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Split-flow injector for capillary zone electrophoresis

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Abstract

A simple construction of a split-flow injector eliminating some common problems connected with the use of such devices is described. It consists of a low-pressure pump, an injection valve and a delivery tube in which the separating capillary inlet is fixed. The sample is injected without moving the separating capillary inlet and without interrupting the applied voltage. The grounded electrophoretic electrode is close to the injection valve so that all metal parts of the injector are kept at a sufficiently low potential. Minimum length and small internal diameter of delivery tube minimizes additional sample zone broadening. The effects of some experimental parameters, such as the position of the separation capillary inlet with respect to the background solution flow direction and background solution flow-rate are experimentally studied. The injector was tested primarily for the electrokinetic injection. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Split-flow injector; Injection methods; Instrumentation; Benzoic acids; Nitrophenols

1. Introduction

Sample introduction in capillary electrophoresis (CE) influences the final result of the analysis to a much greater degree than in other separation methods. Uncontrolled sample introduction may cause serious problems with the accuracy of quantitative results as well as with the repeatability of migration times. The fundamental difference in injection techniques used in CE and other column separation methods is given by the necessity to introduce an extremely low sample volume into the separation capillary. Hydrodynamic and electrokinetic sampling are clearly the most frequently used techniques in CE. Both injection modes require, in principle, interruption of the applied voltage and repeated insertion of the capillary inlet into the vials of background electrolyte and sample solution. This may lead to some undesirable effects decreasing the reproducibility of injection, initiating the formation of system peaks, causing clogging and rupture of the fragile separation capillary.

Several alternative injectors suitable for CE, eliminating at least some of the above mentioned problems, have been described in the literature. Rotary micro-valves made of a non-conductive material with the internal volume in the nanoliter range allow sampling directly into the separation capillary without the necessity to interrupt analysis [1]. Mostly the lack of suitable mechanically and chemically resistant materials and technical problems connected with the fabrication of such miniaturized devices restrain the wider applicability of this type of

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injector. Split-flow injectors [2] allow only a part of the initial sample volume be introduced into the capillary and seem to be promising especially from the point of view of simple fabrication and the possibility of utilizing common HPLC injection valves.

As it is reported in the literature, the use of split-flow injection has several advantages over the conventional means of sampling [3]. Likewise for injection with rotary micro-valves, it is not necessary to interrupt the applied voltage during the injection step. A split-flow injector can be interfaced easily with an autosampler. The analytical capillary is held stationary and its inlet is not exposed to excessive mechanical forces. As a main drawback limiting the use of split-flow injectors, the authors mention the effect of sample zone broadening due to its mixing with the background electrolyte and the problems connected with the location of the high-voltage electrode close to the sampling device. Such configuration causes problems with the flow of the parasitic current through the delivery tube, the generation of bubbles in the metal rotary valve and, accidentally, may cause the exposure of the operator to the high voltage.

In this paper, we describe a split-flow injector with improved operational characteristics. It consists of a low-pressure linear pump, an injection valve and a delivery tube in which the separating capillary inlet is fixed. No specially machined interfacing components were used in the injector construction. The location of the grounded electrophoretic electrode close to the injection valve make it possible to ground all metallic parts of the injection valve which eliminates the problem with the high voltage and makes injection safe for the operator. The injection is done without interruption of the applied voltage and without moving of the capillary inlet. Using a model sample, the basic peak parameters obtained using the test injector were compared with those obtained using a standard commercial sampling device.

The split-flow injector was designed especially for the injection of samples, which are available in sufficient volume, and recovery of the rest of the sample is not required; the split-flow injector ensures that only a small sample portion is introduced into the separation capillary. In the case that a very small sample volume comes in for analysis and/or the recovery of the rest of the sample is required, another sampling method should be employed.

2. Experimental

2.1. Injector construction

The injector scheme is depicted in Fig. 1. Its principal part is an approximately 10-cm long piece of a polyethylene tube (1 mm I.D.×2 mm O.D.) which was bent approximately to the right angle. One end of the tube was connected to the outlet of a standard HPLC six-way sampling valve (Valco Europe, Switzerland); the distance of the tube bend from the valve outlet was about 1 cm. The other end of the tube was immersed into the background electrolyte solution in an auxiliary vessel.

