Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 463–468
Talanta
www.elsevier.com/locate/talanta

Preparation and evaluation of stable coating for capillary electrophoresis using coupled chitosan as coated modifier

Xiaojia Huang [a,*], Qiuquan Wang [b], Benli Huang [b,*]

[a] *Environmental Science Research Center, Xiamen University, P.O. Box 1009, Xiamen city, Fujian province 361005, China*
[b] *Department of Chemistry & the MOE Key Laboratory of Analytical Sciences, Xiamen University, Xiamen 361005, China*

Received 10 July 2005; received in revised form 13 October 2005; accepted 13 October 2005
Available online 28 November 2005

Abstract

A coated capillary modified with a coupled chitosan (COCH) was developed by using a simple and fast (60 min) process that could be easily automated in capillary electrophoresis instrument. The COCH coating was achieved by first attaching chitosan to the capillary inner wall, and then coupling with glutaraldehyde, and rinsing chitosan again to react with glutaraldehyde. The COCH coating was stable and showed amphoteric character over the pH range of 1.8–12.0. When the pH value was lower than 4.5, the capillary surface possessed positive charges, which caused a reversal in the direction of the electroosmotic flow (EOF). The normal EOF direction could be obtained when the pH value was higher than 4.5. The COCH coating showed strong stability against 0.1 mol/L HCl, 0.1 mol/L NaOH and other solvents compared with conventional chitosan coating. The relative standard deviation of the run-to-run, day-to-day and capillary-to-capillary coating was all below 2% for the determination of EOF. The COCH-modified capillary was applied to acidic and basic proteins analyses and high efficiency could be attained. The comparison between unmodified capillary, chitosan-modified and COCH-modified capillary for the separation of real sample, extract from *Elaphglossum yoshinagae* with water, was also studied. Better results could be obtained on COCH-modified capillary than the other two capillaries.

Keywords: Capillary coating; Capillary electrophoresis; Chitosan; Protein separation

1. Introduction

Capillary electrophoresis (CE) has been utilized as a dominant separation technique for biochemical sample analytes, such as proteins and peptides, because of its high efficiency, fast separation, small samples need, etc. [1–3]. However, when proteins were separated by using uncoated fused silica capillary, adsorption of the samples to the capillary wall often occurred, which would result in protein peaks broaden, tailing, decreased reproducibility and even being impossible to be detected. The reasons of low separation efficiencies for protein are complex, but the presence of charged silanol groups on the wall surface plays an important role [4,5].

Several approaches have been proposed in order to decrease protein adsorption on capillary surface, including manipulation of the buffer pH to make the silanol groups fully protonated [6] or fully ionized [7]; using of additive in the sample [8,9]; coating of the inner capillary wall by chemically bonding or physically adsorbing [5,9,10–22]. In the last approach, adsorbed wall coating is an attractive alternative to covalent coating due to the simplicity and speed of the coating process. Many types of reagents, such as polyacrylamide [23,24], poly(vinyl alcohol) [25–27] and poly(ethylene oxide) [28,29], etc., have been used to coat capillary inner surface. There are several reviews focused on wall coating and modification [30–35].

Chitosan, or (1 → 4)-2-amino-2-deoxy-β-D-glucan, is a hydrophilic polyelectrolyte obtained by deacetylation of chitin, which is very abundant in nature [36]. Yao and Li [9] have employed it as both a dynamic and static cationic adsorbed coating for the analysis of basic proteins. Good separation results were obtained and higher 400,000 theoretical plates/m could be achieved. However, only a pH range of 3.0–5.5 was used in the work, which was narrow for the analysis of basic proteins and real samples. At the same time, the stability of chitosan coating needed to be improved. In this study, a simple and stable chitosan coating process was described. Chitosan first adsorbed to the capillary wall, then coupled with glutaraldehyde, and finally

