

A buffer system for capillary electrophoresis

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A fused-silica capillary wall contains silanol groups which, when ionized, bear a negative charge. This charge is normally a function of the pH of the buffer used for separation. The negatively charged wall attracts cations from the buffer, creating an electrical double layer. When voltage is applied, cations in the diffuse portion of the gradient migrate toward the cathode, carrying the buffer along. The result is a net flow of buffer solution in the direction of the cathode. This electroosmotic flow (EOF) is low at low pH and reaches its maximum at about pH 11.

Other parameters also influence the EOF, such as viscosity of the buffer, ionic strength of the buffer, applied voltage, and dielectric constant of the buffer. When performing capillary separations with fused silica, particularly at low pH, differences can be observed in migration time. These differences can be due to surface chemistry and surface heterogeneity. Differences may occur using the same capillary, the same capillary batch, or from batch to batch. Interactions also exist between the capillary wall, the buffer, and the sample, as if the capillary wall had a memory effect or hysteresis effect.

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Therefore, the use of one dedicated capillary for each buffer, or coated capillaries (when sample sticks on the capillary wall or when EOF has to be suppressed) is recommended. Another recommendation is the use of mobility instead of migration time to correct for capillary size, voltage, and temperature fluctuations.

A dynamic double coating (*Figure 1*) of the capillary wall was developed to obtain a highly reproducible separation within the same capillary and from capillary to capillary.

Experimental

The experiments (*Figure 2*) were performed using a P/ACE 5010 CE system with a diode array detector (DAD) or a P/ACE MDQ system with DAD (**Beckman**

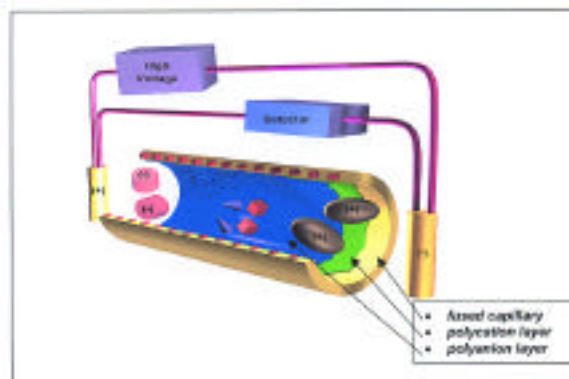


Figure 1 Inner wall of the capillary after coating. On the bare silica wall there is first a layer of polycation (green) and then a layer of polyanion (blue).

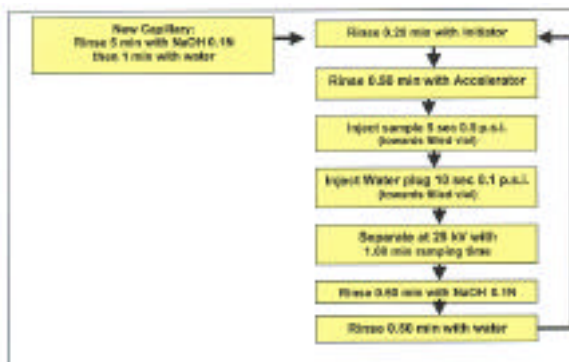


Figure 2 Typical separation procedure for 50- or 75- μm capillary. Separation voltage can be changed according to the length of the capillary.

Coulter, Fullerton, CA). The separation temperature was 25 °C; sample was injected under pressure for 5 sec at 0.5 psi (1 psi = 6894.76 Pa). Separation was performed in a fused-silica capillary obtained from **Polymicro Technologies** (Phoenix, AZ).

CELixir™ buffers (**MicroSolv**, Long Branch, NJ) at different pH containing dynamic coating were produced in the author's laboratory (patent 5.611.903, **Analix**, Namur, Belgium). Other chemicals were purchased from **Sigma Aldrich** (Bornem, Belgium).

