Evaluation of extended light path capillary and etched capillary for use in open tubular capillary electrochromatography

Zhen Liu*, Koji Otsuka, Shigeru Terabe

Faculty of Science, Himeji Institute of Technology, 3-2-1 Kouto, Kamigori, Hyogo 678-1297, Japan

Abstract

Poor sensitivity and low phase ratio are the main drawbacks of open tubular capillary electrochromatography (OTCEC). The poor sensitivity results from the use of narrow bore size capillary, whereas the low phase ratio, which limits the separation capability, is caused by the limited surface area of conventional capillary. Two strategies may be useful to overcome these disadvantages. First, an extended light path (ELP) capillary, which has a bubble cell at the detection point, is used to improve the sensitivity. Secondly, an etched capillary of a 1000-fold increased surface area is used to enhance the phase ratio. In this work, use of an ELP capillary and an etched capillary in OTCEC was evaluated with a chiral stationary phase of avidin prepared with the physical adsorption method. With a 20 μm I.D. ELP capillary with a 150 μm bubble cell, the peak height was enhanced by 4–10-fold and the corrected peak area was increased by 12-fold relative to a 20 μm I.D. conventional capillary. However, the peak efficiency and resolution decreased noticeably. The phase ratio on the etched capillary was slightly enhanced, by a factor of 1.64 relative to an unetched capillary. Consequently, the separation capability was slightly improved. The increase in the phase ratio was much lower than that expected from the increase in surface area, the reason for which is probably the reduced density of surface silanol group and the generation of nitrogen-containing groups due to the etching process. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Extended light path capillary; Electrochromatography; Open tubular capillary electrochromatography; Capillary column; Phase ratio; Etched capillary; Physical adsorption; Enantiomer separations

1. Introduction

Open tubular capillary electrochromatography (OTCEC) [1–14] is one of the main modes of capillary electrochromatography (CEC). Compared to the other two CEC modes, packed column CEC [15–20] and monolithic column CEC [21–26], the main advantages of OTCEC are high efficiency,

simple instrumental handling and the short conditioning time. However, its evident disadvantages are poor sensitivity due to the narrow bore size of the capillary and low separation capability due to the low phase ratio on the limited surface area. To resolve these problems, two tactics may be used. First, an extended light path (ELP) capillary, which is fabricated with a bubble cell (usually 3–5-fold expansion) at the detection point, may yield a 3–5-fold increase in sensitivity relative to conventional capillary. Several applications have been described for the analysis of drugs in biological fluids with

*Corresponding author. Tel.: +81-791-580-171; fax: +81-791-580-493.

E-mail address: zhenliu_001@yahoo.com (Z. Liu).

capillary zone electrophoresis (CZE) [27,28]. Secondly, an etched capillary of a 1000-fold increased surface area is used to enhance the phase ratio. Pesek and co-workers [4–9,29] have developed etched bare fused-silica capillary and a variety of chemically modified etched capillaries with desired selectivity for OTCEC. However, the capability of etched capillary to improve the phase ratio has not yet been investigated.

In this work, we assessed the possibility of ELP and etched capillaries for improving the detection sensitivity and separation capability of OTCEC with a chiral stationary phase of physically adsorbed avidin. The physical adsorption method [10–14,26] is employed to prepare the stationary phase, because of its simplicity of preparation procedure and feasibility of estimation of the amount of the stationary phase on the capillary wall.

2. Experimental

2.1. Instrumentation

All experiments were performed on a Beckman P/ACE 5000 instrument (Fullerton, CA, USA). Data collection and instrument control were realized with a Beckman P/ACE Station on a personal computer. UV detection was carried out at 200 or 214 nm. The slit was 200 μm for observation on ELP capillaries and 800 μm for observation on etched capillaries. The temperature was kept constant at 20 °C. The applied voltage was 20, −20 or −10 kV. Open tubular liquid chromatography (OTLC) was performed by applying a pressure of 3.45 kPa (0.5 p.s.i.) to the capillary from the inlet. The samples were injected by a pressure of 3.45 kPa for 2–8 s.