Using an injection needle, a small hole was made through the tube wall so that the sampling end of the separation capillary could be tightly inserted into the polyethylene tube. Two different geometric orienta-

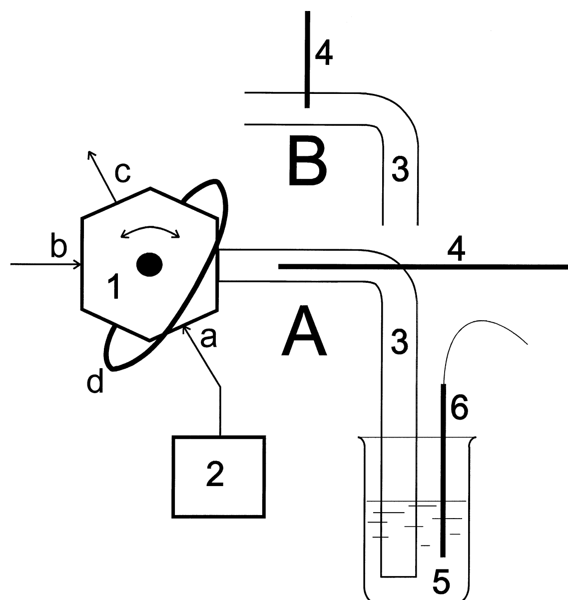

Fig. 1. Schematic diagram of split injector; capillary inlet placed against (A) or perpendicularly (B) to the flow direction. 1, six-way sampling valve (a, inlet from the pump, b, filling of the sampling loop, c, waste, d, sampling loop); 2, pump; 3, polyethylene delivery tube; 4, separating capillary; 5, auxiliary vessel; 6, ground electrophoretic electrode.

tions of the capillary with respect to the background solution flow direction were tested: capillary inlet was oriented either against (Fig. 1A) or perpendicularly (Fig. 1B) to the direction of the solution flow. In both arrangements, the capillary end was fixed in the center of the polyethylene tube and about 5 mm away from the valve outlet. Low sample zone dispersion was achieved in this arrangement as the zone of the sample solution flows only a very short distance between the valve outlet and the capillary inlet.

The sampling valve was equipped with a 30-μl sampling loop that was filled using a hypodermic syringe. Background electrolyte solution was supplied from an autoburette ABU 12 (Radiometer, Denmark); varying the flow-rates in the range from 62 to 1000 μl/min, the sampling time was varied from 29 to 1.8 s.

Individual components used in the injector construction, mainly the internal diameter of the polyethylene delivery tube and the sampling loop volume can be changed purposefully.

2.2. Apparatus and reagents

The experimental setup for electrophoretic measurements consisted of a high-voltage power supply CZE 1000 (Spellman, USA) and an UV Detector LCD 2082 (Ecom, Czech Republic) with optical fibers connecting the detector with the capillary. Optical fibers prevent the detector from being impaired in the case that the capillary is damaged during the measurement, therefore the high-voltage electrode can be placed in the vessel on the detector side of the separation capillary. The grounded electrophoretic electrode is then placed in the auxiliary vessel, i.e., on the inlet capillary side close to the sampling valve. As the sampling valve is also at the ground potential no parasitic current can flow between the electrophoretic electrode and the sampling valve so that the sampling is safe.

The results obtained using the test injector were compared with those obtained using the injecting part of a Crystal 310 CE instrument (ATI Unicam, UK). A standard fused-silica capillary 75 μm I.D.× 375 μm O.D. (Composite Metal Service, UK) was used in all experiments. A benzoic acid (BA) or a mixture of *o*-nitrophenol (ONP) and benzoic acid (Sigma, St. Louis, MO, USA) were employed as a test system. The electroosmotic flow velocity was determined using a 0.01 *M* aqueous solution of thiourea (Lachema, Czech Republic) as a neutral marker. The other reagents used and electrophoretic experimental conditions applied are summarized in Table 1.

2.3. Sampling procedure

A background electrolyte solution flows through the polyethylene tube and a high voltage is switched on. By turning the sampling valve, the sample zone from the sampling loop is introduced into the stream of background electrolyte. At a certain time, the sample zone passes over the capillary inlet and is injected; the remainder of the sample leaves the polyethylene tube into the auxiliary vessel. After sample injection, the background electrolyte solution flow can be stopped. The capillary inlet is in contact with pure background electrolyte. On the contrary to the split injector described in Ref. [3], the com-

Table 1
Capillary electrophoresis experimental conditions

Sample solutions[a]	Benzoic acid in the concentration range from 1 to 14 mg in 100 ml of 10 m*M* Tris or a mixture of 40 mg of *o*-nitrophenol and 14 mg of benzoic acid in 100 ml of 10 m*M* Tris
Background electrolyte	20 m*M* sodium tetraborate
Total length of capillary	76 cm
Length to detector	60 cm
Applied voltage	20 kV (current during analysis 45 μA)
Electrokinetic injection	20 kV
UV detection	200 nm

[a] TRIS was added to increase solubility of both analytes in water.

position of the background electrolyte is not changed due to the possible electrochemical reactions taking place at the electrophoretic electrode as it is placed in an auxiliary vessel far enough away from the capillary inlet.

