Journal of Chromatography, 559 (1991) 57–68
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 461

Influence of operating parameters on reproducibility in capillary electrophoresis

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ABSTRACT

The reproducibility of two migration parameters (retention time and mobility) of a seven-component test mixture was examined under various operating conditions using laboratory-built capillary electrophoresis systems. It was found that the frequency of rinsing the capillary and the solutions used for rinsing had the greatest effect on migration reproducibility. In addition, it was found that the migration behavior of solutes that interact with micelles is not repeatable unless the proper rinse protocol is applied. Inconsistent migration behavior is linked to inconsistent total current of the system. Preliminary investigations indicate that the fluctuation in total current were associated with non-equilibrium conditions between the buffer and the capillary wall.

INTRODUCTION

In order for any analytical technique to be useful, the results obtained must be reproducible. The reproducibility of the results obtained by capillary electrophoresis (CE) has been addressed in the work of several researchers. Some researchers pretreat new columns with caustic rinses before use [1–3]. VanOrman *et al.* [4] investigated the reproducibility of electroosmotic flow over three days and four capillaries. Black [5] presented results on the effects of temperature, ionic strength, pre-run washing and ion depletion in relation to CE system design. Lambert and Middleton [6] compared the effects of acidic and caustic pretreatments on electroosmotic flow.

The migration reproducibility may depend on several operational factors: ionic strength of the buffer, age of the capillary, previous capillary treatments, frequency of capillary treatments, applied voltage and external capillary temperature. The purpose of this study was to observe the influences of these operational factors on the reproducibility of the migration parameters of test solutes. This study was restricted to varying the parameters that did not alter the system hardware; therefore, the type of capillary (*e.g.*, coated *vs.* uncoated) or the capillary diameter, for example, were not altered.

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0021-9673/91/$03.50

The migration reproducibility of any system may also be influenced by the migration parameter used for evaluation. In CE, two parameters are the solute retention times (t_r) and the electrophoretic mobilities (μ). The value for μ is calculated from retention time as follows:

$$\mu = \left(\frac{1}{t_r} - \frac{1}{t_0}\right)\left(\frac{l_t l_d}{V}\right) \quad (1)$$

where t_0 is the retention time of an unretained marker, l_t is the total length of the capillary, l_d is the length from the upstream end of the capillary to the detector window and V is the applied voltage.

EXPERIMENTAL

Apparatus

Experiments were carried out on two laboratory-built CE systems that were patterned after the original system described by Jorgenson and Lucacs [7–9]. Both systems used a 0–30-kV high-voltage power supply (Series EH, Glassman High Voltage, Whitehouse Station, NJ, USA), a variable-wavelength UV detector (Model 200, Linear Instruments, Reno, NV, USA) operating at 210 nm and 50 μm I.D. × 375 μm O.D. fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA). Electropherograms were recorded with an electronic integrator (Model SP4200, Spectra-Physics, San Jose, CA, USA, or Model QA-1, Waters Assoc., Milford, MA, USA). Total current was recorded on a stripchart recorder by measuring the voltage across a 1-kΩ resistor in series with and downstream of the capillary.

Reagents and chemicals

For all experiments, a seven-component test mixture was used. The test compounds were arterenol (Sigma, St. Louis, MO, USA) (a cationic solute), 2-chlorophenol, 2,3-dichlorophenol, 2,4,5-trichlorophenol, *m*-methylbenzoic acid, *p*-hydroxybenzoic acid and 2,4,6-trimethylbenzoic acid (Aldrich, Milwaukee, WI, USA) (anionic or partially dissociated acidic solutes). In the remainder of this paper, these compounds will be referred to as ART, 2CP, 23CP, 245CP, mMBA, pOHBA and 246BA, respectively. Phosphate buffer (pH 7) was used as the mobile phase with sodium chloride present to adjust the ionic strength. For micellar electrokinetic capillary chromatography (MECC) experiments, sodium dodecyl sulfate (SDS) (Sigma) was used in the mobile phase and Sudan III (Aldrich) was used as the micelle mobility marker in the test mixture. All other chemicals were of analytical-reagent grade.

