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# Split injector for capillary zone electrophoresis

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## ABSTRACT

A split injector is described for capillary zone electrophoresis. It consists of a pump for delivery of the medium, a rotary injector, an interface, and a fused-silica capillary connected between the interface and the rotary injector. Several factors affecting split injection are examined, such as geometrical configuration of the interface and sample loop volume. The use of this split injector is demonstrated for both rectangular capillaries with large cross-sectional areas and the more conventional circular capillaries. The present injection method requires no interruption of the applied voltage.

## INTRODUCTION

Although the initial development of capillary zone electrophoresis (CZE) was rather slow [1–5], CZE has become one of the best high-performance separation techniques for the analysis of complex mixtures because of its high resolution [6–8]. Compared with ordinary liquid chromatography (LC), the sample amount used in CZE (with a circular capillary column of 50 $\mu$m I.D.) is very small. To avoid band broadening, the amount of sample injected is limited to a few nl. The development of an improved injection method is a key factor in encouraging further acceptance of CZE.

Several injection methods are currently in use: gravity [2–4], electrokinetic injection [2,3,9], hydrostatic force [9–12], rotary valve [13], partial electric sampling [14], side flow caused by an electric field [15,16], and split-flow sample introduction [17]. Split-flow sample introduction, developed by Tehrani *et al.* [17], interrupts the applied voltage: the procedure is a stepwise method that has the same scheme as gravity injection.

These injection methods are not applicable to capillaries with large cross sections. A rectangular capillary has several advantages in CZE [18], but injecting a sample is difficult because of its low flow resistance. Most of these injection methods require interruption of the applied voltage during injection. This voltage interruption

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may recondition the double layer on the capillary walls, thus potentially reducing reproducibility.

In the present paper, we describe a split-injection method for CZE. This method is done under continuous flow using a pump and a rotary valve. The sample is introduced into the capillary by electrokinetic injection. The injection procedure does not interrupt the applied voltage. Although the present method is developed for rectangular capillaries with large cross-sectional area, it is also applicable to circular capillaries. We describe the construction of this injector and characterize its operation.

## EXPERIMENTAL

Schematic diagrams of the CZE system, the split-injection system, and the CZE interface are shown in Figs. 1 and 2. The present system uses either a circular or a rectangular capillary. The latter has a large cross section, is fragile, and is not flexible. The rectangular capillary terminates inside a reservoir (7 in Fig. 1). The space between the end of the reservoir and the rectangular capillary is sealed with epoxy. Care should be taken to ensure that the epoxy does not extend into the end of the rectangular capillary column.

The capillary column is washed and filled with buffer as follows. Electrode 5 is removed and the mouth of the reservoir is capped; liquid is then supplied from syringe

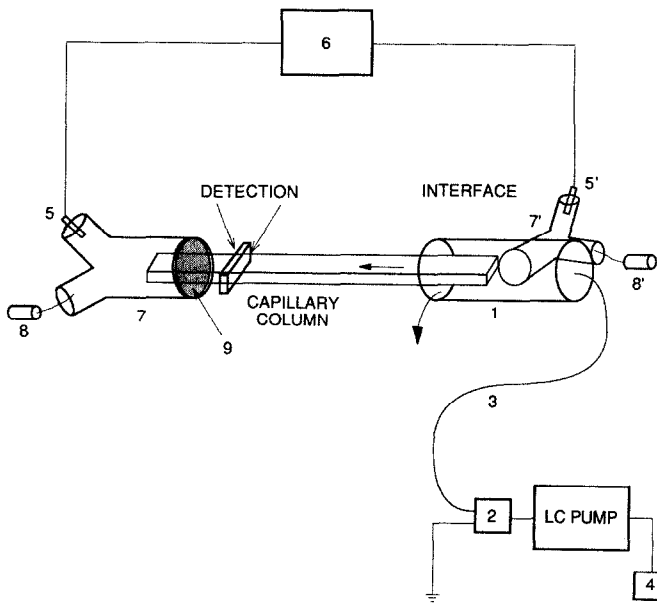


Fig. 1. Schematic diagram of capillary electrophoretic system with split injector. 1 = Interface; 2 = injection valve; 3 = delivery tube of fused-silica capillary, 50 or 100 $\mu$m I.D. and 1.5–2 m in length; 4 = reservoir for LC pump; 5 and 5′ = electrodes; 6 = high-voltage supply; 7 and 7′ = reservoirs using 3-way PTFE connectors; 8 and 8′ = syringes for filling reservoirs; 9 = epoxy seal.