2.2. Capillaries

All the capillaries used had a length of 57 cm (50 cm to detector). Fused-silica capillaries (20 and 50 μm I.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). ELP capillaries (20 μm I.D. with a bubble cell of 150 μm) were gifts from Agilent Technologies (Waldbronn, Germany). Etched bare fused-silica capillaries (50 μm I.D.)

were obtained from Silicon Valley Separation Media (San Jose, CA, USA).

2.3. Preparation of stationary phase and frontal analysis

After the capillaries were activated, the stationary phase of avidin was prepared according to the method proposed previously [13]. The frontal analysis method was the same as described previously [13], except that thiourea was used to measure breakthrough time without any adsorption.

2.4. Chemicals and solutions

All the chemicals used were of analytical or chromatographic grade. Avidin was a gift from Eisai (Tokyo, Japan). Racemic samples of danysl-valine (Dns-Val), dansyl-methionine (Dns-Met), dansyl-threonine (Dns-Thr), dansyl-serine (Dns-Ser) and chrysanthemic acid were purchased from Sigma (St. Louis, MO, USA) while racemic samples of fluribiprofen and ibuprofen were purchased from Wako (Osaka, Japan). The sample solutions were prepared with water. The concentrations of the samples were 0.05 or 0.1 mg/ml. Water was purified with a Milli-Q Labo system (Nihon Millipore, Yonezawa, Japan).

3. Results and discussion

3.1. Use of ELP capillary

With three racemic samples of Dns-Val, Dns-Met, Dns-Thr as test solutes, the detection sensitivity on a ELP capillary was investigated and compared with a conventional capillary of identical inner diameter. As shown in Fig. 1, the sensitivity was effectively improved by the use of ELP capillary. The corrected peak area and peak height were increased by 12-fold and 4–10-fold, respectively. However, the use of the bubble cell gave rise to noticeable losses in efficiency and resolution. As can be seen from Table 1, the losses in efficiency and resolution are related to the efficiency: the higher the efficiency, the larger the losses. These losses are caused by the mismatch of the detection slit length with the bubble cell size. When a sample band enters the bubble cell, it
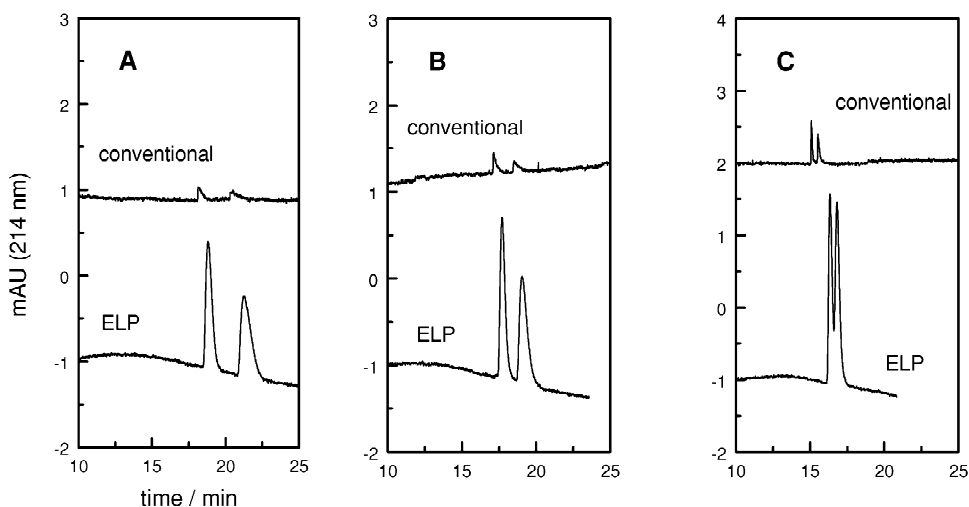
Fig. 1. Comparisons of OTCEC separations of racemic samples on ELP and conventional capillaries. Sample (0.1 mg/ml): A, Dns-Val; B, Dns-Met; C, Dns-Thr. Conventional capillary, 57 cm (50 cm to detector)×20 μm I.D.; ELP capillary, 57 cm (50 cm to detector)×20 μm I.D. with a bubble cell of 150 μm; slit length for detection, 200 μm; mobile phase, 10 mM phosphate buffer, pH 6.00; applied voltage, −10 kV; temperature, 20 °C; injection: 3.45 kPa×8 s.