Procedure

Gravity injection was used for all runs. The test mixture was held 2–5 cm above the level of the downstream buffer for 5–20 s. This technique does not provide the reproducible injection volumes possible with electroinjection techniques; however, our interest was in the reproducibility of migration parameters rather than peak quantification. The capillary was rinsed by applying vacuum from a water aspirator to the sealed downstream buffer flask. When not under vacuum, the flask was open to air.

The time for each rinse was measured in column volumes, which was determined by aspirating an air bubble through the capillary and noting the time required for the bubble to be detected. Typically, one column volume passed through the capillary in 15 s while under aspiration vacuum.

RESULTS AND DISCUSSION

Influence of rinsing solution

As mentioned above, the preconditioning of the silica capillary surface has been described by many researchers; however, with the exception of Lambert and Middleton's recent study of acidic and alkaline preconditioning [6], little attention has been paid in the literature to the effects of various rinsing solutions. For this study, three solutions were chosen: phosphate buffer, pure methanol and 0.1 *M* sodium hydroxide. The buffer was chosen because its means of preconditioning would be mechanical; that is, it has no chemical effect on the capillary wall, but would rinse away any particulate matter and fill the capillary with fresh buffer. Methanol was chosen because it would remove organic solutes that may have attached to the silica capillary wall. Sodium hydroxide was chosen for its ability to remove older silica and expose a fresh surface.

Tests were carried out on two capillaries from the same capillary stock. Comparisons of the rinsing solutions were made by considering the average retention time and mobility for each component in each set and also the 95% confidence intervals (CI) of the means. For this study, *ca.* 1600 runs were made. In order to keep track of this large number of runs, individual runs were grouped into "sets" in which each set contained runs performed under similar conditions. Sets were grouped into "blocks", in which sets varied in one or two operational parameters. The data sets used are presented in Table I. These data are from two blocks (1 and 2); different capillaries were used for each block.

Before examining the influence of particular rinsing solutions, it is interesting to observe the behaviour of retention times over all sets. The retention time for the longest

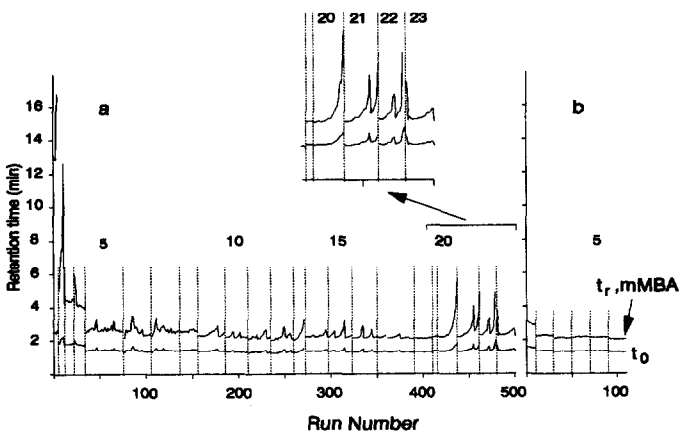


Fig. 1. Retention times of mMBA (top trace) and methanol (t_0; bottom trace) for (a) capillary 1 and (b) capillary 2. Vertical dotted lines delineate sets; numbers indicate the set number.

TABLE I

EXPERIMENTAL CONDITIONS: INFLUENCE OF RINSING SOLUTION

Conditions for all sets: 10 m*M* ionic strength phosphate buffer, no SDS, 15 kV.

