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ELECTRIC SAMPLE SPLITTER FOR CAPILLARY ZONE ELECTROPHORESIS

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SUMMARY

A method for reproducible sampling of small quantities of sample for high-performance capillary electrophoretic separations was developed. The method is based on the principle of the splitter. The sample migrates electrophoretically in two electrical circuits and the splitting ratio is given by the ratio of the corresponding electric currents. Very small aliquots of the sample in the form of short zones (pulses) can be introduced corresponding to direct sampling of nanolitre volumes.

INTRODUCTION

Free zone electrophoresis[1] as an analytical method has shown promising analytical possibilities and was successfully used for analysis of inorganic, organic and biological samples[2].

Later attempts to perform capillary zone electrophoresis[3,4] fall aproximately into the same period as the development of isotachophoresis[5]. At present, capillary isotachophoresis is a highly instrumentally developed technique and several instruments are commercially available, while capillary zone electrophoresis is still at an early stage of development[6–11].

The prerequisite for high-performance separation and quantitative analysis in capillary separation methods is the sampling of very small amounts in the form of sharp pulses[12]. In capillary GC, the splitter has been used for such sampling for several years. The injection of small sample amounts is not a critical problem in capillary isotachophoresis because the self-sharpening effect of the boundary and the concentrating effect (adjustment of concentrations) enable one to inject diluted samples. However, the method of sampling seems to be one of the key problems in capillary free zone electrophoresis[6].

This situation led us to develop a sampling method for capillary zone electrophoresis which is an electric analogy of the splitter in chromatography. Considering that during the electromigration process substances move with various speeds, it was necessary to determine theoretically and verify experimentally whether the electric splitter allows sampling of real aliquots of sample, *i.e.*, whether the sample taken for separation is truly representative of the original one.

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EXPERIMENTAL

The principle of the splitter is shown in Fig. 1. The current, I_1, flows through the dosing capillary and drives the original sample, n_1. This current is then split into I_2, which drives part, n_2, of the original sample into the separation capillary and I_3 which drives the rest of the sample, $n_3 = n_1 - n_2$, to the drain.

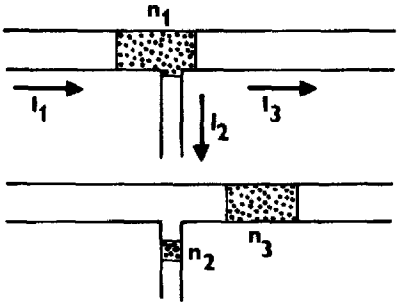

Fig. 1. Principle of the electric splitter.

Let us consider the separation capillary of cross-sectional area S_2 through which the electric current I_2 flows. The electric field strength at the rim of this capillary is

$$E = I_2/\kappa S_2 \tag{1}$$

where κ is the specific conductivity of the electrolyte at the splitting point. An individual ionic species of the electrolyte in this electric field moves at the velocity v

$$v = uE \tag{2}$$

where u is its mobility.

The total mass flux of any component i into the separation capillary is

$$dn_2/dt = S_2cv \tag{3}$$

where c is the concentration of this component. By substituting eqns. 1 and 2 into eqn. 3 we obtain:

$$\frac{dn_2}{dt} = I_2 \cdot \frac{cu}{\kappa} \tag{4}$$

From eqn. 4 it follows that the mass flux is independent of the cross-section of the separation capillary.

Similarly, for the mass flux into the drain we can write:

$$\frac{dn_3}{dt} = I_3 \cdot \frac{cu}{\kappa} \tag{5}$$

The ratio of the mass fluxes is:

$$\frac{dn_2/dt}{dn_3/dt} = \frac{I_2}{I_3} \tag{6}$$

Hence, this ratio is independent of the conductivity of the electrolyte at the splitting point and of the concentration and the mobility of the component. Thus, if we consider that the composition of the electrolyte in the dosing capillary is a function of time, $\kappa = \kappa(t)$, $c = c(t)$, where c relates to any component of the sample, then:

$$\frac{dn_2/dt}{dn_3/dt} = \frac{I_2(t)uc(t)/\kappa(t)}{I_3(t)uc(t)/\kappa(t)} = \frac{I_2}{I_3}(t) \tag{7}$$