expands in the radial direction to fill the increased area but compresses in the axial direction. The radial expansion is essential for the enhancement in sensitivity while the axial compression requires an accordingly reduced slit length to preserve the original peak efficiency. Provided that there is no mixing or band broadening process, the band compression is proportional to the square of the diameter at a given section of the bubble cell, leading to a band that is compressed by a factor of 9 to 25 for typical bubble cells expanded 3–5-times relative to the capillary inner diameter. Therefore, to keep the detected peak efficiency the same, the slit should be reduced to 1/9 to 1/25 of its original length. For example, to retain the efficiency detected with typical slits of 200 and 800 μm on the Beckman CE instrument, the slit lengths for an ELP capillary with

a bubble cell of five-times larger inner diameter should be 8 and 32 μm, respectively. Clearly, such slit lengths are too limited to be practical, whereas use of any larger slit length will be accompanied with loss in efficiency and thereby loss in resolution. On the other hand, the slit length required depends on the width of the sample band; a shorter slit is needed for a narrower sample band. As the slit length is usually fixed, the loss in efficiency on the ELP capillary depends on the width of the sample band. This explains the above dependence of the losses in efficiency and resolution on the peak efficiency. Clearly, the ELP capillary is not suitable for very efficient peaks with low resolutions. As high efficiency is a main advantage of OTCEC, the ELP capillary is unfavorable to maintain such an advantage. However, it is useful for highly retentive

Table 1
Comparisons of the peak efficiency and resolution on conventional and ELP capillaries[a]

Compound	Conventional capillary			ELP capillary			Ratio		
	N_1	N_2	R_s	N_1'	N_2'	R_s'	N_1'/N_1	N_1'/N_1	R_s'/R_s
Dns-Val	64 000	17 000	2.6	25 000	13 000	2.3	0.39	0.76	0.88
Dns-Met	85 000	44 000	3.5	31 000	17 000	1.6	0.36	0.39	0.46
Dns-Thr	350 000	230 000	2.7	38 000	35 000	1.0	0.11	0.15	0.37

[a] Unit of the efficiency: theoretical plates/m.

analytes, which usually encounter low efficiency in OTCEC.

3.2. Use of etched capillary

Because the surface matrix of an etched capillary is different from an unetched capillary after being treated with etching reagent, it is necessary to find out a proper activation procedure to offer maximum silanol density. Since the electroosmotic flow (EOF) on a fused-silica capillary is generated mainly due to the presence of ionized surface silanol groups, monitoring the change in EOF may shed light on the change of the surface silanols on the etched capillary. With a mobile phase of 10 mM phosphate buffer, pH 6.00, the EOF on an etched capillary was found faster than that on an unetched one; however, the enhancement of the EOF depended on the activation conditions. After the capillaries were rinsed with 0.1 M NaOH for 30 min, followed with water for 10 min and the mobile phase for 20 min, the t_0 value on the etched capillary under a voltage of 20 kV was 7.9 min, being 0.7 min shorter than that on the unetched capillary. As a contrast, after rinsing the capillaries with 0.1 M NaOH for 12 h followed with water for 10 min and the mobile phase for 20 min, the t_0 value on the etched capillary under the same voltage was reduced to 5.0 min, 0.3 min faster than that on the unetched capillary. The EOF at acidic pH condition was also investigated. After the

capillaries were treated with 0.1 M NaOH for 30 min followed with water for 10 min and a mobile phase of 10 mM phosphate buffer (pH 2.42) for 20 min, the t_0 value on the unetched capillary under a voltage of 20 kV was 55.3 min while no peak for the t_0 marker (thiourea) was observed on the etched capillary within 90 min under 20 kV and within 60 min under −20 kV. The weak cathodic EOF on the unetched capillary indicates that there were still some ionized silanol groups at the capillary inner surface under an acidic pH as low as 2.42. The disappearance of EOF on the etched capillary suggests that there were some functional groups of opposite charge at the capillary inner surface, generating a weak opposite potential to offset the weak potential produced by the ionized silanols. The EOF measurement was repeated with three etched capillaries, the trends were found the same. These results indicate that a long duration of activation procedure was favorable for increasing the ionized silanol number on the etched capillary and that the etched capillary had some positively charged groups.