* Corresponding authors. Tel.: +86 592 2186477 804; fax: +86 592 2183052.
E-mail addresses: hxj@yanan.xmu.edu.cn (X. Huang), blhuang@xmu.edu.cn (B. Huang).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.10.015

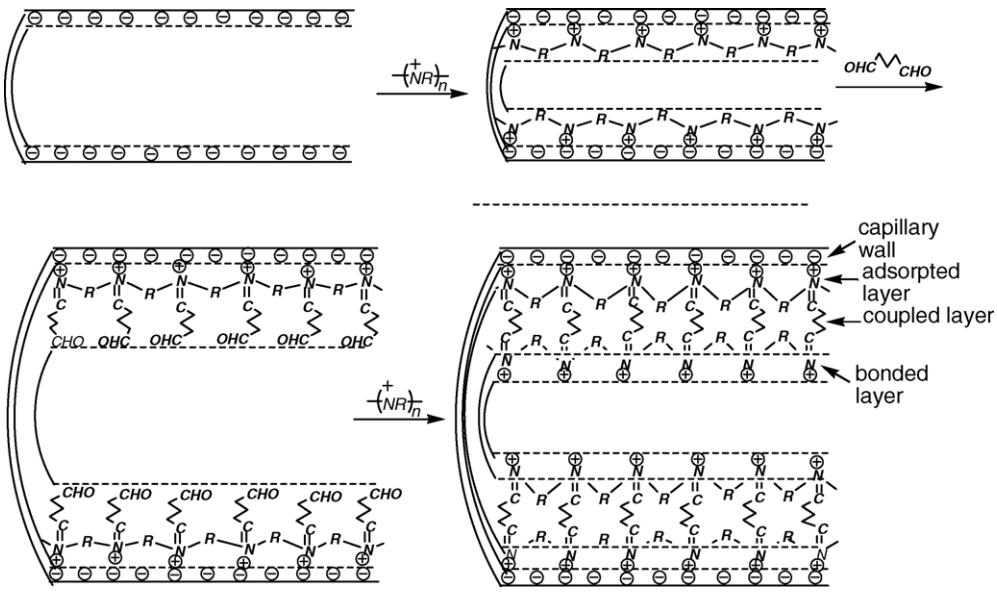

Fig. 1. Scheme of the capillary profile of COCH coating.

coated chitosan again to obtain multiple polymer layers, which thickly coat the capillary wall like a "carpet". The characters of coupled chitosan (COCH) coating and chromatographic performance for basic and acidic proteins were evaluated. Comparison between unmodified, chitosan-modified and COCH-modified capillaries for the separation of a real sample was also investigated.

2. Materials and methods

2.1. Chemicals and materials

Chitosan (average molecular mass ca. 300,000 and degree of deacetylation, 92%) was obtained from Yuhuan Chemical Factory (Zhejiang, China). Its stock solution, ca. 1% (w/v) in 1% aqueous acetic acid was prepared by stirring at room temperature then filtering through a 0.45 μm membrane. Bovine serum albumin (BSA) and cytochrome *c* were purchased from Sigma (St. Louis, MO, USA) and their samples were diluted in deionized water at a concentration of 0.2 g/mL. Glutaraldehyde (25% water solution), benzyl alcohol, which was used as an EOF marker, and other reagents were obtained from Shanghai Chemical Reagents Co. (Shanghai, China). Fused silica capillary (75 μm i.d. × 375 μm o.d.) used in this study was obtained from Yongnian Ruifeng Instrumental Co. (Hebei, China).

2.2. Apparatus

CE was performed with a "Backman" P/ACE MDQ system (Fullerton, CA). Thirty-two karat workstation was used for data processing (Fullerton, CA). The theoretical plate numbers (N) were determined from peak widths at half height ($w_{0.5}$) using the formula $N = 5.54(t_R/w_{0.5})^2$. The capillaries were thermostated at 25 °C by using liquid coolant and the samples were injected by pressure (0.5 psi) for 5 s. All samples were detected by UV absorbance at 214 nm.