3. Results and discussion

3.1. Amount of analyte introduced into capillary

The theoretical amount of analyte, m (ng), electrokinetically injected from the sample solution into the capillary by a standard method (using an injection part of a Crystal 310 CE instrument) was calculated from the equation [4]:

$$m = 2.5 \cdot 10^{-3}(\mu_{ep} + \mu_{eo})\pi d^2 Utc/L, \quad (1)$$

where μ_{ep} and μ_{eo} ($cm^2\ s^{-1}\ V^{-1}$) are the electrophoretic and electroosmotic mobility, respectively, d (μm) is the capillary internal diameter, U (kV) is the applied voltage, t (s) is the time of injection, c ($mg\ l^{-1}$) is the concentration and L (cm) is the total capillary length. Using the experimental data, $\mu_{ep}(ONP) = -33.4 \cdot 10^{-5}\ cm^2\ s^{-1}\ V^{-1}$, μ_{ep} (BA) $= -33.6 \cdot 10^{-5}\ cm^2\ s^{-1}\ V^{-1}$, $\mu_{eo} = 67.9 \cdot 10^{-5}\ cm^2\ s^{-1}\ V^{-1}$ $d = 75\ \mu m$, $U = 20$ kV, $t = 1.8$ s, c (ONP) $= 400\ mg\ l^{-1}$, c (BA) $= 140\ mg\ l^{-1}$, $L = 76$ cm and $l = 60$ cm[1], the injected amount of 2.89 ng ONP and 1.01 ng BA was calculated. The peak areas obtained using the standard method (supposing that the peak areas correspond to the calculated injected amount of individual analytes) were then compared with those obtained when the tested injector was used. From the comparison, the amounts of ONP and BA injected into capillary using the test injector were estimated.

The amount of analyte injected from the sample solution into the capillary depends on the injection voltage and time of sampling. The time of sampling equals the time at which the sample zone passes over the capillary inlet. This time is controlled by the background solution flow-rate, the volume of the sample injected by the injection valve and by the internal diameter of the polyethylene delivery tube. At a constant injection voltage, a constant sample volume and a fixed internal diameter of the delivery tube, the sampling time can be controlled only by the background solution flow-rate.

3.1.1. Effect of capillary orientation

It was found that, at the constant injection voltage, the amount of analyte injected into the capillary depended on the geometric orientation of the capillary inlet with respect to the direction of the background solution flow.

For the capillary inlet oriented against the buffer flow, the total amount of analyte injected was substantially higher then the amount of analyte injected into the capillary oriented perpendicularly to the solution flow direction; it is illustrated in Fig. 2. It is also seen from this figure that, for this capillary orientation, the analyte was injected even if no voltage was applied. Obviously, a complex mechanism of the injection determines the amount of analyte injected in this case. The total amount of injected analyte is the sum of two contributions, the amount injected electrokinetically and the amount introduced into capillary hydrodynamically. The hydrodynamic injection stems from the convection in the buffer solution flowing against the capillary inlet. The amount of analyte injected hydrodynamically exceeded substantially that injected electrokinetically and increased linearly with the buffer solution flow-rate.

For the capillary oriented perpendicularly to the solution flow direction, the analyte was injected primarily by electrokinetic injection. The contribution of hydrodynamic injection was negligible. The injector with the capillary inlet in this geometric arrangement was mainly tested.

The amount of analyte injected purely electrokinetically is not dependent on the capillary orientation. An equal amount of analyte was injected both for the capillary oriented perpendicularly to and against the flow direction, provided that the amount of analyte injected hydrodynamically was subtracted from the total amount in the latter case, see Fig. 2.

3.1.2. Dependence on sampling time and analyte concentration

According to the above theoretical equation, the amount of analyte electrokinetically injected into the

[1] l is the capillary length to the detector.