After the capillaries were activated with 0.1 M NaOH for 12 h, followed by a rinse with water for 10 min and 10 mM phosphate buffer (pH 6.00) for 20 min, the adsorbed amounts of avidin on the etched and unetched capillaries were measured by using the frontal analysis method and compared with each other. As shown in Table 2, the adsorbed amount was 7.13 μmol/l on the etched capillary and

Table 2
Comparisons of the adsorbed amounts of avidin, the retention factors and the separation factors observed on etched and unetched capillaries

	Etched capillary	Unetched capillary	Ratio (etched/unetched)
Adsorbed amounts (μmol/l)	7.13	4.34	1.64
Retention factor (OTLC mode)			
Flurbiprofen	0.186	0.108	1.72
	0.336	0.178	1.88
Ibuprofen	0.101	0.054	1.89
	0.192	0.093	2.07
Dns-Thr	0.023	0.015	1.51
	0.042	0.026	1.61
Separation factor			
Flurbiprofen	1.80	1.65	1.09
Ibuprofen	1.90	1.74	1.09
Dns-Thr	1.87	1.75	1.07

Conditions: capillary, 57 cm (50 cm to detector)×50 μm I.D.; mobile phase, 10 mM phosphate buffer, pH 6.00; temperature, 20 °C; applied pressure, 3.45 kPa.

4.34 μmol/l on the unetched capillary. The increase in phase ratio was only 1.64-fold, very slight compared to the large increase of the surface area. The retention factors and separation factors of a few test compounds on the resulting stationary phases on the etched and unetched capillaries were investigated under OTLC mode by applying a pressure to the inlet of the capillary with the same instrument. As shown in Table 2, the retention factors of the enantiomers of flurbiprofen, ibuprofen and Dns-Thr on the etched capillary were slightly higher than those on the unetched capillary, by 1.51–2.07-fold. This is in agreement with the slight increase in phase ratio on the etched capillary. The separation factors on the etched capillary were almost the same as those on the unetched capillary, which means that there was nearly no change in selectivity. The separation capability of the etched capillary is illustrated with the CEC separations of Dns-Thr, Dns-Ser and chrysanthemic acid. Being consistent with the increase in the phase ratio, the improvement of the separations on the etched capillary was minute, as shown in Fig. 2. The enantiomers of Dns-Thr, which could be baseline separated on the unetched capillary, were separated with a little better resolution on the etched capillary. Dns-Ser and chrysanthemic acid, which could be partly separated on the un-

etched capillary, were resolved somewhat better on the etched capillary.

The low phase ratio on the etched capillary may have resulted from three aspects of reason: (1) low accessible surface area, (2) low density of surface silanol groups at the capillary wall, and (3) the presence of oppositely charged groups. The surface topography of etched and unetched capillaries was investigated with atomic force microscopy (AFM) [30]. It was found that on etched capillaries small (width<50 nm) and shallow (depth<50 nm) pits occur frequently, providing the main contribution to the high surface area. As the one-dimensional size of avidin is only 5 nm approximately [31], most of such pits should be accessible for the molecules of avidin. The etching process involves two main reactions, (1) and (2), and two subsidiary reactions, (3) and (4), of surface silica as follows [32]:

$$Si_sOH + NH_4F = Si_sF + NH_3 + H_2O \tag{1}$$

$$Si_sOH + HF = Si_sF + H_2O \tag{2}$$

$$2Si_sOH + NH_3 = Si_s\langle{}^{NH}_{O}\rangle Si_s \tag{3}$$
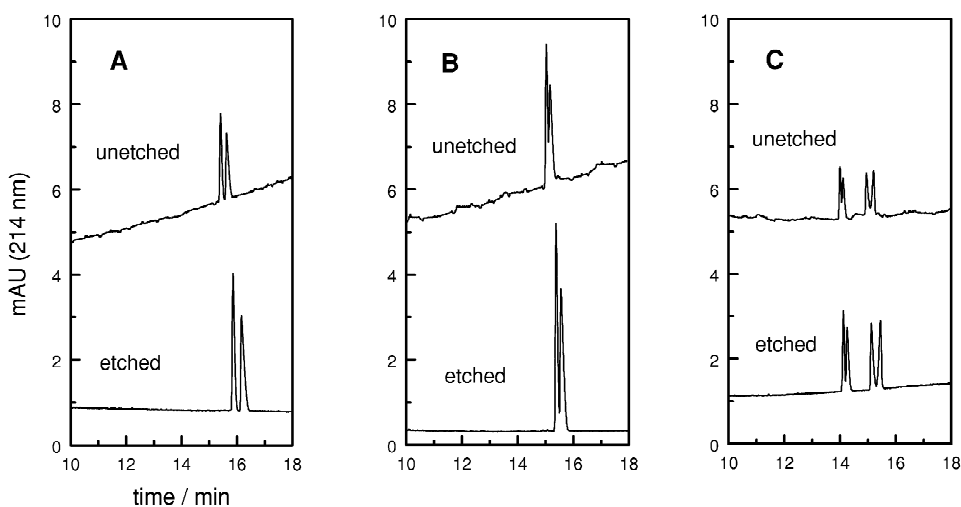
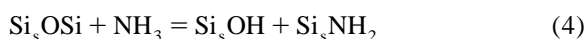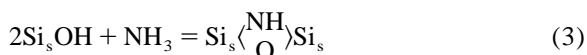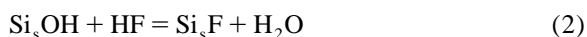$$Si_sOSi + NH_3 = Si_sOH + Si_sNH_2 \tag{4}$$

Fig. 2. Comparisons of OTCEC separations of racemic samples on unetched and etched capillaries. Sample: A, Dns-Thr (0.1 mg/ml); B, Dns-Ser (0.1 mg/ml); C, chrysanthemic acid (0.05 mg/ml). Columns, 57 cm (50 cm to detector)×50 μm I.D.; slit length for detection, 800 μm; mobile phase, 10 m*M* phosphate buffer, pH 6.00; applied voltage, −10 kV; temperature, 20 °C; injection, A and B, 3.45 kPa×2 s, C, 3.45 kPa×5 s.

Therefore, the surface matrix of etched capillary should be Si_sF instead of Si_sOH, whereas $\langle{}^{NH}_{O}\rangle$ and Si_sNH_2 are possible by-products. The change of the surface matrix is supported by the evidence of photoelectron spectrometry that fluorine and nitrogen are incorporated into the surface structure of etched capillary [8]. The groups of $-NH_2$ and $=NH$ may be protonated at acidic condition, bring positive charge, and then counteract with the ionized silanol groups. This explains the disappearance of EOF under acidic pH. In addition, the presence of the groups of $-NH_2$ and $=NH$ is detrimental for the adsorption of avidin due to the electrostatic repulsion. Therefore, the main reason for the low adsorbed amount of avidin on the etched capillary should be the reduced density of silanol groups and the presence of positively charged groups.

4. Conclusion

The ELP capillary can effectively improve the sensitivity of OTCEC, with an enhancement factor of up to 10 for peak height. However, it is only suitable for separations with low efficiency and high resolution, due to the accompanying loss in efficiency. In the case of physically adsorbed stationary phase, etched capillary can slightly improve the phase ratio and separation capability of OTCEC. When etched capillary is used for silanol-based chemically bonded stationary phase, it seems true that the phase ratio obtained is also much lower than that expected from the increase in surface area, due to the reduced silanol groups and abundant inert Si_sF groups at the inner surface. Although the improvement in phase ratio by using etched capillary in this work is not so high, it is comparable with the way reducing the bore size, which may offer a phase ratio two-times higher by reducing the inner diameter by twofold. Therefore, etched capillary is still helpful for improving the phase ratio in OTCEC.

