ELSEVIER

Journal of Chromatography A, 802 (1998) 391–394

JOURNAL OF CHROMATOGRAPHY A

Short communication

Comparison of the reproducibility in migration times between a constant-current and a constant-voltage mode of operation in capillary zone electrophoresis

Yasuyuki Kurosu*, Yasuyo Satou, Yoshiko Shisa, Tetsuo Iwata

JASCO Technical Research Laboratories Corporation, 2097-2, Ishikawa-cho, Hachioji-shi, Tokyo 192, Japan

Received 16 September 1997; received in revised form 11 November 1997; accepted 24 November 1997

Abstract

A comparative study of the reproducibility in migration times between constant-current (CC) and a constant-voltage (CV) mode of operation in capillary zone electrophoresis was carried out. We found that the CC mode gave better reproducibility than the CV mode in both successive injections (5 or 6 times) and day-to-day analyses (16 measurements throughout 4 days).

Keywords: Migration times; Capillary electrophoresis; Reproducibility

1. Introduction

In spite of the recent progress in capillary zone electrophoresis (CZE) – developed rapidly as a separation tool – several problems to be overcome still remain [1–4]. One of the crucial problems in CZE is its poor reproducibility in migration times. In order to improve the reproducibility in CZE, Lee and Yeung have introduced two kinds of novel indices instead of the conventional migration times and have demonstrated their usefulness [5]. One is defined as a migration index (MI) and the other as an adjusted migration index (AMI). The MI is defined by integrating the current density divided by the effective capillary length as a function of the time from injection beginning to the migration times. Whereas the conventional migration times depends upon many separation conditions, the MI depends on only two parameters: the zeta potential between the buffer solution and the capillary inner wall and that between the buffer solution and the analyte. The AMI is the sophisticated version of the MI and we do not discuss it here. The MI is conceptually the same to use with a power supply in a constant-current (CC) mode, when we use the same capillary column with the same length and inner diameter [5–7]. In other words, migration times in the CC mode are proportional to the MI. Analysis with the CC mode, therefore, can be expected to give better reproducibility in migration times compared with that under the constant-voltage (CV) mode. However, only the CV mode of operation has been widely reported. A comparative study between the two modes has rarely been carried out. This motivated us to carry out this study. We show some experimental data and discuss

*Corresponding author. Fax: 81 426464707.

0021-9673/98/$19.00
PII S0021-9673(97)01221-1

the CC mode of operation with respect to the CV mode.

2. Experimental

2.1. Chemicals and materials

We used the following reagents and materials: pyridoxal (PL), benzyl alcohol (BA) and adenosine-5′-monophosphate (AMP) (Wako, Osaka, Japan); pyridoxamine (PM) and adenosine3′:5′-cyclic-monophosphate (cAMP) (Sigma, St. Louis, MO, USA). Water was purified by deionization, followed by distillation. All other regents were of analytical grade. The uncoated capillary (60 cm total length, 45 cm effective length and 50 μm internal diameter) was purchased from GL Science (Tokyo, Japan).

2.2. Apparatus

Capillary electrophoresis (CE) experiments were carried out in a fully automated CE-900 system (Jasco, Tokyo, Japan), equipped with a UV–Vis detector, CE-970 (Jasco). The system enabled us to select two separation modes: CV and CC modes. For the CV mode, the value of the voltage across the capillary with a 0.1 kV step between 0 and 30 kV, and for the CC mode, that of the current through the capillary with a precision of 0.1 μA between 0 and 200 μA, can be set through a menu panel. For both, actual voltage across the capillary and current through it can be monitored analogously by a chart recorder. The system also enables temperature- and time-programs to carry out a series of CE analyses. The detector wavelength was fixed at 265 nm. This is because the Vitamin B_6 group (PM and PL) were expected to be unstable under the illumination of the shorter wavelength although the absorption maximum for each analyte is below 265 nm. A part of the capillary, about 20 cm in the effective length of 45 cm, was temperature-controlled by forced air. The temperature of the capillary was about 32°C. Samples set in an autosampler were maintained at 10°C.