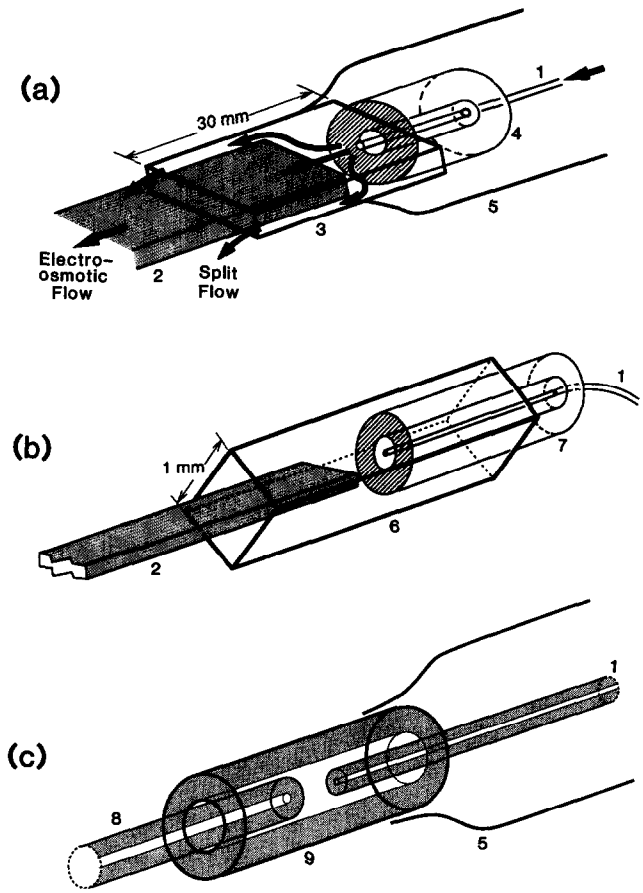


Fig. 2. Interface for split injector having (a) "rectangular" interface for rectangular capillary (50 × 1000 μm); (b) "square" interface for rectangular capillary (50 × 1000 μm); (c) "circular" interface for circular capillary (50–100 μm I.D.). Arrows show directions of sample and solvent flow. 1 = Fused-silica capillary from injection valve (2 in Fig. 1); 2 = rectangular capillary; 3 = rectangular 200 × 2000 μm capillary; 4 = PTFE tubing, 0.5 mm I.D. , 2.0 mm O.D., and 10 mm in length; 5 = cover; 6 = square tubing (side 1 mm long); 7 = PTFE tubing, 0.5 mm I.D. and 1 mm O.D.; 8 = circular capillary; 9 = circular capillary tubing, 0.5 mm I.D. × 0.8 mm O.D.

8 by pressing the cylinder by hand. Syringe 8′ is also used for filling reservoir 7′ and interface 1. The latter also serves as a reservoir.

Another possible procedure is to use suction. Here one end of the rectangular or circular capillary is inserted into the reservoir, and the other end is inserted a few millimeters into a larger capillary, which is connected by vacuum tubing to a pump.

When voltage is applied, the nature of the media in the reservoirs changes continuously due to the gain and loss of specific ions and possible electrochemical reactions at the electrodes. Although the reservoir (7 in Fig. 1) has a volume of only 0.2 ml, no effect is observed for the first hour of continuous operation. After 1 h, we observe a variation in the elution time of a solute. Therefore, the buffer must be

changed in the reservoir every hour. This problem can also be overcome by supplying a fresh buffer from another reservoir with gravity flow via a small PTFE tube.

The sample is introduced with a pressurized flow using split injection. This device is similar in principle to that used for microcapillary liquid chromatography [19]. The transfer line consists of an LC pump (Beckman solvent delivery module 112), an injection valve (Model 7030 for 1-$\mu$l and 5-$\mu$l loops; Model 7410 for 10-$\mu$l loop; Rheodyne, Cotati, CA, USA), a long delivery tube (fused-silica capillary, 50 or 100 $\mu$m I.D. and 1.5–2.0 m or 41 cm long), and an interface. A large electric voltage drop occurs between the interface and the injection valve because the injection valve is grounded and high voltage is applied at electrode 5′. The delivery tube (3 in Fig. 1) acts as an insulator. The parasitic current is less than one-third the current of the round (50 $\mu$m I.D.) analytical column and one-fifteenth the current of the rectangular (50 × 100 $\mu$m) analytical column. Although the parasitic current is relatively small compared to that of the analytical column, the injection valve should be grounded. If not, the voltage at the injection valve will approach that of the interface.