Block and set	No. of runs	Rinsing solution	Rinsing protocol[c]
1/1[a]	5	Buffer	60 c.v., start of set
1/2	6	CH_3OH	2 c.v., start of set
1/3	11	CH_3OH	100 c.v., start of set
1/4	12	CH_3OH	20 c.v., start of set
1/5	41	NaOH and CH_3OH	2 c.v. of each, start of set
1/6	30	NaOH and CH_3OH	2 c.v. of each, start of set
1/7	25	NaOH and CH_3OH	30 c.v. NaOH, 2 c.v. CH_3OH, start of set
1/8	20	NaOH and CH_3OH	2 c.v. of each, start of set
1/9	30	Buffer	10 c.v., start of set
1/10	25	Buffer	10 c.v., start of set
1/11	25	Buffer	10 c.v., start of set
1/12	25	CH_3OH	10 c.v., start of set
1/13	12	CH_3OH	10 c.v., start of set
1/14	26	CH_3OH	10 c.v., start of set
1/15	25	Buffer	10 c.v., start of set
1/16	27	NaOH	10 c.v., start of set
1/17	40	Buffer	2 c.v., between each run
1/18	20	Buffer	2 c.v., between each run
1/19	5	Buffer	20 c.v., start of set
1/20	22	NaOH and CH_3OH	2 c.v. of each, start of set
1/21	24	MeOH	10 c.v., start of set
1/22	18	NaOH	10 c.v., start of set
1/23	19	Buffer	2 c.v., between each run
2/1[b]	10	Buffer	60 c.v., start of set
2/2	20	CH_3OH	10 c.v., start of set
2/3	20	CH_3OH	10 c.v., start of set
2/4	20	Buffer	60 c.v., start of set
2/5	20	Buffer	10 c.v., start of set
2/6	20	NaOH	10 c.v., start of set

[a] Block 1: l_t = 40 cm, l_d = 26.5 cm, capillary 1.
[b] Block 2: l_t = 40 cm, l_d = 26.5 cm, capillary 2.
[c] c.v. = Column volumes.

retained compound (mMBA) and t_0 are plotted *versus* the run number in Fig. 1a (capillary 1) and 1b (capillary 2), where vertical dotted lines mark the starting and ending runs for each set. It is apparent that the retention behavior of the two capillaries is different. The number of runs required to obtain consistent retention times was much smaller for capillary 2 (10 runs; set 2/1) compared with capillary 1 (34 runs; sets 1/1–1/4), even though both were from the same stock. This may be due to contamination of the inner surface of capillary 1 occurring during its installation in the system.

The initial runs with capillary 1 show very unstable behavior. As described in Table I, the capillary was rinsed with buffer or methanol for the first four sets. It may be that the inner capillary surface was initially contaminated and the buffer and

methanol did not clean the surface. After treatment with the combined sodium hydroxide–methanol rinse (1/5–1/8), the retention time stabilized. Subsequent rinses with either buffer or methanol (1/9–1/15) showed similar retention times and similar behavior with respect to minor retention time fluctuations.

Sodium hydroxide was again used in 1/16, 1/20 and 1/22. When used by itself (1/16), it has no more effect than buffer or methanol. However, note that in sets 1/20–1/22 the retention time increased exponentially and did not decrease until rinsed again. This was accompanied by a similar increase in current. Flushing the capillary with any rinsing solution returned the retention time (and current) to a reasonable level again, as shown in 1/21, 1/22 and 1/23.

It appears that the combination of a sodium hydroxide rinse followed immediately by a methanol rinse may lead to the anomalous behavior of retention time and current. This may be due to the formation of cavities by the sodium hydroxide in the capillary wall that become filled with methanol. As the methanol diffused from the cavities, the zeta potential near the wall may be reduced, causing the electroosmotic mobility to decrease.

It is important to note that the fluctuations in retention time coincide with those in t_0. Identical observations were made for all compounds in the test mixture; therefore, the fluctuation is due to irregularities in the electroosmotic flow and not to any characteristic of the test compounds. If this is the case, then a figure of merit that compensates for electroosmotic mobility fluctuation (such as mobility) should show greater stability than retention time.

