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Short communication

Influence of the sample volume and the position of the electrode and the capillary-end in the sample vial on the electrokinetic injection in capillary electrophoresis

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Abstract

The influence of the positions of the electrode and the capillary-end and volume in the sample vials on the electrokinetically injected amount of the analytes in capillary electrophoresis was studied. The influence may be reduced by using a more or less fixed position of the electrode and the capillary. © 1997 Elsevier Science B.V.

Keywords: Sample volume; Injection methods; Electrode position; Propranolol hydrochloride

1. Introduction

In capillary electrophoresis (CE) it is of great importance to introduce the sample analytes in a minimum volume in a very short time in order to maintain maximum resolution. This may be achieved by injecting the analytes in a buffer with a low conductivity and using a buffer with a high(er) conductivity for the separation [1–8]. The analyte ions migrate under the influence of the applied electric field towards the concentration boundary. Once they cross the boundary and move into the buffer with the higher conductivity, they slow down, forming a concentration stack.

Using electrokinetic injection instead of hydrodynamic injection results in an electric field over the sample vial which increases the concentration stack.

When the electroosmotic flow is eliminated, the amount of material injected is a function of the electrophoretic mobility of the analyte(s) and the conductivity of the sample and the running buffer. However, two important features must be kept in mind. First, because the amount of material injected is a function of several parameters which are hard to control, it may be difficult to maintain adequate reproducibility in the injection of the sample. Such parameters can, for instance, be the pH of the electrolyte and the sample solution as this affects the charge on the compound and that is related to the amount injected. Other factors affecting electrokinetic injections include depletion of the sample and changes of pH of the sample solution due to electrolysis during injection [11].

Second, the amount of each sample component loaded onto the capillary will be a function of the mobility of each sample component [6,9] and is related to the sample and buffer viscosity.

It is also important that the column should be free

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of vibrations during the injection process, because this may produce a physical disturbance at the end of the capillary, causing improper field amplification at the injection point [10].

This paper shows that there are some additional parameters that influence the amount of analyte migrating into the capillary during electrokinetic injection, namely the positions of the electrode and the capillary-end and the volume in the sample vial.

Two electrophoresis systems were used to investigate the impact of these parameters.

2. Experimental

2.1. Apparatus

2.1.1. System 1

The CE system was a Model Prince with a four-position sample tray and a programmable injector system from Lauerlabs (Emmen, The Netherlands). Detection at 210 nm was carried out with a Lambda 1000 UV–Vis VWL detector (Bischoff, Leonberg, Germany). The bare fused-silica capillary with an outer polyimide coating (50 μm I.D.×375 μm O.D.) was from Polymicro Technologies (Phoenix, AZ, USA). Data acquisition of CE–UV was performed by the Maclab system (ADinstruments, Castle Hill, Australia) using the Chart program (version 3.3, ADinstruments) for recording the electropherograms. For interpretation of the electropherograms, the Peaks program (ADinstruments) was used. The vials used were 4-ml glass vials with a 0.7-ml plastic insert for a Waters 96 and were obtained from Phase Sep.

2.1.2. System 2

The second CE system was a HP 3D capillary electrophoresis system (Hewlett-Packard, Amstelveen, The Netherlands) with a carousel and a programmable injector system. Detection was carried out with the built-in diode array detector at 210 nm. The bare fused-silica capillary with an outer polyimide coating (50 μm I.D.×375 μm O.D.) and the polypropylene vials (1 ml/11 mm) were from Hewlett-Packard. Data acquisition was performed by the HP 3DCD ChemStation Software (Rev. A. 04.01, Hewlett-Packard).

2.2. Solutions

The run-buffer was a 100 m*M* phosphate buffer with a pH of 2.5 and a conductivity of 0.7 mS/cm. It was prepared by dissolving sodium dihydrogenphosphate monohydrate (Merck, Darmstadt, Germany) to a concentration of 100 m*M* and adjusting the pH with concentrated *ortho*-phosphoric acid (85%, Merck).

The analyte solution for system 1, propranolol hydrochloride (pharmacopoeial quality), was dissolved in water to a final concentration of 25 μg/ml.

The analyte solution for system 2, propranolol hydrochloride, was dissolved in a solution containing 1 part run-buffer and 9 parts of water to a final concentration of 50 μg/ml.

Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). The conductivity of the purified water was always less than 2 μS/cm.

All solutions were filtered (0.45 μm) through a membrane filter and degassed for 5 min in an ultrasonic bath (50 kHz, Branson Europa, Soest, The Netherlands), immediately prior to use.