A test mix was set up containing pheniramine, brompheniramine, ephedrine, nortryptiline, hydroxy-

Table 1

Peak no.	Reproducibility on migration time and mobility										Marker
	1	2	3	4	5	6	7	8	9	10	
P/ACE 5010:											
Mean migration time (min)	1.96	2.04	2.33	2.40	2.48	2.61	2.66	2.80	2.89	4.10	4.81
RSD (%) on migration time	0.15	0.17	0.16	0.17	0.15	0.15	0.17	0.14	0.16	0.16	0.13
RSD (%) on mobility	0.12	0.16	0.12	0.12	0.13	0.12	0.11	0.16	0.00	1.43	—
P/ACEMDQ:											
Mean migration time (min)	4.92	5.18	6.11	6.35	6.60	6.93	7.18	7.62	7.92	11.42	14.04
RSD (%) on migration time	0.13	0.08	0.17	0.17	0.18	0.35	0.12	0.14	0.11	1.68	0.16
RSD (%) on mobility	0.13	0.11	0.17	0.17	0.19	0.80	0.07	0.10	0.00	9.62	—

Table 2

Peak no.	Reproducibility from capillary to capillary										Marker
	1	2	3	4	5	6	7	8	9	10	
Same batch ($n = 3 \times 220$)											
Mean migration time (min)	1.96	2.04	2.32	2.40	2.48	2.61	2.66	2.80	2.89	4.10	4.81
RSD (%) on migration time	0.28	0.31	0.29	0.28	0.28	0.22	0.28	0.27	0.27	0.25	0.22
Different batches ($n = 3 \times 22$)											
Mean migration time (min)	1.97	2.06	2.34	2.42	2.50	2.63	2.69	2.83	2.92	4.15	4.87
RSD (%) on migration time	0.73	0.72	0.79	0.81	0.82	0.84	0.86	0.86	0.89	1.13	1.36

zine, metoprolol, verapamil, loperamide, phenylglycine, and benzylalcohol (EOF marker).

The capillary was initialized with NaOH and water; two successive rinse steps were then performed, first with a polycation (Initiator™, **Microsolv**), and then with the running buffer containing a polyanion (Accelerator™, **Microsolv**). After the separation, the coating was removed, first by rinsing with NaOH (0.1 M/L), and then with water.

To optimize the separation, about 1 mm of polyimide coating was removed from both ends of the capillary. Separation was also improved by injecting a water plug after the sample, and by ramping up the voltage for 1 min at the beginning of the separation.

General maintenance of the P/ACE 5010, MDQ, and computer hard disk was performed before the experiments.

Results and discussion

Several experiments were performed to validate the dynamic double coating technique. Experiments were performed on the following test mix: pheniramine, brompheniramine, ephedrine, ketamine, nortryptiline, hydrazine, metoprolol, verapamil, loperamide, phenylglycine, and benzylalcohol (EOF marker). This was also the order of separation when separation was done at pH 2.5, as shown in *Figure 3*.

Within-run reproducibility on migration time and mobility

To control the EOF of the capillary, the migration time RSD for an EOF marker (benzylalcohol) was determined. The same was done for the other molecules from the test mix and the results were compared with mobility RSD.

Mobility calculation was done for peak 6, loperamide (*Figure 3*). This peak was then used as a reference to calculate the mobility for the other peaks.

Table 1 shows the separations performed on a P/ACE 5010 with DAD and 50 $\mu\text{m} \times 37$ cm capillary, CELixir, pH 2.5 (70 mM phosphate buffer), at 25 kV; current = 69 μA and $n = 22$. Also shown are the separations of the same test mix on a P/ACE MDQ with DAD and 75 $\mu\text{m} \times 60$ cm capillary CELixir, pH 2.5, at 25 kV; current = 79 μA and $n = 22$.

The RSDs for migration time were below 0.20% with two exceptions, which may be due to the fact

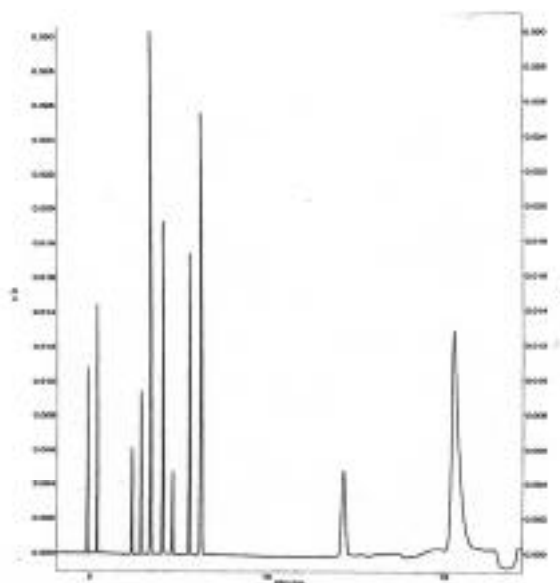


Figure 3 Separation of test mix with CELixir, pH 2.5.