The average and 95% CI for retention time and mobility are presented in Fig. 2a and b, respectively, for the longest retained compound, mMBA, for capillary 1. On initial inspection, it is clear that mobility is more stable and shows less error than retention time for the first four sets. Closer examination also shows that sets which

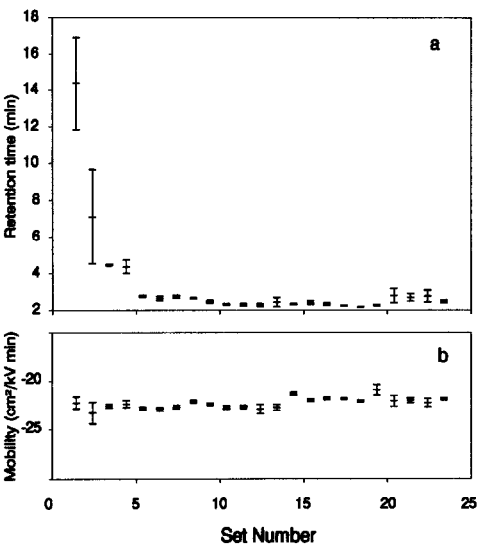


Fig. 2. Average and 95% CI of (a) retention time and (b) mobility of mMBA for the sets of data illustrated in Fig. 1 for capillary 1.

experienced exponential increases in retention time (1/20, 1/21 and 1/22) show no difference in mobility. It may be concluded that the increase in retention time was due to a reduction in electroosmotic flow and that mobility is a much more rugged migration parameter than retention time.

From this information, it appears that the type of rinsing solution to be used depends on the situation. For cases where retention time is initially unstable, a rinse with sodium hydroxide is helpful. After achieving stable retention time, rinses with either sodium hydroxide, methanol or buffer work equally well, but the combination of sodium hydroxide and an organic rinsing solution such as methanol sometimes appears to alter retention time and should be avoided.

Influence of rinsing frequency

As with the question of the type of rinsing solution to use, there is no consensus on how frequently the capillary should be rinsed. This study follows two rinsing protocols: 1, rinse only at the beginning of a set, and 2, rinse between each run in a set. The experimental conditions for this analysis are presented in Table II.

TABLE II

EXPERIMENTAL CONDITIONS: INFLUENCE OF RINSING FREQUENCY

Conditions for all sets: buffer rinse solution, 15 kV.

Block and set	No. of runs	[SDS] (m*M*)	Rinsing protocol[d]
1/9[a,b]	30	0	10 c.v., start of set
1/10	25	0	10 c.v., start of set
1/11	25	0	10 c.v., start of set
1/15	25	0	10 c.v., start of set
1/17	40	0	2 c.v., between each run
1/18	20	0	2 c.v., between each run
3/1[c]	20	10	60 c.v., start of set
3/2	20	10	2 c.v., between each run

[a] Block 1: l_t = 40 cm, l_d = 26.5 cm, capillary 1.
[b] Block 1 data are also listed in Table I and are recompiled here for comparison with Block 3 data.
[c] Block 3: l_t = 40 cm, l_d = 26.5 cm, capillary 2.
[d] c.v. = Column volumes.

In studying the influence of rinsing frequency on reproducibility, three factors were considered: the magnitude of the errors in retention time and mobility, the total current of the system and its correlation with migration behavior and the variation in selectivity. First, the errors in retention time and mobility will be discussed.

To compare errors from several sets of experiments, relative confidence intervals (RCI) were used (confidence intervals have been used throughout this work because they reflect the number of measurements used to determine the average). The RCI was the 95% CI divided by the average retention time or mobility. These RCI are presented in Fig. 3a for retention time and in Fig. 3b for mobility. The averages and 95% CI from which these graphs were generated are presented in Table III for retention time and mobility for five of the compounds.