2.3. CE conditions used for experiments

System 1 used a capillary with a total length of 70.0 cm and an effective length of 55.0 cm. System 2 used a capillary with a total length of 38.0 cm and an effective length of 28.5 cm. An optical viewing window with a length of 0.5 cm, obtained by burning off the polyimide coating, was aligned with the UV detection cell. The coating of the first 2 mm of the capillary was also stripped.

A new capillary was rinsed with 1 *M* sodium hydroxide for 10 min at 1000 mbar, with water for 10 min at 1000 mbar and with the run-buffer for 10 min at 1000 mbar. Electrokinetic injection was carried out with different voltages and injection times. The injection voltage was ramped by 5 kV/s. In system 1, electrokinetic injections were carried out from vials containing 200, 300, 400, 500, 600 and 700 μl of a 25 μg/ml propranolol solution. In system 2, electrokinetic injections were carried out from vials containing 200, 300, 400, 500, 600 and 700 μl of a 50 μg/ml propranolol solution. Separation was carried out at 30°C after the electrode and the capillary-end were dipped in a vial containing

water and began when the ground electrode and the other capillary-end were placed into the vial containing the run-buffer and the high voltage was switched to 30 kV.

For each volume a different sample was used during injection. Each sample then was injected five times to exclude outliers.

2.4. Statistical methods

One-way analysis of variance (ANOVA), the paired two-sample *t*-test and the independent two-sample *t*-test were performed with an Origin 3.0 (MicroCal Software, Northampton, MA, USA) program.

3. Results and discussion

3.1. System 1

In Fig. 1, the peak areas are shown as a function of the sample volume. The liquid level of the outlet buffer was the same as the level in a sample vial containing 500 μl of a solution. One-way analysis of variance of the data given in Tables 1 and 2 confirm that there is a significant difference between the means at a 95% reliability level (α=0.05; F=23.5, $P=1.60\times10^{-8}$): where α=the error of the first kind (unreliability in accepting the null-hypothesis); F= probability distribution (the ratio of two independent variance estimates obtained from the sample normal distribution); and P=probability factor (the factor that indicates the chance that the given test-statistic is not correct).

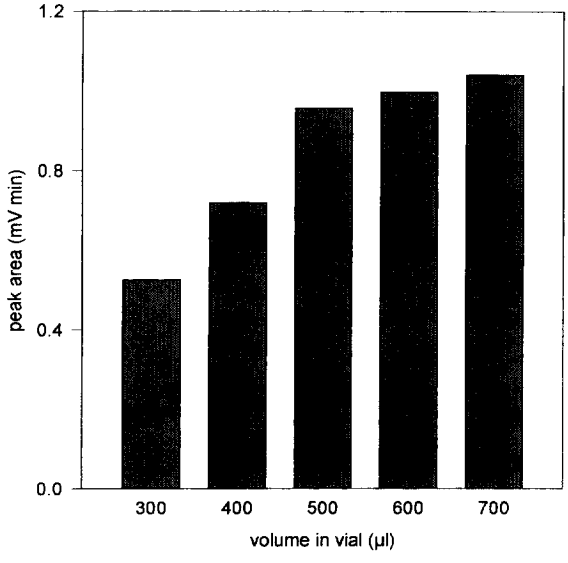

Fig. 1. Influence of sample volume on peak area (means±S.D.) using system 1. Electrokinetic injection at 10 kV for 3 s.

Table 1
Influence of sample volume on the peak area for different sample volumes using system 1 with electrokinetic injection at 10 kV for 3 s

Volume (μl)	Peak area (mV min)	R.S.D. (%)
300	0.53	16 (n=6)
400	0.72	26 (n=4)
500	0.96	4.3 (n=5)
600	1.00	4.4 (n=5)
700	1.04	4.0 (n=4)

3.2. System 2

In Fig. 2, the peak areas are shown as a function of the sample volume. The liquid level of the outlet buffer was the same as the level in a sample vial containing 500 μl of a solution. One way analysis of variance of the data given in Table 3 confirms that there is a significant difference between the means of the peak area at a 95% reliability level (α=0.05; F=19.6, $P=8.8\times10^{-8}$).