A metal-free automatic injector (Model 8126 with 4- or 5-$\mu$l loop, operated by a compressed-gas controller Model 7163, Rheodyne) was also tried. It is located close to the interface. The automatic injector is connected to the interface with a short fused-silica capillary, 11.5 cm in length. Because the automatic injector is at high voltage, the supply of sample to the injection loop is via a long fused-silica tubing (*ca.* 1 m), the end of which was connected to a microsyringe and grounded.

Three interfaces are shown in Fig. 2, which will be termed (a) "rectangular", (b) "square", and (c) "circular". The sample to be separated is placed inside the injection valve. The LC pump provides pressure to the supply buffer and forces the sample through the delivery tube and into the interface. At the interface some portion of the sample is injected into the capillary.

A delivery tube (3 in Fig. 1) of variable length (11.5, 41, and 150 cm) connects the interface with the injection valve and acts as an insulator as well as a transfer tube for the sample. For a delivery tube 50 $\mu$m I.D. and 1.5 m long, the sample remains in the capillary for 0.2–0.6 s (flow velocity is estimated to be 7.5–2.5 m/s), and the flow-rate of the medium is between 0.3 and 0.7 ml/min. At the interface, the flow velocity of the sample decreases because the inner diameter is larger than the delivery tube. The sample zone is opposite to the inlet of the capillary column.

The inlet of the capillary must be carefully aligned with the outlet of the delivery tube. Both the capillary and the delivery tube must be centered in the tubing of the interface. In the square interface, centering of the rectangular capillary is easily accomplished. In the rectangular interface, however, the end of the analytical capillary is frequently off axis. Therefore, the square interface is more reliable than the rectangular one. The length between the inlet of the capillary and the outlet of the delivery tube was varied from 0 to 10 mm; the optimum length was approximately 2 mm. Glass, PTFE, and polyethylene have been tested as possible materials for the interface. Glass is the superior material because the inner surface of the interface should be smooth and have good wettability.

The split flow leaves the interface, shown by the arrows in Fig. 2a. In some cases the split flow generates a back pressure because of the narrow path between the interface and the inserted portion of the capillary. For these situations, the surface level of the buffer at the position of electrode 5′ in Fig. 1 should be sufficiently high

compared to the level in the interface to maintain the flow in the forward direction. The concentration of the sample at the inlet of the analytical capillary column is crudely estimated to be one-seventh or one-fifth of its original concentration, depending on whether the 1.5-m or the 41-cm delivery tube is used. These values are obtained with the square interface, using the same experimental conditions as in Fig. 4. The dilution factor is very dependent on the experimental setup; it could be reduced in the future. The equipment and reagents used, experimental setup, and conditions are similar to those described previously [18], except as noted above.

## RESULTS AND DISCUSSION

After the solute passes from the rotary value through a delivery tube, it travels a short distance between the end of the delivery tube and the inlet of the capillary in the interface, as shown in Figs. 1 and 2. Then a small part of the solute is transferred into the capillary by electrokinetic injection. The remainder exits the interface and is removed. The amount injected depends on the electroosmotic flow velocity, $v_{osm}$, the electrophoretic velocity of the solute, $v_{el}$, the cross-sectional area of the capillary, $A_{column}$, and the rate of flow (volume per unit time) pumped through the delivery tube, $V$. The split ratio, $R_{split}$, for a solute is given by

$$R_{split} = V/[(v_{osm} + v_{el}) A_{column}]$$

Of course, when the solute is uncharged, $v_{el}$ vanishes in the above expression. Split injection introduces a bias in quantitation, as previously discussed [20]. The split ratio for a neutral compound is calculated from the pressurized flow volume divided by the estimated electroosmotic flow volume. The electroosmotic flow is determined by measuring the elution time of a neutral compound, such as benzene or water. Values of the split ratio used in the present experiment range from 25 to 500.