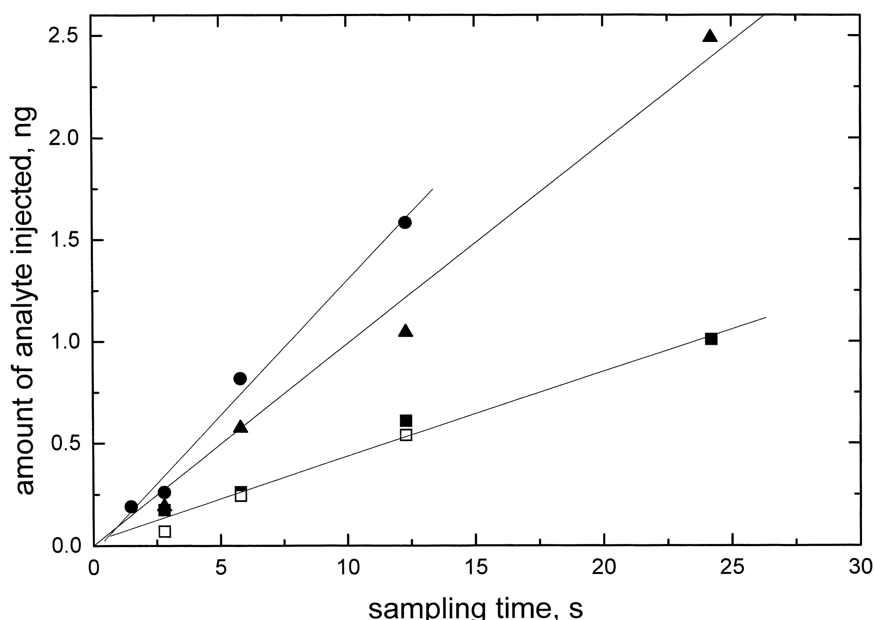

Fig. 2. Dependence of the injected amount of analyte on the sampling time for different geometric orientations of the capillary inlet with respect to the background solution flow direction: ●, against; ▲, against, no voltage applied (hydrodynamic injection); ■, perpendicularly to, □, against, amount of analyte injected hydrodynamically was subtracted from the total injected amount. Sample: 3.5 mg of BA in 100 ml of 10 m*M* Tris, i.e., 1050 ng of BA in a 30-μl sampling loop, electrokinetic injection at 20 kV.

capillary is linearly dependent both on the time of injection and concentration of analyte in the sample solution.

The experimental dependence of the amount of BA, $m(t)$ (ng), injected into the capillary on the sampling time, t (s), was proved to be linear (see the corresponding points in Fig. 2) and could be described by the regression equation, $m(t) = 0.021(0.029) + 0.042(0.002)t$, with correlation coefficient of 0.997 (standard deviations of the intercept and the slope are in the parentheses). The zero value of the intercept is within the 95% confidence interval.

Likewise, the amount of analyte injected into capillary at a constant sampling time, $m(c)$ (ng), was linearly dependent on the concentration, c see Fig. 3; the same dependence obtained using the Crystal injector is given in Fig. 3 for comparison. The regression line describing the dependence obtained using the test injector is: $m(c, \text{test}) = 0.002(0.007) + 4.84(0.11)c$ with a correlation coefficient of 0.999, and that for the Crystal injector: $m(c, \text{Crystal}) = 0.033(0.016) + 5.69(0.22)c$ with a correlation coefficient of 0.998 (in both equations, the standard deviations of the intercept and the slope are in the parentheses). The dependence obtained for the test injector passes through the origin, as the intercept value is not significantly different from zero value (0.95% confidence interval). This is not true for the Crystal injector. In this case, a small amount of analyte is injected into capillary probably as a result of diffusion and convection transport processes occurring during the immersion of the sampling end of capillary into the solution of the sample before the sampling voltage is switched on. These vague transport processes are avoided using the test injector.