Keeping constant $I_2/I_3(t) = I_2/I_3$, we obtain:

$$\frac{dn_2}{dt}(t) = \frac{dn_3}{dt}(t) \cdot \frac{I_2}{I_3} \tag{8}$$

The total amount of component trapped in the separation capillary is

$$\frac{dn_2}{dt} \cdot dt = \frac{I_2}{I_3} \cdot \frac{dn_3}{dt} \cdot dt \tag{9}$$

and thus:

$$n_2 = \frac{I_2}{I_3} \cdot n_3 \tag{10}$$

Similarly it is possible to extend this treatment to the current I_1. For this current, $I_1 = I_2 + I_3$, and for the total sample amount, $n_1 = n_2 + n_3$.

Obviously:

$$n_2 = \frac{I_2}{I_1} \cdot n_1 \tag{11}$$

The quantity sampled by the electric splitter into the separation capillary, n_2, is thus proportional to the total sampled amount and to the ratio of the electric currents passing through the separation and dosing capillaries, I_2 and I_1, respectively, for any component. Hence, the sample in the separation capillary is a true aliquot of the original sample, the ratios of the constituents being unchanged.

EXPERIMENTAL

Two types of equipment were used for verifying the operation of the splitter. The home-made equipment (Fig. 2) consisted of a monolithic block (200 × 50 × 20 mm) of polyester resin formed by casting[13]. It comprises the dosing and separation

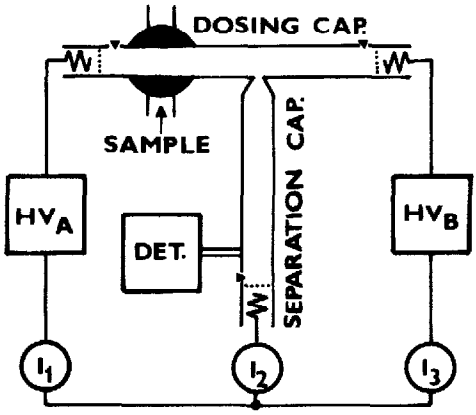

Fig. 2. Scheme of the home-made splitter. ▼, Operating valve; ....., Cellophane membrane.

capillaries, dosing valve, inlet and outlet channels, operating valves and detection cell. The electrolyte chambers of Plexi-glass separated by Cellophane membranes are attached to the block. The volume of the dosing valve was about 1 μl, the inner diameters of the dosing and separation capillaries being 0.5 and 0.2 mm, respectively. The distance of the dosing valve from the splitting point was about 10 mm. The sensing electrodes of the potential gradient detector penetrated through the wall into the separation capillary to a distance of 80 mm from the splitting point. The power supply for isotachophoresis was used as the high voltage source (HV_A)[14]. Equipment with manually stabilized current was used as the power supply HV_B.

The commercial instrumentation (CS-Isotachophoretic Analyser; ÚRVJT, Spišská Nová Ves, Czechoslovakia) was equipped with a preseparation PTFE capillary (200 × 0.8 mm I.D.) coupled to the separation capillary (170 × 0.3 mm I.D.). The preseparation capillary is equipped with the sampling valve and an injection port for microsyringes. Both the preseparation and the separation capillaries are provided with a conductivity detector.

Histidine (Reanal,, Budapest, Hungary), MES (N-morpholineethanesulphonic acid), Triton X-100 (Serva Feinbiochemica, Heidelberg, F.R.G.) and the other chemicals used (Lachema, Brno, Czechoslovakia) were of p.a. quality. The distilled water was deionized by a mixed bed ion exchanger.

The sample was introduced via a dosing valve. The appropriate current ratio, I_2/I_1, was set by operating the power supplies HV_A and HV_B. After the sample had passed the splitting point the supply HV_b was disconnected and the appropriate current on the power supply HV_A was set for the analysis.