Band broadening caused by the split injector degrades the quality of the electropherogram. Solute and buffer mix in both the delivery tube and the interface. Shortening the delivery tube minimizes the band broadening caused by mixing. Tube lengths of 150, 41 and 11.5 cm gave $1.0 \cdot 10^5$, $1.2 \cdot 10^5$ and $1.4 \cdot 10^5$ theoretical plates,

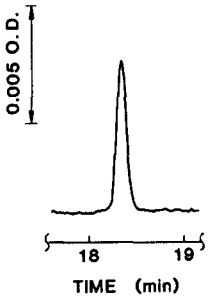


Fig. 3. Electropherogram with a short delivery tube. Delivery tube = 11.5 cm; interface is for a rectangular capillary (200 × 2000 μm); split flow = 0.4 ml/min; split ratio = 133; rectangular capillary = 50 μm × 100 μm × 75 cm (length between column inlet and detector = 55 cm); sample = 5 μl of dansyl-L-serine ($4.7 \cdot 10^{-4}$ *M*); medium = 5 m*M* phosphate buffer (pH 6.55) with 5% ethylene glycol; applied voltage = 9 kV; and current = 71 μA. The theoretical plate number is $1.4 \cdot 10^5$.

respectively, for the separation of dansyl-L-serine. Fig. 3 shows a typical peak obtained using a short delivery tube and the rectangular interface. Note the symmetry of this peak.

Assessing the band broadening in the interface is difficult, but an estimation can be made by comparing the separation efficiency obtained by gravity to that by split injection. With gravity injection, $1.6 \cdot 10^5$ and $2.1 \cdot 10^5$ theoretical plates are obtained using rectangular capillaries with small cross-sectional areas of 16 × 195 μm and 27 × 340 μm, respectively [18]. With a circular capillary (51 μm I.D.), $2.1 \cdot 10^5$ theoretical plates are obtained by gravity injection [18]. Using split injection, $1.4 \cdot 10^5$ theoretical plates are obtained using a rectangular capillary with a large cross-sectional area (50 × 1000 μm). Although these capillary geometries differ, we can compare their theoretical plate numbers to judge the band broadening caused by the split injector. We conclude that split injection causes 1.2–1.5 times more band broadening than gravity injection.

Fig. 4 shows electropherograms obtained with three injector loops of different volumes using a rectangular capillary and a rectangular interface. The peak height is proportional to the volume of the sample loop. The relation is given as $y = ax + b$, where $x$ and $y$ are the injection volume (μl) and the peak height (mm), respectively. The constants $a$ and $b$ are estimated to be 7.89 and 7.0, respectively.

For dansyl-L-serine, the peak height is recorded as a function of sample concentration. The plot is a straight line with a correlation coefficient of 0.990 in the range $10^{-4}$–$10^{-6}$ $M$ of sample solution. Application of the split injector to a 50 × 1000 μm rectangular capillary using a square interface is shown in Fig. 5. The long optical path length (1000 μm) provides about 7 times higher sensitivity than obtained in the electropherogram shown in Fig. 4b. Fig. 6 shows a typical electropherogram using a circular capillary with a circular interface.

Table I lists the run-to-tun reproducibilities of 8 injections of dansyl-L-serine and 3 injections of dansyl-L-cysteic acid and pyridoxamine. A square interface is more

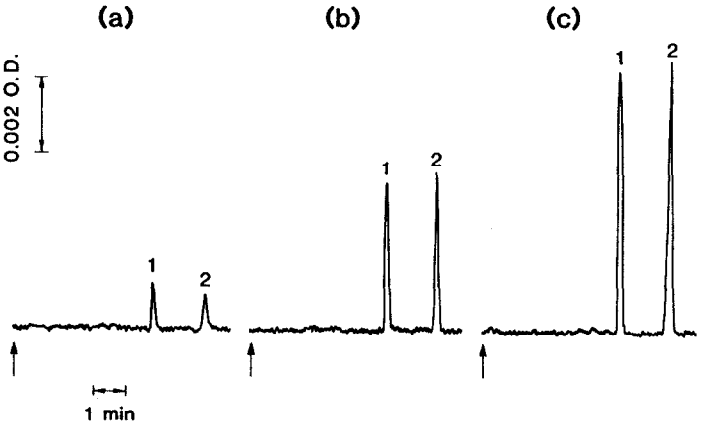


Fig. 4. Effect of loop volume: (a) 1 μl, (b) 5 μl and (c) 10 μl. Conditions are: rectangular capillary, 57 cm long, 50 × 1000 μm; rectangular interface; split flow = 0.5 ml/min; split ratio = 70; sample = (1) pyridoxamine ($1.6 \cdot 10^{-3}$ $M$), (2) dansyl-L- serine ($1.8 \cdot 10^{-3}$ $M$); medium = 5 m$M$ phosphate buffer (pH 6.8) with 5% ethylene glycol; applied voltage = 7.8 kV; current = 76 μA; detection wavelength = 310 nm; and cell path length = 50 μm.

