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Note

Extra-column effects in high-performance capillary electrophoresis

KOJI OTSUKA*
Department of Industrial Chemistry, Osaka Prefectural College of Technology, Saiwai-cho, Neyagawa, Osaka 572 (Japan)
and
SHIGERU TERABE
Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto 606 (Japan)

High-performance capillary electrophoresis (HPCE), which was first introduced by Mikkers *et al.*[1], Jorgenson and Lucaks[2] and Hjertén[3], has been widely employed in various fields. Although samples to be analysed by HPCE are in principle limited to ionic compounds, the development of electrokinetic chromatography (EKC)[4] has permitted the separation even of electrophoretically neutral compounds in addition to charged compounds. In particular, EKC with micellar solutions of ionic surfactants (micellar EKC)[5,6] has become one of the most efficient methods for the analysis of small neutral or ionic compounds with HPCE instruments.

We have previously reported band broadening in micellar EKC[7], and discussed extra-column effects with respect to the instrumental conditions when using on-column injection and detection. The injection volume, detection volume and time constant of the detection system were extensively investigated in order to obtain an electrokinetic chromatogram without any loss of the performance characteristics attained in the column. As on-column injection and detection are usually employed also in HPCE, the discussion is equally applicable to HPCE.

In this paper, we briefly describe the extra-column effects in HPCE according to our previous paper[7] and calculate the maximum limits of the amount of sample injected, the length of the detector cell and time constants of the detection system required to avoid adverse effects due to extra-column band broadening.

THEORY

As a detailed derivation of the equations for the instrumental requirements were given previously[7], only a brief explanation is presented below.

Extra-column band broadening in HPCE can be ascribed to two factors, the injection volume of sample solutions and the cell volume of the detector, as long as on-column injection and detection are employed. Then, the observed total peak variance, σ^2_{tot}, is represented by

$$\sigma^2_{tot} = \sigma^2_{inj} + \sigma^2_{col} + \sigma^2_{det} \quad (1)$$

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where σ^2_{col} is the variance generated in the column and σ^2_{inj} and σ^2_{det} are extra-column variances originated in the injection and detection systems, respectively. As the column efficiency and resolution are not seriously impaired by a 5% or 10% increase in peak width[8], σ^2_{tot} is required to satisfy the following conditions:

$$\sigma^2_{tot} \leqslant (1.050\sigma_{col})^2 = 1.103\sigma^2_{col} \qquad \text{(for a 5\% increase)} \tag{2}$$

or

$$\sigma^2_{tot} \leqslant (1.100\sigma_{col})^2 = 1.210\sigma^2_{col} \qquad \text{(for a 10\% increase)} \tag{3}$$

Here, σ^2_{col} is equal to HL, where H and L are the plate height and the column length, respectively, and hence the total extra-column variance, σ^2_{ext}, must be kept smaller than 10.3% (for a 5% increase in peak width) or 21.0% (for a 10% increase) of HL. If we assume that the contribution of σ^2_{inj} is equal to half of that of σ^2_{ext}, in other words $\sigma^2_{inj} = \sigma^2_{det}$, the injection length, l_{inj}, or the length of the column occupied by a sample solution is required to be

$$l_{inj} \leqslant (12 \cdot 0.103/2)^{1/2}\,\sigma_{col} = 0.786(HL)^{1/2} \qquad \text{(5\% increase)} \tag{4}$$

or

$$l_{inj} \leqslant (12 \cdot 0.210/2)^{1/2}\,\sigma_{col} = 1.120(HL)^{1/2} \qquad \text{(10\% increase)} \tag{5}$$

by assuming that a certain length of the inside of the column is completely displaced by a plug of the sample solution.

Similarly, limits of the cell length required, l_{det}, or the length of the slit along the column axis, are also given by eqn. 4 or 5 but with l_{inj} replaced with l_{det}.