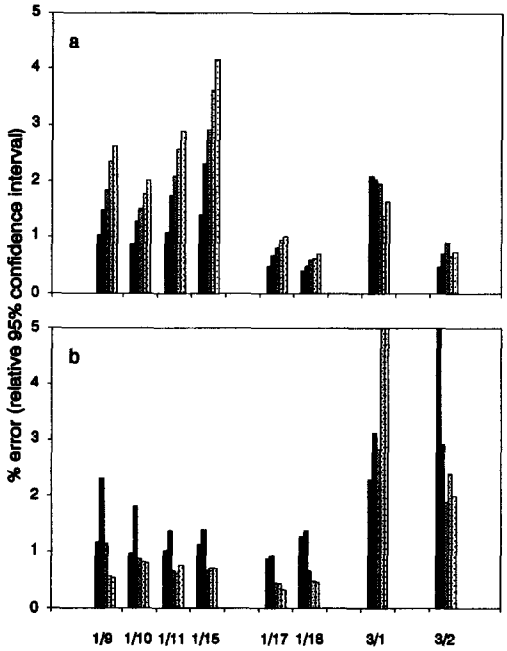


Fig. 3. Relative percentage error based on the 95% CI in (a) retention time and (b) mobility for ART, 23CP, 245CP, 246BA and mMBA under four conditions: no SDS, rinsing before sets (1/9, 1/10, 1/11, 1/15); no SDS, rinsing between runs (1/17, 1/18); SDS, rinsing before sets (3/1); and SDS, rinsing between runs (3/2).

Comparison of the retention time error in sets with rinsing before each set (1/9–1/11, 1/15, 3/1) *versus* sets with rinsing between each run (1/17, 1/18, 3/2) shows that rinsing before each run leads to less error. This was true regardless of the presence of SDS micelles (3/1 *vs.* 3/2). Both the relative error (Fig. 3a) and actual error (Table III) increase with increased retention, characteristic of random error.

The mobility data in Table III show a relatively constant absolute error, which leads to decreasing relative error with increasing retention as shown in Fig. 3b. The calculation of mobility (eqn. 1) reduces the systematic error by removing the influence of the variation of the electroosmotic flow. However, the random error increases because mobility is based on two measurements (t_r and t_0) instead of one (t_r). Without micelles present, the reduction in systematic error is greater than the increase in random error. The presence of micelles makes the measurement of t_0 less certain; therefore, the random error is greater with micelles present.

In CZE, mobility is preferred as the relative error is much less than the error in retention time (1% *vs.* 2–2.5%), especially for sets with rinsing at the beginning of the set. In MECC, retention time appears to be better than mobility as the figure of merit when SDS is present (1–2% *vs.* 2–5%). However, as with runs without SDS, rinsing between runs leads to lower error.

The second factor considered in comparing the rinsing protocols was the fluctuation in total current and its correlation with fluctuations in migration behavior. As shown in Fig. 4a, the current for 1/9–1/11 gradually increases with time and

TABLE III

COMPARISON RUNS WITH DIFFERENT RINSING FREQUENCIES

Block and set	ART	23CP	245CP	246BA	mMBA
Retention times (min) and 95% confidence intervals					
1/9[a]	1.073 ± 0.011	1.563 ± 0.023	1.856 ± 0.034	2.218 ± 0.052	2.451 ± 0.064
1/10[a]	1.041 ± 0.009	1.506 ± 0.019	1.779 ± 0.027	2.088 ± 0.037	2.296 ± 0.046
1/11[a]	1.032 ± 0.011	1.498 ± 0.026	1.771 ± 0.037	2.075 ± 0.053	2.278 ± 0.066
1/15[a]	1.072 ± 0.015	1.564 ± 0.036	1.852 ± 0.054	2.167 ± 0.078	2.387 ± 0.099
1/17[b]	1.045 ± 0.005	1.500 ± 0.010	1.760 ± 0.014	2.027 ± 0.019	2.217 ± 0.022
1/18[b]	1.024 ± 0.004	1.463 ± 0.007	1.711 ± 0.010	1.975 ± 0.012	2.154 ± 0.015
6/3[c]	1.434 ± 0.030	2.304 ± 0.047	3.125 ± 0.061	2.913 ± 0.040	3.348 ± 0.055
6/4[d]	1.542 ± 0.007	2.449 ± 0.017	3.273 ± 0.029	2.747 ± 0.018	3.129 ± 0.023
Mobilities (cm^2/kV cm) and 95% confidence intervals					
1/9[a]	14.60 ± 0.17	−6.05 ± 0.14	−13.17 ± 0.15	−19.35 ± 0.11	−22.36 ± 0.12
1/10[a]	14.35 ± 0.14	−6.62 ± 0.12	−13.79 ± 0.12	−19.67 ± 0.16	−22.73 ± 0.18
1/11[a]	14.68 ± 0.15	−6.55 ± 0.09	−13.82 ± 0.09	−19.64 ± 0.12	−22.66 ± 0.17
1/15[a]	14.18 ± 0.16	−6.50 ± 0.09	−13.50 ± 0.09	−19.00 ± 0.13	−21.96 ± 0.15
1/17[b]	13.97 ± 0.12	−6.57 ± 0.06	−13.53 ± 0.06	−18.81 ± 0.08	−21.79 ± 0.07
1/18[b]	14.12 ± 0.18	−6.57 ± 0.09	−13.57 ± 0.09	−19.10 ± 0.09	−22.08 ± 0.10
6/3[c]	4.56 ± 0.10	−14.08 ± 0.44	−22.15 ± 0.63	−20.5 ± 1.2	−23.7 ± 1.2
6/4[d]	4.30 ± 0.35	−12.67 ± 0.37	−19.93 ± 0.38	−15.80 ± 0.38	−18.94 ± 0.38