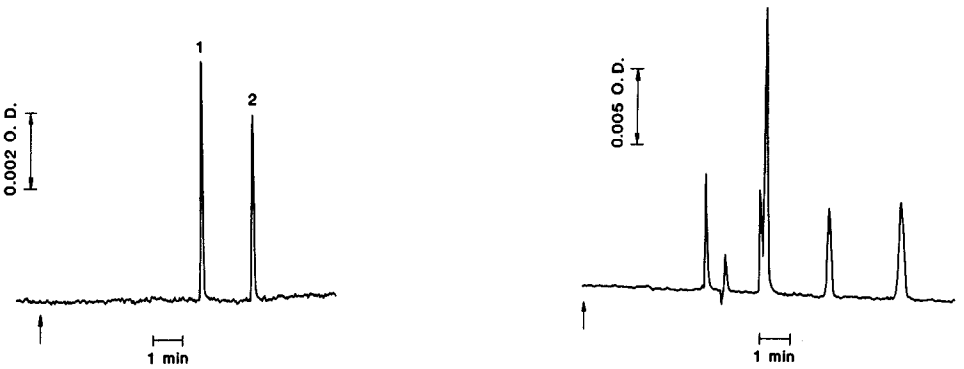


Fig. 5. Typical electropherogram using square interface. Conditions are: loop = 5 $\mu$l; split flow = 0.7 ml/min; split ratio = 70; applied voltage = 9.8 kV; current = 77 $\mu$A; detector wavelength = 310 nm; and cell path length = 1000 $\mu$m. Other experimental conditions are the same as in Fig. 3. Sample = (1) pyridoxamine ($1.6 \cdot 10^{-4}$ *M*), (2) dansyl-L-serine ($2.9 \cdot 10^{-4}$ *M*); split flow = 0.7 ml/min; applied voltage = 9.8 kV; current = 77 $\mu$A; detector wavelength = 310 nm; path length = 50 $\mu$m. Capillary and medium are the same as in Fig. 3.

Fig. 6. Typical electropherogram using circular interface. Conditions are: loop = 5 $\mu$l; capillary column = circular fused-silica tubing, 100 $\mu$m I.D. and 65 cm long; split flow = 0.5 ml/min; split ratio = 550; sample = quinine, guanosine, 5-bromouracil, dansyl-L-serine, dansyl-L-cysteic acid and flavianic acid, eluted in order, each of $1–2 \cdot 10^{-3}$ *M* except quinine ($3 \cdot 10^{-4}$ *M*); medium = 20 m*M* phosphate buffer (pH 6.45) with 5% ethylene glycol; applied voltage = 12 kV; current = 54 $\mu$A; and detection wavelength = 310 nm.

TABLE I

REPRODUCIBILITY OF PEAK HEIGHTS

| | Interface | | |
|---|---|---|---|
| | Rectangular | Square | Square |
| Flow-rate (ml/min) | 0.2 | 0.5 | 0.3 |
| Sample | Dansyl-L-serine, $10^{-3}$ *M* | Dansyl-L-cysteic acid, $6 \cdot 10^{-4}$ *M* | Pyridoxamine, $2 \cdot 10^{-4}$ *M* |
| Peak height | 23.0<br>23.1<br>23.0<br>23.2<br>21.3<br>21.1<br>22.3<br>21.4 | 43.0<br>42.0<br>45.0 | 37<br>37.5<br>40.0 |
| Mean peak height | 22.0 | 43.3 | 38.2 |
| R.S.D. (%) | 6.07 | 3.52 | 4.2 |

reliable than a rectangular one. Relative standard deviations (R.S.D.) are 3–4% for dansyl-L-cysteic acid and pyridoxamine using the square interface. Fluctuations of the pressurized flow affect the reproducibility. In this experiment we used a syringe-type pump. If we had used a pulseless pump, the R.S.D. values would have been smaller.

Compared with other injection methods for CZE separations, the split injector described here has definite advantages and disadvantages. By their nature, split injectors are wasteful of sample. Moreover, the concentration of the sample is diluted by this injection method. Consequently, the split injection method is not the method of choice in ultra-trace analysis or when the sample volume is extremely limited.

The advantage of the present split injector is that it offers convenient sample introduction without the need to interrupt the applied voltage during the injection step. Using the split injector, the separation efficiency ranges from 20 000 to 150 000 theoretical plates, which is more than adequate for most separations. Additionally, this injection method can be interfaced easily to an autosampler. Note that the analytical capillary is held stationary during injection. This feature allows large cross-sectional capillaries to be used, unlike other injection methods. In particular, it enables use of rectangular capillaries of large cross-sectional area.

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