Figs. 1 and 2 show that variations in sample volume may cause significant variations in the peak areas. It seems that the impact on the peak area is proportional to the volume in the sample vial. The

Table 2
Significant differences between the sample volumes using system 1 with electrokinetic injection at 10 kV for 3 s calculated with an independent two-sample *t*-test

Volume (μl)	Significantly different from:	t	P
300	500	10.48	2.427×10^{-6}
	600	11.34	1.242×10^{-6}
	700	11.28	3.429×10^{-6}
400	500	2.768	0.0278
	600	3.229	0.0145
	700	3.311	0.0162
500	700	3.021	0.0194

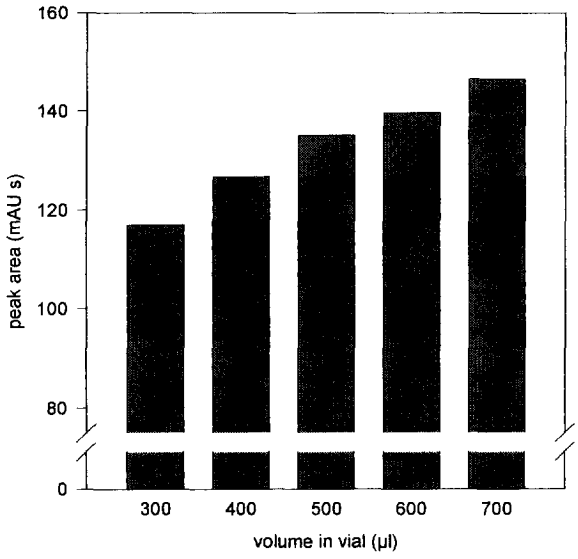

Fig. 2. Influence of sample volume on peak area (means±S.D.) using system 2. Electrokinetic injection at 10 kV for 3 s.

latter may be explained by the fact that the applied electric field over the sample solution in the vial decreases with the volume of the sample solution in the vial. This leads to a reduced amount of sample in the capillary.

The relatively high coefficients of variation obtained with sample injection from the 300- and 400-µl sample vials with system 1 and the sample injection from the 200-µl sample vial with system 2 cannot be explained at the moment.

Table 2 gives the significant differences between the tested sample volumes calculated with an independent two-sample *t*-test. The results imply that not only the peak areas obtained by injection from the low volume sample vials with system 1 differ from the rest, but also the peak areas obtained by injection from the higher volumes significantly differ from each other.

Table 3
Influence of sample volume on the peak area for different sample volumes using system 2 with electrokinetic injection at 10 kV for 3 s

Volume (µl)	Peak area (mAU s)	R.S.D. (%)
300	116.994	2.1 (n=5)
400	126.665	0.62 (n=5)
500	135.058	0.17 (n=5)
600	139.646	1.1 (n=5)
700	146.515	0.77 (n=5)

According to Tables 1–3 the effect is larger for the Lauerlabs system, where peak areas range from 0.53–1.04 mV min (~95%), than for the Hewlett-Packard system where peak areas range from 90.7 to 146.5 mAU s (~62%). Also the precision of the Hewlett-Packard system was usually better than that of the Lauerlabs system as can be concluded from the relative standard deviation (R.S.D.). The latter may be due to the fact that the systems differ in their electrode capillary configuration as shown in Fig. 3. In the Lauerlabs system (system 1) the electrode is placed parallel to the capillary-end, whereas in the Hewlett-Packard system (system 2) the capillary is placed inside the electrode at a fixed position, 5 mm up to the electrode. The first system causes a lot more friction of the electrode which can easily lead to a change in alignment of the electrode and the capillary.

In order to assess the possible contribution of siphoning, some additional tests were carried out using system 2. Two tests injections were made at 0 KV for 3 and 9 s, respectively. Under these circumstances, the analytes can enter the capillary only by siphoning or diffusion which is expected to increase with injection time. The peak areas are given in Table 4. One-way analysis of variance shows, for both injection times, that the mean peak areas for

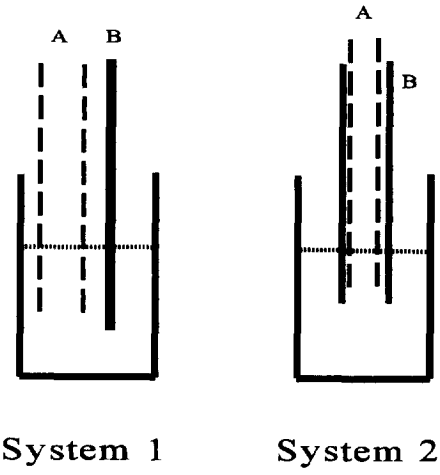

Fig. 3. Positioning electrode (B) and capillary-end (A) in systems 1 and 2.