[a] Buffer rinse before each set; no SDS.
[b] Buffer rinse each run; no SDS.
[c] Buffer rinse before each set; 10 m*M* SDS.
[d] Buffer rinse before each run; 10 m*M* SDS.

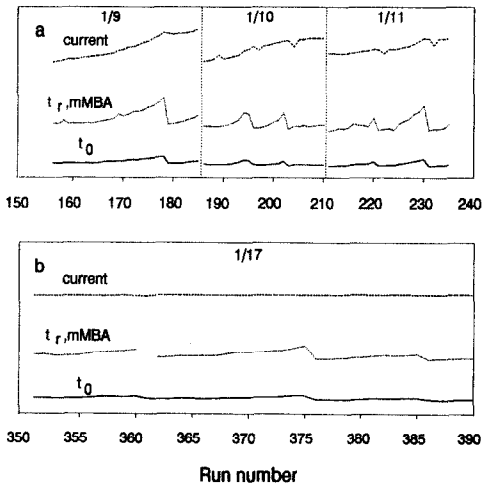


Fig. 4. Relationship between (dashed lines) current, (dotted lines) retention time of a typical solute (mMBA) and (solid lines) the retention time of methanol (t_0) for (a) sets 1/9, 1/10 and 1/11 (rinsing before sets) and (b) set 1/17 (rinsing between runs). Currents and retention times are not to scale.

contains many fluctuations. The cause of this increase may be temperature equilibration or non-steady-state capillary wall interactions; these will be addressed further in a later section. This behavior has been observed to correlate to fluctuations in retention time and t_0, as illustrated in Fig. 4 for mMBA (again, mMBA was representative of the behavior of all solutes).

Rinsing returns the system to "first run" conditions. The current for the first runs of 1/9, 1/10 and 1/11 were all about 6.7 μA. This was also the current of each run in 1/17 (Fig. 4b) and 1/18 (not shown), in which rinsing was done between runs. In addition to keeping the current constant from run to run, rinsing between runs also reduced the fluctuations in retention time and t_0. It was the reduction in these fluctuations by rinsing between runs that led to the lower error shown in Fig. 3 and Table III.

Set 1/23 is another example of the benefit of rinsing between runs. In the inset in Fig. 1, 1/23 follows three consecutive sets that experienced exponential, anomalous increases in retention (and also current). The initial retention time in 1/23 was high, but quickly reduced to normal levels. Later in the set the retention time began to climb, but decreased again before becoming too large. This small rise late in the set can be seen for several sets in Fig. 1 (1/9–1/16), so it is not uncommon. It is noteworthy that rinsing before each run in set 1/23 eventually returned the system to normal operation. Without rinsing between each run, it would seem necessary to replace the column based on the observed behavior in 1/20–1/22.

The third factor considered in comparing the influence of rinsing frequency is the variation in separation selectivity (*i.e.*, relative retention of compounds). Rinsing between runs will maintain the selectivity in an MECC separation as described below.