References

[1] S. Mayer, V. Schurig, J. High Resolut. Chromatogr. 15 (1992) 129.
[2] D.W. Armstrong, Y. Tang, T. Ward, M. Nichols, Anal. Chem. 65 (1993) 1114.
[3] H. Hofstetter, O. Hofstetter, V. Schurig, J. Microcol. Sep. 10 (1998) 287.
[4] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 255.
[5] J.J. Pesek, M.T. Matyska, J. Cap. Electrophoresis 4 (1997) 213.
[6] J.J. Pesek, M.T. Matyska, S. Cho, J. Chromatogr. A 845 (1999) 237.
[7] J.J. Pesek, M.T. Matyska, S. Menezes, J. Chromatogr. A 853 (1999) 151.
[8] M.T. Matyska, J.J. Pesek, A. Katrekar, Anal. Chem. 71 (1999) 5508.
[9] M.T. Matyska, J.J. Pesek, J.E. Sandoval, U. Parkar, X. Liu, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 97.
[10] Z. Liu, H. Zou, J. Ni, Y. Zhang, Anal. Chim. Acta 378 (1999) 73.
[11] Z. Liu, H. Zou, M. Ye, J. Ni, Y. Zhang, Electrophoresis 20 (1999) 2891.
[12] Z. Liu, H. Zou, M. Ye, J. Ni, Y. Zhang, Chin. J. Chromatogr. 17 (1999) 245.
[13] Z. Liu, K. Otsuka, S. Terabe, J. Sep. Sci. 24 (2001) 17.
[14] Z. Liu, K. Otsuka, S. Terabe, Electrophoresis 22 (2001) 3791.
[15] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[16] C. Yan, D. Schaufelberger, F.J. Erni, J. Chromatogr. A 670 (1994) 15.
[17] M.M. Dittmann, K. Masuch, G.P. Rozing, J. Chromatogr. A 887 (2000) 209.
[18] R. Stol, W.T. Kok, H. Poppe, J. Chromatogr. A 853 (1999) 45.
[19] M. Ye, H. Zou, Z. Liu, J. Ni, Y. Zhang, Anal. Chem. 72 (2000) 616.
[20] Z. Liu, H. Zou, M. Ye, Electrophoresis 22 (2001) 1298.
[21] C. Fujimoto, Anal. Chem. 67 (1995) 2050.
[22] J.L. Liao, N. Chen, C. Ericson, S. Hjertén, Anal. Chem. 68 (1996) 3468.

Z. Liu et al. / J. Chromatogr. A 961 (2002) 285–291

291

[23] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 69 (1997) 3646.

[24] I. Gusev, X. Huang, Cs. Horváth, J. Chromatogr. A 855 (1999) 273.

[25] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, N. Nagayama, K. Hosoya, N. Tanaka, Anal. Chem. 72 (2000) 1275.

[26] Z. Liu, K. Otsuka, S. Terabe, M. Motokawa, N. Tanaka, Electrophoresis (2002) in press.

[27] G. Hempel, D. Lehmkuhl, S. Krümpelmann, G. Blaschke, J. Boos, J. Chromatogr. A 745 (1996) 173.

[28] J. Olgemöller, G. Hempel, J. Boos, G. Blaschke, J. Chromatogr. B 726 (1999) 261.

[29] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 887 (2000) 31.

[30] P.E. Pallen, J.J. Pesek, M.T. Matyska, J. Frommer, Anal. Chem. 72 (2000) 2751.

[31] L. Pugliese, A. Coda, M. Malcovati, M. Bolognesi, J. Mol. Biol. 231 (1993) 698.

[32] P.K. Iler, in: The Chemistry of Silica (Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry), Wiley, New York, 1979, p. 677.