Table 4
Peak areas for different sample volumes using system 2 without applying a voltage for 3 and 9 s, respectively

Volume (μl)	Peak area±S.D. (mAU s) (0 kV, 3 s)	R.S.D. (%)	Peak area±S.D. (mAU s) (0 kV, 9 s)	R.S.D. (%)
300	1.61±0.23	14	1.89±0.36	19 (n=5)
400	2.48±0.23	9.1	2.45±0.47	19 (n=5)
500	2.28±0.22	9.7	2.34±0.47	20 (n=5)
600	3.10±0.35	11	3.57±0.37	10 (n=5)
700	4.27±0.27	6.3	4.39±0.70	16 (n=5)

different sample volumes are significantly different at a 95% reliability level (α=0.05; F=56.6, P=1.68×10^{-12} and F=22.7, P=2.28×10^{-8}, respectively). When we compare the two tests with a paired two-sample t-test, the two means are *not* significantly different at a 0.05 confidence level (t=−2.34, P=0.0664).

The average peak areas obtained for both injection times are very small, so siphoning can be excluded. The peak areas given in Table 4 therefore indicate that the phenomena observed in Figs. 1 and 2 cannot be explained by siphoning.

Finally, we compared the results of an electrokinetic injection at 10 kV for 9 s with an electrokinetic injection at 30 kV for 3 s using system 2. When siphoning is negligible one should expect that the peak areas would be the same. The results are presented in Table 5 and in Fig. 4. One-way analysis of variance shows for both that the means are significantly different for all sample volumes at a 95% reliability level (α=0.05; F=276, P=0 and F=260, P=0, respectively). When we compare both tests with a paired two-sample t-test, the two means are significantly different at a 0.05 confidence level (t=5.20, P=0.00345). The latter can not be explained at the moment.

When we compare the results of the electrokinetic injection with 10 kV for 9 s in Table 5 with the results of the electrokinetic injection with 10 kV for 3 s in Table 3, we can conclude, as expected, that the results with the former are a factor of 3 higher.

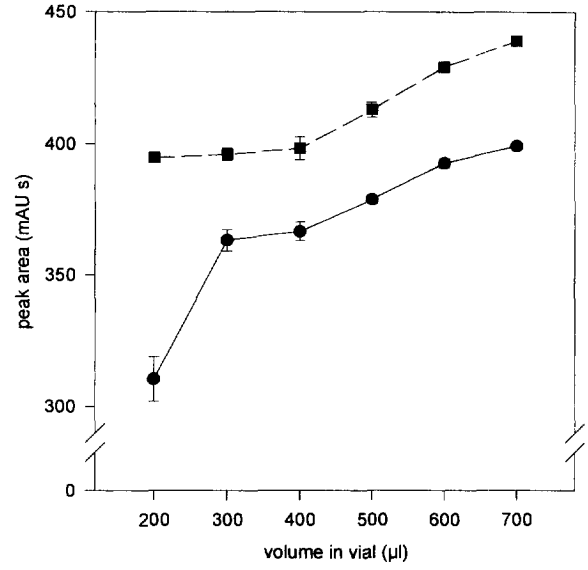

Fig. 4. Influence of sample volume on peak area (means±S.D.) using system 2. Electrokinetic injection at 30 kV, 3 s (●) and 10 kV, 9 s (■), respectively.

4. Conclusion

Electrokinetic injection can be used to increase the

Table 5
Peak areas for different sample volumes using system 2 with electrokinetic injection at 10 kV for 9 s and 30 kV for 3 s, respectively

Volume (μl)	Peak area±S.D. (mAU s) (10 kV, 9 s)	R.S.D. (%)	Peak area±S.D. (mAU s) (30 kV, 3 s)	R.S.D. (%)
300	396.10±2.34	0.59 (n=4)	363.17±4.08	1.1 (n=5)
400	398.31±4.34	1.1 (n=4)	366.51±3.59	0.98 (n=5)
500	412.92±2.86	0.69 (n=5)	378.69±1.30	0.34 (n=5)
600	429.08±0.59	0.14 (n=5)	392.63±1.78	0.45 (n=5)
700	439.02±1.62	0.37 (n=5)	399.20±0.45	0.11 (n=4)

sensitivity of capillary electrophoresis. However, the positions of the electrode and the capillary-end and the volume in the sample vials may effect the injected amounts of the analytes and hence the sensitivity. Using an injection system with a more or less fixed position of the electrode and the capillary, as in the Hewlett-Packard system, may reduce this effect. A constant volume in the sample vial should be maintained to warrant reproducible electrokinetic injections.

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