The mobilities for 3/1 and 3/2 are presented in Fig. 5 for 245CP, 246BA, pOHBA and mMBA. The mobilities of 3/2 (rinsing between runs) show relatively stable behavior, although not as stable as the behavior seen in CZE. The fluctuations were constant for all compounds, so the selectivity did not alter from run to run. On the other hand, the mobilities of 3/1 (rinsing before sets) show a constant increase. (The sign convention dictates that migration toward the positive electrode is in the negative direction; negative mobility would be expected for anionic solutes.) More critical than

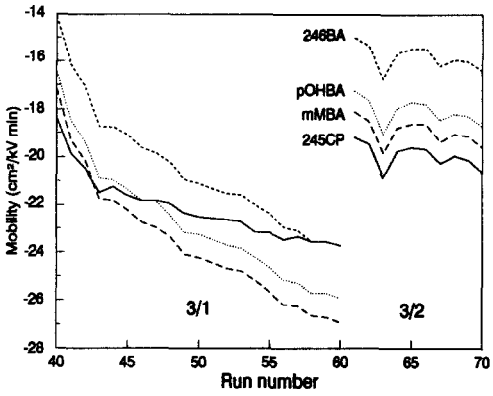


Fig. 5. Dependence of mobility on run number. Runs 40–60 were performed with rinsing before the set; runs 61–70 were performed with rinsing between runs.

the magnitude of the change in mobility is the change in selectivity that occurred. For the initial runs in both protocols, 245CP was the longest retained. As the run number in 3/1 increased, 245CP shifted position and, after twenty runs, co-eluted with 246BA. This is further evidence that rinsing before each run restored the system to the initial conditions and that rinsing should be performed before every run.

Influence of other operating parameters

The influence of ionic strength and the use of Sudan III were also tested. Ionic strength was examined by performing runs with phosphate buffer at three levels of ionic strength: 10, 50 and 100 m*M*. Rinsing was performed at the beginning of the set in order to test the ionic strength contribution under "worst case" conditions. The variance in mobility within a set was compared with the variance between sets in the standard ANOVA format [10,11]. In general, the sets at low ionic strength were most similar to one another, and the similarity between sets decreased with increasing ionic strength.

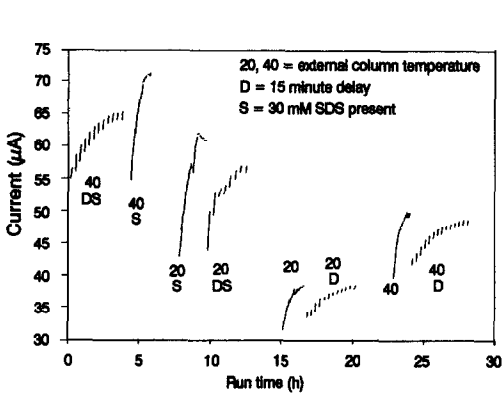
The presence of Sudan III in MECC was also investigated as a possible source of irreproducible behavior. It was thought that Sudan III, used in the sample as the micelle retention marker, precipitated in the column or interacted with the wall due to its low solubility. Analysis of sets run in a two-day period with and without Sudan III showed that the variation in mobility as a result of Sudan III was less than the day-to-day variation.

Examination of increase in total current

It was shown in Fig. 4 and has been a long-standing observation in this laboratory that the total current increases as the number of runs increases if rinsing is performed between sets, and the current ultimately reaches a plateau after several runs. Two factors were thought to contribute to this behavior. The first factor was slow warming of the capillary to an equilibrium temperature. Since a temperature gradient exists from the center of the capillary to the outer surface, it was thought that the warming of the capillary might contribute to the rise in current. The second factor was slow equilibration of the inner capillary surface. The surface of the capillary may be altered during the rinse and its alteration may result in the depletion of current carriers during the initial runs of a set until a steady-state condition was reestablished.

To study this behavior further, a 15-min delay was introduced between runs. This delay would allow the capillary temperature to re-equilibrate to an initial value before every run within a set. The initial and final current readings for each run are combined and illustrated in Fig. 6.

