 8 = micropipette; 9 = electroosmotic pump; 10 = leading electrolyte; 11 = sample reservoirs.

After the electric current had been switched on, the rate of movement of the boundary between the leading and terminating electrolytes was decelerated to 5% and the sample was pumped into the injection port. The pumping rate was set experimentally so that the sample was not flushed out of the capillary into the terminator compartment. By using the classical sampling procedure (microsyringe), a model mixture of 1 m*M* 2-(*p*-sulphophenylazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid, trisodium salt (SPADNS) + 5 m*M* acetate + 5 m*M* sulphate was injected and then separated. The maximal volume injected was 10 μl. This model mixture was then diluted 20-fold, *i.e.*, the resulting concentrations were 50 μM SPADNS + 250 μM acetate + 250 μM sulphate, and separated by means of the continuous sampling technique. In this instance, the maximal volume injected was of 200 μl.

The continuous sampling technique was also used with real samples, *viz.*, for the simultaneous analyses of chlorides and sulphates in mineral waters taken from Bohemian sources. The analyses of mineral waters were performed successfully in the operational system containing bivalent cadmium as a counter ion[7,8].

RESULTS AND DISCUSSION

A comparison of the step lengths for SPADNS and acetate obtained for the same amounts sampled into the LKB 2127 Tachophor by using the classical procedure and the continuous sampling technique shows that the agreement is excellent (Figs. 2 and 3). Similar results were obtained for sulphate step lengths. Naturally, the accuracy of the measurements of the step lengths of the zones of SPADNS was higher with UV detection (measurement of peak widths at half-height) owing to the low resolution of the thermal detector.

The continuous sampling technique was applied to the analyses of chlorides and sulphates in mineral waters. By using microsyringe sampling, the analysis of Velešice mineral water with chloride and sulphate concentrations of 0.70 and 0.95 m*M* (which were determined by titration[9]), respectively, was difficult and for a 43-cm capillary almost impossible[10] in the operational system used[8]. The maximal volume that could be injected was 10–15 μl for the 43-cm capillary. Therefore, 62-cm capillary tubes with an available injection volume of 20–25 μl were used for the analysis of Velešice water[8]. Nevertheless, the amounts injected were close to the limit of quantitative evaluation. In such a situation, the use of the continuous sampling technique may be beneficial. The plot of step lengths *versus* the volume of Velešice water plus SPADNS (200 μl of 0.005 *M* SPADNS per 10.0 ml of the mineral water) is shown in Fig. 4. The step lengths for 3 μl of the standard mixture of 0.01 *M* chloride and 0.01 *M* sulphate were 26.5 and 52.2 mm, respectively. Consequently, the concentrations of chlorides and sulphates are 0.72 and 0.97 m*M* with standard deviations of 1.1 and 1.3%, respectively. The corresponding values previously determined by microsyringe-sampling isotachophoresis were 0.68 m*M* for chloride and 0.93 m*M* for sulphate with standard deviations of 1.4 and 1.0%, respectively.

The continuous sampling technique can be used advantageously for the

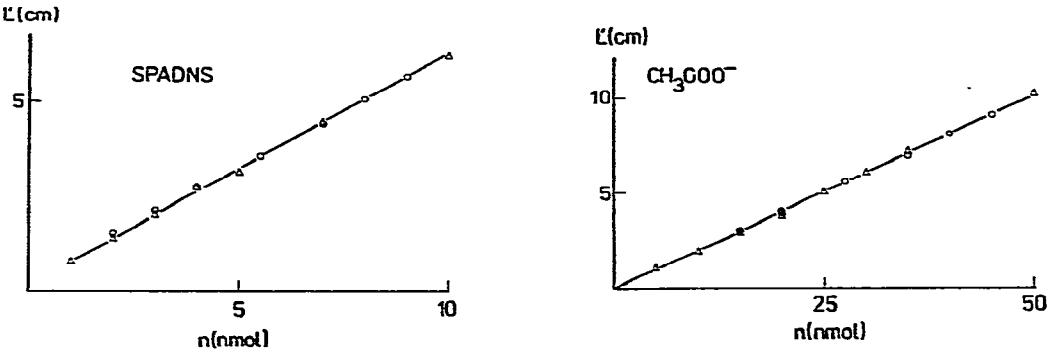

Fig. 2. Dependence of the step lengths (L') on the sampled amounts (n) of SPADNS by using a microsyringe (△) and the continuous sampling technique (○) with a UV detector. Leading electrolyte: 0.01 *M* HCl + histidine, pH 6.0 (20°) + 0.1% Triton X-100. Terminating electrolyte: 0.005 *M* glutamic acid. Electric current, 250 μA for sampling and separation and 100 μA for detection. Chart speed, 12 cm/min.

Fig. 3. Dependence of the step lengths (L') on the amounts (n) of acetate ions sampled by using a microsyringe (△) and the continuous sampling technique (○) determined with a thermometric detector. Operational system, conditions of separation and chart speed as in Fig. 2.

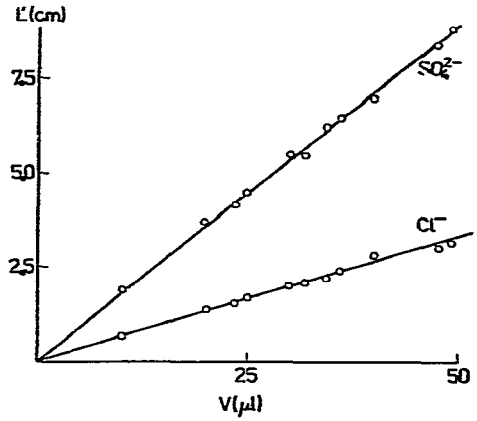

Fig. 4. Dependence of the step lengths (L) on the sampled volume (V) of Velešice mineral water determined by using a thermometric detector. Leading electrolyte: 0.008 M $Cd(NO_3)_2$ with $Cd(OH)_2$ suspended in the leading electrolyte compartment. Terminating electrolyte: 0.005 M citric acid. Electric current, 230 μA for the sampling and separation and 130 μA for detection. Chart speed, 4 cm/min.

analysis of samples that are difficult owing to the low concentrations of the components in question. The disadvantage of the continuous sampling technique is that a longer time is required, although an increase in current density reduces both the dosing and analysis times. For the analysis of Velešice water, the time required (15–20 min) was comparable to that of the previous analysis performed by using discrete sampling, as a shorter capillary tube (43 cm) could be used. For the application of this technique, it is important to set the electric current and the pumping rates of the counter flow and the sample solution properly. These settings are facilitated by preliminary experiments with coloured ions present in the sample or terminator. Their presence, however, is not necessary once the pumping rates have been set. There is the possibility of pumping a sample without a counter flow. An additional focusing by using a counter flow is necessary and the results are worse for higher sample volumes than the aforementioned ones.

The use of the continuous sampling technique may also be advantageous in continuous flow isotachophoresis[11], in connection with counter flow flushing of fractions and in analytical and preparative isotachophoresis with an instrument that has been described earlier[12].

CONCLUSIONS

The continuous sampling technique has proved to be a quantitative method of sampling. Its main benefit is that the operational capability of isotachophoresis is increased towards lower concentrations (by 1–2 orders of magnitude). The method has been applied to the analysis of chlorides and sulphates in mineral waters with satisfactory reproducibility and reliability.

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