For the commercial apparatus, only one high voltage power supply was used and the sampling current, I_2, was derived by connection of the electrodes in the electrolyte chambers to the ends of the dosing and separation capillaries. The splitting ratio could not be selected in this case. It was given by the actual electrical resistances of both channels; however, even in this case the performance of the splitter could be tested.

RESULTS AND DISCUSSION

To check the splitter operation quantitatively, isotachophoresis experiments were performed. The reason for this stems from the well known way of quantitating the isotachophoretic records by measuring the step lengths. Prior to each experiment the separation and dosing capillaries up to the sampling valve were filed with the leading electrolyte and the rest of the dosing capillary with the terminating electrolyte. Then the sample was introduced and the current ratio selected. Fig. 3 illustrates the relationship of the isotachophoretic zone length and the current ratio, I_2/I_1. It is seen that the electrically introduced quantity is directly proportional to the splitting current ratio.

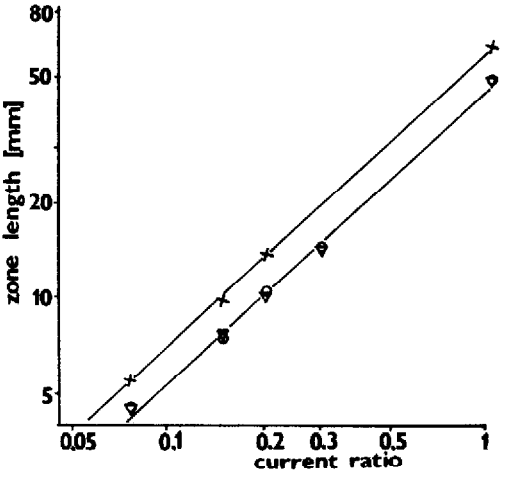

Fig. 3. Dependence of the isotachophoretic zone length in the separation capillary on the electric current ratio (splitting ratio) during the electrical sampling. Leading electrolyte: 10^{-2} *M* HCl + β-alanine, pH = 3.9, containing 0.5% Triton X-100. Terminating electrolyte: acetic acid. Sample: Oxalate; (▽) fumarate (○) and citrate (×), each *ca.* 10^{-2} *M*.

In practice, the accuracy, *i.e.*, the agreement of the splitting ratio with the electric current ratio is important. In the measured range of the splitting current ratio the accuracy was found to be better than 2%, and the relative standard deviation of measurement was better than 3%. In Fig. 4 the relative contents of some components in the sample (expressed as the ratio of the zone length to the total length of all zones) is plotted *vs.* the splitting ratio. Obviously, no enrichment was observed, *i.e.*, the splitter always provided a representative aliquot of the original sample. To verify the absence of uncontrolled introduction of the sample into the separation capillary during the migration to the splitting point, some experiments were carried out where the current I_2 was switched off, *i.e.*, the sampling ratio was zero: no sample was detected in the separation capillary.

To test the performance of the splitter for capillary zone electrophoresis, a model mixture was analyzed in the zonal mode both in the home-made and in the commercial isotachophoretic instrumentation. The detectors available (potential gra-

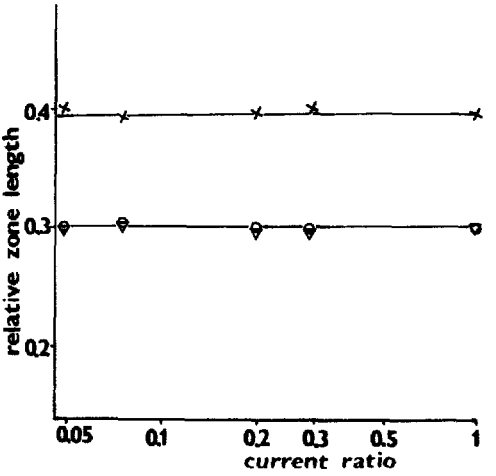

Fig. 4. Dependence of the relative contents of oxalate (▽) citrate (×) and fumarate (○) in the split sample on the current ratio. For the electrolyte system and sample, see Fig. 3.

dient or contact conductivity) are not very suitable for zone electrophoresis because of their considerable baseline drift and irregular instabilities[7], however, they do serve for our qualitative purposes.