2.3. Electrophoretic procedures

The separation buffer was 40 m*M* sodium phosphate, pH 8.0. BA was used as a neutral marker. The separation run was curried out at a CV mode of +15 kV or a CC mode of 40 μA.

Prior to the first use, a new capillary was subjected to a standard wash cycle: (1) separation buffer for 1 min under 2000 mbar; (2) 0.1 *M* NaOH for 1 min under 2000 mbar; (3) separation buffer for 1 min under 2000 mbar; (4) run for 0.5 min under +5 kV; steps 1–4 were repeated four times. The capillary was also washed with the standard wash cycle once, before the first injection on the day and between individual injections.

The samples were injected at the anode side with a hydrodynamic mode (20 mbar, 0.1 min) by using a dynamic compression injection technique ([1], Chapter 4, p. 103). The concentration of samples were 2.5 mg/ml (PM), 2% (v/v) (BA), 0.83 mg/ml (PL), 0.33 mg/ml (cAMP) and 0.33 mg/ml (AMP), respectively. The mixture of the samples were dissolved in the separation buffer.

3. Results and discussion

The same electrophoretic patterns were obtained under both the CC and the CV modes (Fig. 1). The voltage and current fluctuations as a function of the time for the CC mode during a typical analytical run are shown in Fig. 1a, and those for the CV are shown in Fig. 1b, respectively. For the CC mode (40.0 μA with a peak-to-peak fluctuation of 0.4 μA), the average voltage was 15.0 kV with a peak-to-peak fluctuation of 270 V. For the CV mode (15 kV with a peak-to-peak fluctuation of 90 V), the average current was 40 μA with a peak-to-peak fluctuation of 0.8 μA.

3.1. Comparison of the reproducibility in migration times in successive injections between CC mode and CV mode

The reproducibilities of successive injections ($n=5$ or 6) were studied. This measurements were repeated three times throughout 4 days. The results are summarized in Table 1. The smaller relative standard deviations (R.S.D.s) were obtained at the CC mode than at the CV mode for individual peaks and measurements. For example, an average value of the

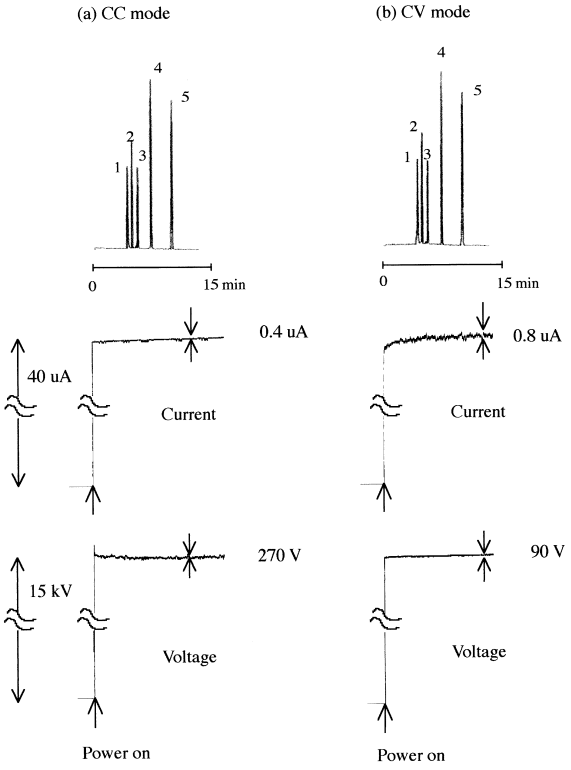

Fig. 1. Typical electropherograms under (a) the CC mode and (b) the CV mode. The magnitude of a peak-to-peak fluctuation of current (middle) and voltage (bottom) during the run at each mode is indicated. The electrophoretic conditions were as described in Section 2. Peak assignment: (1) PM; (2) BA; (3) PL; (4) cAMP; (5) AMP.