Examination of Fig. 6 shows that the current increased with increasing run number for all sets, regardless of the presence of the delay period. This shows that temperature equilibration was not the cause of the increase in total current. It is interesting to note two observations regarding the role of SDS micelles with respect to the equilibrium current level. First, with SDS present, the current for sets with delays was less than that for sets without delays. Second, the contribution of SDS to the total curent was not as great as might have been expected. With SDS present, the current increased by 32% on average. This increase can be attributed to the addition of 30 m*M* Na^+ to the buffer, which increased the ionic strength from 50 to 65 m*M*, or by 30%. The contribution of the free dodecyl sulfate would increase the ionic strength to 67



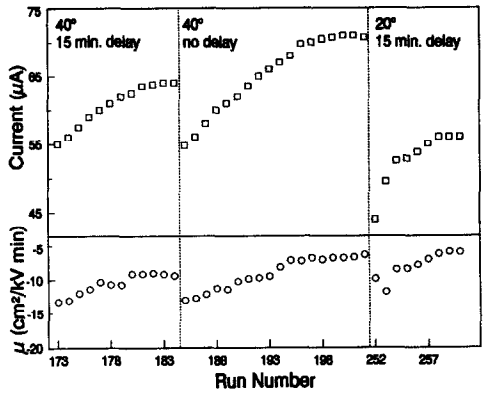


Fig. 6. Comparison of sets with and without 15-min delays between injections. The run time excludes times in which the system was not operating, *i.e.*, nights and weekends. 20, 40 = External column temperature (°C); D = 15-min delay; S = 30 m*M* SDS present.

Fig. 7. Relationship between total current and mobility of ART for sets 4/1, 4/2 and 4/3.

m*M*, assuming that the critical micelle concentration (CMC) is 4 m*M*, raising the increase in ionic strength to 34%.

It appears that the micelles contribute very little to the increase in current. If 50 dodecyl sulfate monomers comprise one micelle [12], the effective concentration of micelles is (30−4)/50, or 0.52 m*M*, and the effective charge of each micelle would be −50, making the contribution of micelles to the ionic strength (0.5)(0.52)(-50^2), or 650 m*M*. If the micelles contributed to the total current, one would expect the current to be much larger than the value observed.

The change in mobility of one of the solutes may shed light on the increase in current. Examination of the behavior of ART in 4/1, 4/2 and 4/3 in Fig. 7 shows a correlation between the behavior of mobility and total current. The increase in current may be due to an increase in charge carriers as the number of runs increases, or may be due to a depletion of charge carriers in the first run and a gradual increase with subsequent runs. It has been shown that phosphate will bind to the silica surface of the capillary with a coverage of *ca.* 23 phosphates per silanol [12]. The presence of the electric field may cause a change in the steady-state coverage of the silica by phosphate. As the phosphate re-establishes a steady-state condition, the solute mobility would level off and the current would rise to an equilibrium value.

From the experiments above, it appears that the increase in current was more likely to be due to interactions of the buffer with the capillary wall than to temperature effects.

CONCLUSIONS

The reproducibility in capillary electrophoresis depends initially on the migration parameter used; the use of mobility is generally, but not universally, better than retention time. When a term such as mobility is used as the basis for comparison, the greatest reproducibility occurred when the capillary was rinsed between each run, when low ionic strength buffers were used and when SDS was not present in the buffer.

The nature of the rinsing solution appears to be of little importance, however, the combination of NaOH and an organic rinsing solution seems to destabilize the migration behaviour. Sodium hydroxide solutions may be required, however, if the new capillary demonstrates poor behavior that may be the result of contamination.

There is evidence that the surface of the capillary undergoes modification until a steady-state condition has been established. At this steady state, the total current and the retention times reach steady values. It may require several runs to reach this condition, however. By rinsing the capillary before each injection, the initial, unmodified conditions can be reproducibly achieved.

ACKNOWLEDGEMENTS

We thank David M. Smith and Chang-yu Quang for their assistance in collecting data and Helen K. Smith for assistance with the manuscript. We also thank Burroughs Wellcome (Research Triangle Park, NC, USA) for the use of the SP4200 integrator. A research grant from the National Institutes of Health (FIRST Award, GM38738) is gratefully acknowledged.

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