On the other hand, the time variance generated in the column, $\sigma^2_{t,col}$, is given by

$$\sigma^2_{t,col} = \sigma^2_{col}/v_s^2 \tag{6}$$

where v_s is the velocity of the solute. As eqns. 2 and 3 are applicable to time variances, the total time variance of the detection system, $\sigma^2_{t,det}$, should not exceed 10.3% (for a 5% increase) or 21.0% (for a 10% increase) of $\sigma^2_{t,col}$. Therefore, the following conditions are required for the time constant of the detection system, τ, which is equal to $\sigma_{t.det}$:

$$\tau \leqslant (0.103HL)^{1/2}/v_s = 0.321(HL)^{1/2}/v_s \qquad \text{(5\% increase)} \tag{7}$$

or

$$\tau \leqslant (0.210HL)^{1/2}/v_s = 0.458(HL)^{1/2}/v_s \qquad \text{(10\% increase)} \tag{8}$$

As H and v_s are equal to L/N and L/t_R, respectively, where N and t_R are theoretical plate number and the retention time of the solute, respectively, eqns. 7 and 8 are rewritten as

$$\tau \leqslant 0.321 t_R/N^{1/2} \qquad \text{(5\% increase)} \tag{9}$$

or

$$\tau \leqslant 0.458 t_R/N^{1/2} \qquad \text{(10\% increase)} \tag{10}$$

TABLE I

CALCULATED MAXIMUM INJECTION (DETECTION) LENGTH AND INJECTION VOLUME

Values for the use of a 50-μm I.D. capillary.

Parameter	*N*								
	100 000			*500 000*			*1 000 000*		
L (mm)	150	250	500	150	250	500	150	250	500
H (μm)	1.50	2.50	5.00	0.30	0.50	1.00	0.15	0.25	0.50
5% increase:									
l_{inj} (l_{det}) (mm)	0.37	0.62	1.24	0.17	0.28	0.56	0.12	0.20	0.39
V_{inj} (nl)	0.73	1.22	2.43	0.33	0.55	1.09	0.23	0.38	0.77
10% increase:									
l_{inj} (l_{det}) (mm)	0.53	0.89	1.77	0.24	0.40	0.79	0.17	0.28	0.56
V_{inj} (nl)	1.04	1.75	3.48	0.47	0.78	1.55	0.33	0.55	1.10

[a] Assumed limit of the increase in peak width.

RESULTS AND DISCUSSION

Maximum l_{inj} values calculated according to eqns. 4 and 5 are listed in Table I for various column lengths L and theoretical plate numbers N or various plate heights H. For a 50-μm I.D. capillary, corresponding limits of injection volume, V_{inj}, for each l_{inj} are also given in Table I. As shown above, it should be noted that the maximum l_{det} are the same as the maximum l_{inj}. Obviously, the higher the column efficiency becomes, the smaller l_{inj} (l_{det}) or V_{inj} should be kept.

The required time constants for the detection system according to eqns. 9 and 10 are given in Table II for retention times, t_R, of 5, 10 and 30 min.

As shown in Table I, the sample volume should be kept very small so as not to cause adverse effects on band broadening, and this volume is directly proportional to

TABLE II

CALCULATED MAXIMUM OF THE REQUIRED TIME CONSTANT (s)

Retention time (min)	*N*		
	100 000	*500 000*	*1 000 000*
5% increase[a]*:*			
5	0.30	0.14	0.10
10	0.61	0.27	0.19
30	1.83	0.82	0.58
10% increase[a]*:*			
5	0.43	0.19	0.14
10	0.87	0.39	0.27
30	2.61	1.17	0.82

[a] Assumed limit of the increase in peak width.

the square of the tube diameter, provided that the column efficiency is independent of the diameter, which roughly holds for tubes of I.D. less than 100 μm. When the on-column UV absorption method is employed for detection, the relationship mentioned above is also applied to the detection volume, in which case the light-path length is approximately equal to the tube diameter. Consequently, the use of a small-diameter tube seems advantageous from the viewpoint of detector sensitivity against the sample volume. However, it is generally difficult to employ a commercial UV detector with a tube of I.D. less than 25 μm because of the reduced amount of light passing across the tube.

It should be noted that the absolute amount of an analyte injected or detected in HPCE is extremely small, as shown in Table I, compared with conventional high-performance liquid chromatography, but the concentration should be high, as calculated in the following example. If we assume that l_{det} = 0.5 mm, tube diameter = 50 μm, molecular mass = 200, molar absorption coefficient = 5000 and minimum detectable absorbance = 10^{-4}, then the cell volume V_{det} = 1 nl, the minimum detectable concentration = $4 \cdot 10^{-6}$ M and the minimum detectable amount = 0.8 pg.

Although the present discussion is based only on calculation, we believe that the results will be helpful in evaluating extra-column effects and in designing HPCE systems.

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