R.S.D.s at the CC mode for Peak 2 (a neutral marker) was 0.15% whereas that at the CV mode was 1.00%; which means an improvement in the reproducibility in migration times by a factor of 6.7.

Table 2
Comparison of relative standard deviations (R.S.D.s, %) in migration times in day-to-day analyses between CC mode and CV mode

Mode:	CC	CV
The number of trials:	$n=16$	$n=16$
Peak 1[a]	1.68	2.08
Peak 2[b]	1.06	2.22
Peak 3[c]	1.99	2.10
Peak 4[d]	1.49	2.29
Peak 5[e]	1.95	2.38

The electrophoretic conditions were as described in Section 2.
[a] PM; [b] BA; [c] PL; [d] cAMP; [e] AMP.

3.2. Comparison of the reproducibility in migration times in day-to-day analyses between CC mode and CV mode

The reproducibilities in day-to-day measurements throughout 4 days (where $n=16$) were studied. Four runs a day at each mode were performed. Current or voltage was set independently every day. The results are summarized in Table 2. Again, we are able to obtain smaller values of R.S.D.s at the CC mode than at the CV mode. Furthermore, effect of constituents in sample solutions (e.g., organic solvents or salts) on the reproducibility in migration times was investigated. As a result, we confirmed that the CC mode was less sensitive to the constituents of the sample solution than the CV mode (data not shown).

4. Conclusion

We have verified experimentally that the reproducibilities in migration times of the CC mode were

Table 1
Comparison of relative standard deviations (R.S.D.s, %) of migration times in successive injections between CC mode and CV mode

Mode:	CC				CV			
Experimental No.:	1	2	3		1	2	3	
The number of trials:	$n=6$	$n=5$	$n=6$	(Average)	$n=6$	$n=5$	$n=6$	(Average)
Peak 1[a]	0.58	0.42	0.57	(0.52)	1.71	1.27	0.60	(1.09)
Peak 2[b]	0.09	0.17	0.18	(0.15)	1.52	1.17	0.30	(1.00)
Peak 3[c]	0.64	0.50	0.60	(0.58)	1.71	1.27	0.68	(1.22)
Peak 4[d]	0.20	0.17	0.11	(0.16)	1.59	1.16	0.28	(1.01)
Peak 5[e]	0.39	0.27	0.22	(0.29)	1.62	1.22	0.42	(1.09)

The electrophoretic conditions were as described in Section 2.
[a] PM; [b] BA; [c] PL; [d] cAMP; [e] AMP.

better than those of the CV mode both in successive and day-to-day analyses. These results are reasonable because the index MI introduced by Lee and Yeung is conceptually the same as using a power supply with a CC mode [5–7]. The CC mode analysis, however, has some problems in practical use: (1) it is difficult to derive the electrophoretic mobility of an analyte; (2) it is difficult to perform a gradient potential in order to speed up the separation; (3) it is difficult to construct a high-precision version and is troublesome to deal with because of the high impedance of the buffer solution in a capillary column.

We have to use the CC or the CV mode appropriately and to utilize the MI as recommended in either mode. This is because even the slight fluctuation of voltage under CV mode or of current under CC mode on MI is expected to compensate. Further investigation are in progress.

Acknowledgements

The authors thank Mr. Masao Bounoshita and Mr. Akihiro Satou for providing valuable advice.

References

[1] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis—Principle and Practice, Springer, Berlin, Heidelberg, New York, 1993.
[2] R.L. St Claire III, Anal. Chem. 68 (1994) 569R.
[3] S. Honda, S. Terabe, Capillary Electrophoresis: Basis and Practice (in Japanese), Koudansya, Tokyo, 1995.
[4] A. Kunke, M. Degenhardt, B. Schirm, H. Watzig, J. Chromatogr. A 768 (1997) 17.
[5] T.T. Lee, E.S. Yeung, Anal. Chem. 63 (1991) 2842.
[6] T. Tsuda, J. Liq. Chromatogr. 63 (1991) 2842.
[7] T. Iwata, J. Koshoubu, Y. Kurosu, submitted.