Table 3

Peak no. <i>n</i> = 5 × 3	Between-day reproducibility										Marker
	1	2	3	4	5	6	7	8	9	10	
Mean migration time (min)	4.95	5.21	6.15	6.39	6.64	6.97	7.23	7.67	7.97	11.49	14.17
RSD (%) on migration time	0.48	0.39	0.54	0.53	0.55	0.50	0.49	0.51	0.50	2.68	0.76

Table 4

Peak	CELixir 2.5 compared with phosphate buffer							
	Migration time (min)	Peak height	Peak asymmetry	Theoretical plates	Migration time (min)	Peak height	Peak asymmetry	Theoretical plates
1	8.23	21,635	0.86	585,160	10.63	16,807	1.41	400,032
2	8.69	38,856	1.12	596,025	11.57	21,283	1.28	216,131
3	10.29	10,462	1.17	467,215	15.02	6331	1.38	236,568
4	10.72	29,089	1.20	497,487	16.07	18,134	1.38	211,786
5	11.15	37,479	1.41	493,118	17.67	24,937	1.49	187,266
6	11.85	37,199	1.25	561,799	19.36	22,973	1.87	143,956
7	12.18	23,289	1.38	499,889	19.90	11,944	1.43	194,900
8	12.96	32,139	1.24	557,859	23.35	16,307	1.71	121,879
9	13.48	77,806	1.57	374,499	24.23	36,341	1.68	115,430
10	20.33	13,404	1.11	159,623	61.69	6928	1.08	—
11	24.30	34,636	1.29	77,306	>120	—	—	—

that the test mix was not stable for these molecules. The RSD for mobility for molecules migrating before the reference peak are in the same range as the values obtained for migration time. This similarity indicates that the coating technique controls the EOF very well.

Reproducibility with different capillaries

To attain reproducibility from capillary to capillary, two experiments were performed (Table 2). First, three capillaries from the same batch were separated on a P/ACE 5010 with DAD and a 50 μm × 37 cm capillary CELixir, pH 2.5, at 25 kV; *n* = 3 × 22. The currents observed were 69 μA , 69 μA , and 69 μA . Later, three capillaries from different batches were separated on the P/ACE 5010 with DAD, 50 μm × 37 cm CELixir capillary, pH 2.5, at 25 kV; *n* = 3 × 22 (Table 2). Currents were 69 μA , 66 μA , and 66 μA , indicating a difference in capillary inner diameter.

It can be seen that the RSD on migration time for the same batch was below 0.30%, while with different batches, the RSD was below 1%. This difference from batch to batch may be a result of differences in the capillary inner diameter, as indicated by the differences in current.

Between-day reproducibility

To check for between-day reproducibility (Table 3), separations were done on the same instrument: a P/ACE MDQ with the same 75 μm × 60 cm capillary at 25 kV for five days, including a weekend, with CELixir 2.5. The first three runs of each day were taken to calculate the RSD for *n* = 5 × 3. The RSD on migration time for between-day reproducibility remained around 0.50%.

Scouting at various pH

To determine the absence of memory effect or hysteresis effect on the capillary wall, separation was performed at a different pH with the standard procedure (Table 4). The test mix was injected and separated on the P/ACE MDQ consecutively with the same 75 μm × 60 cm capillary at 25 kV with CELixir, pH 2.5 (phosphate 70 mM); CELixir, pH 4.3 (malic 60 mM); CELixir, pH 6.2 (phosphate 50 mM); CELixir, pH 8.2 (phosphate 40 mM); and CELixir, pH 9.2 (borate 150 mM), at 25 kV. The currents observed were 79, 75, 69, 79, and 72 μA , respectively.

After separation at pH 9.2, a new separation at pH 2.5 was performed and the same separation and migration time was obtained as with the first run (Figure 4). The migration time was 15.33 min for the EOF marker for the first separation at pH 2.5, compared to 15.27 min for the last separation at pH 2.5.

It is also possible to perform the same separation but inject the sample at the short side. Here, the separation is much shorter, and the EOF marker comes out at around 4 min. This feature is also interesting for method development when optimizing pH.