3.2. Effect of sample injection on the peak parameters

Basic parameters of the peaks, namely the area, the height, the baseline width and the peak asymmetry, obtained using the test injector were compared with those obtained using the Crystal 310 CE device. A test solution of a mixture of BA and ONP was used to check the possible effect of

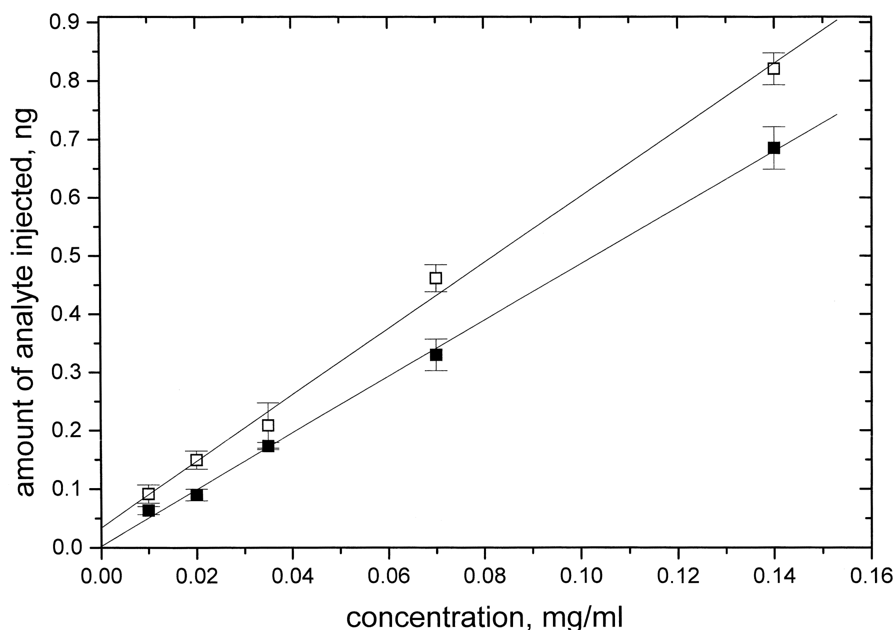

Fig. 3. Dependence of the injected amount of analyte on the concentration for the capillary inlet oriented against the background solution flow direction: ■, test injector, sampling time, 3.6 s, □, Crystal 310 CE injector, sampling time, 1.8 s. Each point is the average of five measurements (error bars = standard deviation).

splitting injection on the separation parameters (number of plates, resolution). The absolute values of the peak parameters could not be compared directly because it was practically impossible to inject exactly the same amount of analyte into the capillary by these two methods. Therefore, to test whether the tested and the standard injection methods differ in their precision, the variances of the corresponding sets of the parameters were compared using the two-tailed F-test [5].

The results of the comparison are summarized in Table 2. For both analytes tested, the peak parameters for which no statistically significant difference between the two variances was obtained are shown. It is seen that the tested injector enables sample injection with precision quite comparable in many respects with those obtained using the standard method. Splitting injection proved favorable in the peak width and, consequently, in the separation efficiency and the peak resolution, see the last two rows in Table 2. An illustrative electrophoregram of the separation of the ONP–BA mixture is shown in Fig. 4.

The data obtained for the injector with the capillary oriented against the flow direction are also presented in Table 2 to demonstrate that this geometrical arrangement of the injector can also be employed for the sample injection.

3.3. Split ratio

Only part of the analyte in the sampling loop is transferred into the separation capillary from the background solution stream. The split ratio, e.g., the ratio of the amount of analyte injected into capillary to the amount of analyte in the sampling loop, depended linearly on the sampling time, t. From the slope of this linear dependence obtained for injection of BA, the split ratio of $0.00004t$ was determined. It corresponds to the split ratios from ca. 1:13 000 to 1:900 for the sampling times tested.

Obviously, the dispersion of the sample zone in the flowing background solution affects the split ratio. As the dispersion depends on the geometric arrangement in the injector, the change in the injector geometry will result in a change of the split

Table 2
Comparison of the selected parameters obtained using the tested injector and the injecting part of a Crystal 310 CE instrument[a]

Parameter	Injector			
	A	B	C	D
Number of points	10	11	7	10
Peak area (V s)	0.107 (0.011)[b] 0.163 (0.019)	0.039 (0.006)[b] 0.062 (0.006)[b]	0.136 (0.012)[b] 0.203 (0.016)	0.160 (0.006) 0.260 (0.007)
Peak height (V)	0.037 (0.002)[b] 0.056 (0.004)[b]	0.016 (0.002) 0.024 (0.002)[b]	0.042 (0.003) 0.065 (0.005)	0.038 (0.001) 0.061 (0.002)
Asymmetry	1.27 (0.18) 1.46 (0.18)[b]	1.23 (0.15) 1.16 (0.08)[b]	1.21 (0.24) 1.76 (0.18)[b]	0.85 (0.03) 1.05 (0.11)
Baseline peak width (s)	6.8 (0.8)[b] 7.7 (0.9)[b]	5.1 (0.6)[b] 5.5 (0.6)[b]	7.7 (0.3)[b] 9.1 (0.6)[b]	10.8 (0.4) 12.6 (0.7)
Number of plates	118 100 96 500	192 900 183 700	884 00 66 100	44 500 34 600
Resolution	1.07	1.56	1.02	0.92