Records of the separation of the model mixture by zone electrophoresis in the home-made equipment are given in Fig. 5. Fig. 5a shows the separation with direct sampling by the valve, Fig. 5b the separation of the same sample with the splitter. It is evident that use of the electric splitter can significantly improve the separation.

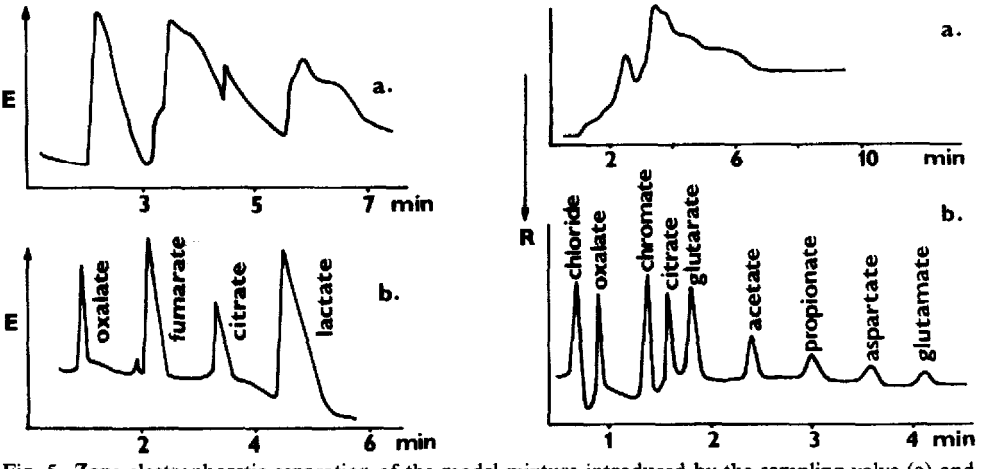

Fig. 5. Zone electrophoretic separation of the model mixture introduced by the sampling valve (a) and by the electric splitter (b). Background electrolyte: 10^{-2} *M* HCl + β-alanine, pH = 3.9, containing 5% Triton X-100. Sample: oxalate, fumarate, citrate and lactate, erach *ca.* 10^{-3} *M*, dissolved in background electrolyte. Electric field strength: 170 V/cm.

Fig. 6. Zone electrophoretic separation of the model mixture with utilization of column coupling. Background electrolyte: 10^{-2} *M* MES + histidine, pH = 6.05, containing 0.5% Triton X-100. Sample: chloride, oxalate, chromate, citrate, glutarate, acetate, propionate, aspartate and glutamate, each 0.00005 *M*, dissolved in water. Electric field strength: 170 V/cm. Conductivity detection before splitting point (a) and analysis of a split aliquot (b). For further explanation see text.

Fig. 6 shows a zone electrophoretic record of the separation of the model mixture performed with the commercial instrument. The sample (25 μl) was introduced by the valve into the preseparation capillary (0.8 mm I.D.) The splitting ratio was 1:40. Fig. 6a shows the signal of the conductivity detector in the pre-separation capillary before the splitting point. Although the zone was not separated after passing the pre-separation capillary, the electric dosing enabled the aliquot to be easily separated. Fig. 6b shows the zone electrophoresis of a split aliquot of the original sample. The improved separation obtained in the latter case is evident.

CONCLUSION

The electric splitter is a useful sampling system for capillary electrophoresis, especially for capillary free zone electrophoresis. It enables one to introduce adjustable, very small aliquots of the initial sample into the separation capillary in the form of very short pulses, which is hardly possible by direct injection. Thus, the splitter allows one to exploit the separation possibilities of the capillary arrangement and to perform quantitative analysis. The splitting ratio can be set and measured as the electric current ratio.

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