Comparison with conventional buffer

To compare CELixir buffer system with a conventional buffer, separation was done on the same instrument (P/ACE 5010) with two capillaries (75 μm × 77 cm)—one with CELixir pH 2.5 (75 mM phosphate buffer), and the other with freshly prepared phosphate buffer (70 mM at pH 2.56) and the same test mix at 25 kV. The current was lower when using CELixir 60 μA as compared with conventional phosphate buffer: 75 μA for CELixir and conventional

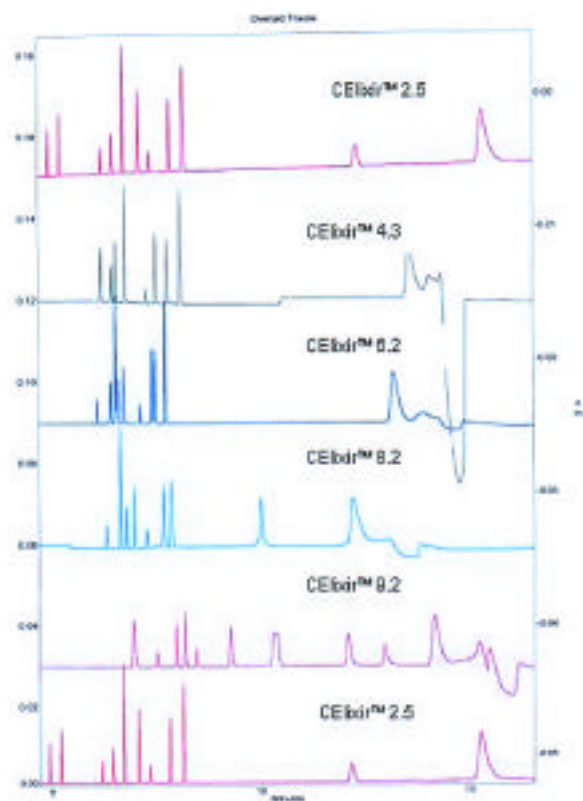


Figure 4 Successive separation at different pHs with CELixir.

buffer. This means that one can use a shorter capillary at the same voltage or use higher voltage with the same capillary.

The migration time with CELixir was shorter compared with the conventional phosphate buffer (Figure 5). Also, after 120 min, the EOF marker still was not seen when it came out around 24.30 min with CELixir. All peaks were resolved with CELixir, while with the conventional phosphate buffer, metoprolol (peak 6) and verapamil (peak 7) were not baseline resolved.

The corrected peak area was almost the same with both systems, but peak height was one-third to twice as high with CELixir as with phosphate buffer.

Based on the USP calculation method, the peak asymmetry and number of theoretical plates were determined. For all molecules, peak asymmetry was better with CELixir than with phosphate buffer. The number of theoretical plates was higher as well.

Conclusion

The following can be concluded from the above experiments:

1. The dynamic double coating results in reproducible migration time RSD below 0.20%.
2. When using different capillaries, the migration

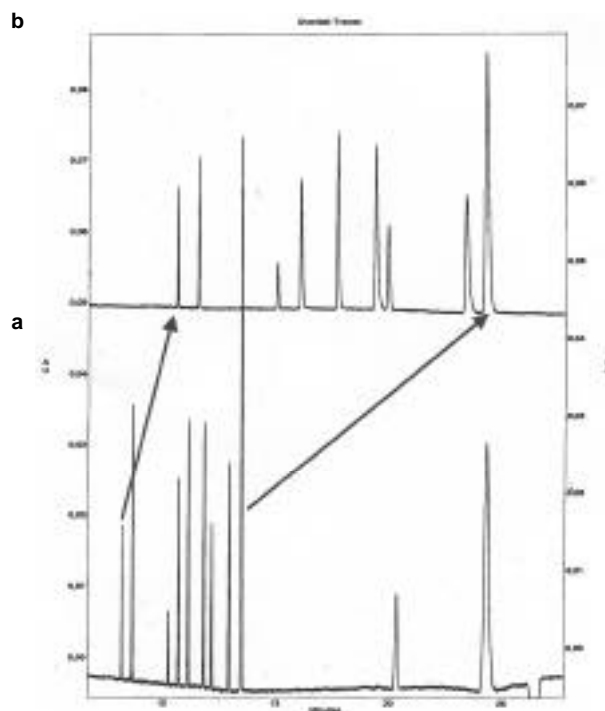


Figure 5 Separation with a) CELixir 2.5 and b) conventional phosphate buffer 2.5.

time stays below 1% RSD, depending on the inner diameter of the capillary.

3. At low pH, CELixir provides shorter migration times, higher peaks, better resolution, and better peak shape than conventional buffer.

4. CELixir allows scouting at different pH without an observed memory effect on the same capillary. The system is therefore well suited for method development.

The buffers will be most beneficial at low pH, where there is normally very low EOF, or where the EOF is difficult to control, and also for molecules that adhere to the capillary wall.

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