[a] A, B and C: tested injector, A, capillary oriented perpendicularly to the flow, sampling time 3.6 s; B, C, capillary oriented against the flow, sampling time 1.8 s and 3.6 s, respectively; D, Crystal 310 CE sampler, sampling time 1.8 s. Upper value in the column corresponds to ONP, lower value to BA. Standard deviations are in the parenthesis.

[b] The peak parameters for which no statistically significant difference between the variances of the tested detector and the Crystal 310 CE sampler was obtained.

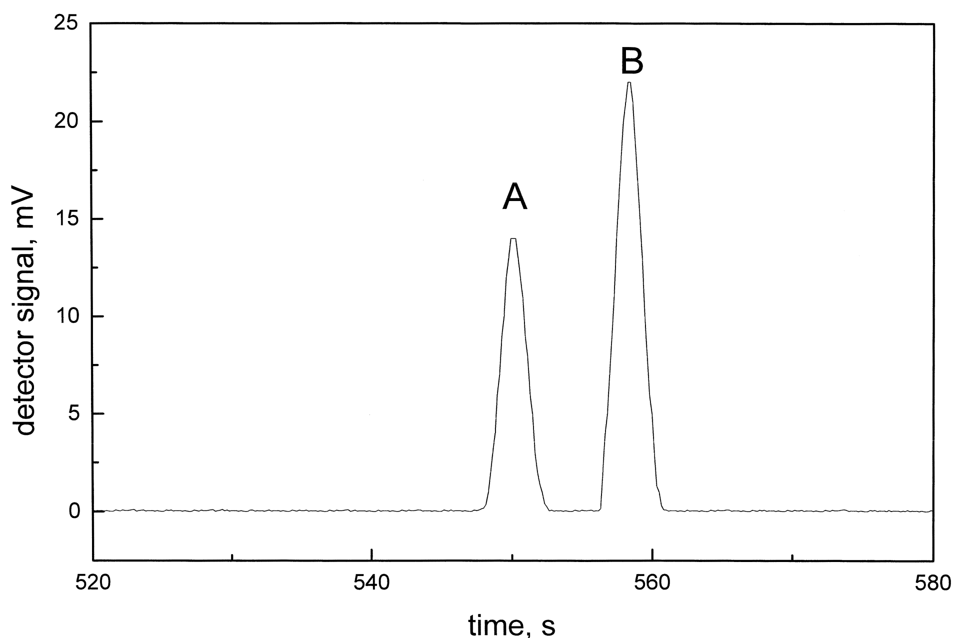

Fig. 4. Illustrative electrophoregram of the separation of benzoic acid (A) and *o*-nitrophenol (B) test mixture using split-flow injection; sampling time, 1.8 s (additional experimental conditions, see Table 1), the inlet of the separating capillary was oriented against flow direction.

ratio. To obtain reproducible injection, all parameters having an influence on the dispersion must be kept constant. Particularly, the inlet end of the capillary is very prone to the motion and must be carefully fixed at a constant position in the delivery polyethylene tube.

4. Conclusions

A very simple split flow injector has been constructed and tested. It consists only of a low-pressure pump and a sampling valve equipped with a piece of polyethylene tube at its outlet. A sampling end of the separation capillary enters this polyethylene tube through the tube wall and is fixed in the centre. No special interfacing and positioning elements are necessary. The sample is injected with a precision comparable with those obtained using the standard electrokinetic sampling device but compared to the standard method, the split flow injector has following advantages:

(i) Electrokinetic sample injection requires no interruption of the applied voltage;

(ii) Both during the sample injection and at the separation, the sampling end of the capillary is permanently immersed in a solution at a constant position. It is not moved several times between the vessels containing either a background buffer or a sample solution as it is with classical electrokinetic sampling;

(iii) The split ratio is simply adjusted in the range of several orders of magnitude by changing the background solution flow-rate;

(iv) The same sampling system can possibly be used for both the electrokinetic and the hydrodynamic injection (the hydrodynamic injection mode was not studied in detail).

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