2.3. Procedure for coupled chitosan (COCH) coating

COCH coating was achieved by the procedure described below. All of the rinsing employed the rinsing function of the "Beckman" P/ACE MDQ system under the rinse pressure of 20 psi. The capillary was rinsed with 1 mol/L NaOH for 30 min, and then with deionized water for 15 min in order to clean the capillary and enhance the dissociation of the silanol groups. After preconditioning, the capillary was rinsed with chitosan for 10 min and allowed to contact statically with the capillary for 10 min, so that more chitosan was adsorbed by silanol groups, then rinsed with 12.5% glutaraldehyde solution 5 min and contacted statically 15 min for coupling with chitosan. Finally, the same concentration chitosan solution was used to rinse over the glutaraldehyde 10 min and contacted statically 10 min to form the outer cationic coating. The scheme of the COCH-coated capillary was shown in Fig. 1.

3. Results and discussion

3.1. Effect of chitosan concentration on the EOF

The polycationic chitosan can be adsorbed to the negatively charged surface of fused silica capillary because chitosan exhibits high density of positive charges in aqueous acidic solution [9]. Effect of chitosan concentration on the EOF was shown in Fig. 2. It could be seen from the figure that the concentration of chitosan was just at 0.005% (w/v) an inversion in the direction of the EOF was exhibited, which indicated that the interfacial double layer had been modified by the adsorption of chitosan. As the concentration of chitosan increased further, more chitosan molecules were adsorbed to the surface, which results in increasing ζ potential of the electric double layer at the capillary surface, and faster EOF was observed. With above 0.15% of chitosan, the capillary exhibited relatively constant EOF, which indicated a saturation of chitosan coverage at the capillary surface.

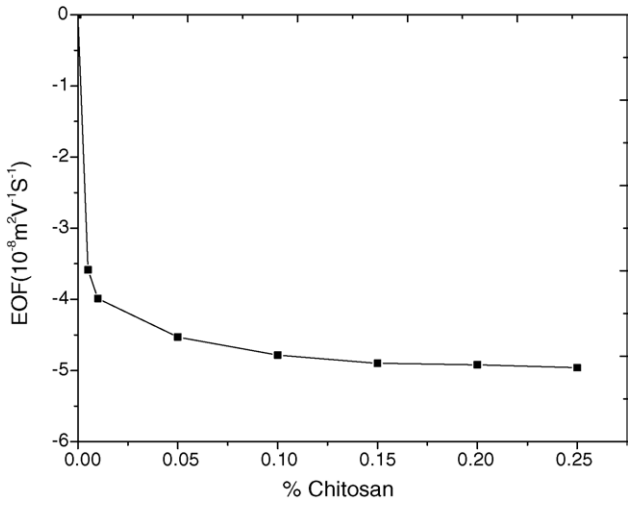

Fig. 2. Influence of chitosan concentration on the EOF. Conditions: applied voltage, −8 kV; buffer, 0.05 moL/L phosphate buffer at pH 1.8; capillary, 75 μm i.d. × 31.2 cm (21 cm effective length).

The concentration of chitosan 0.2% was chosen for further research.

3.2. Comparison of EOF on uncoated capillary and COCH-coated capillary

The EOF of uncoated capillary and COCH-coated capillary over the pH range of 1.8–12 was investigated. The results were shown in Fig. 3. The EOF of the uncoated capillary was generated from anode to cathode, and was stable above pH 9. However, the EOF started to decrease when the pH was lower than 9, and was almost suppressed below pH 3.5. On the other hand, the COCH-coated capillary exhibits positive charges because of the existence of NH_2 groups on the chitosan molecules when the pH below 4.5, so the EOF reversed from cathode to anode below pH 4.5. At the same time, EOF decreased when the pH value increased from 1.8 to 4.5 because the number of positive

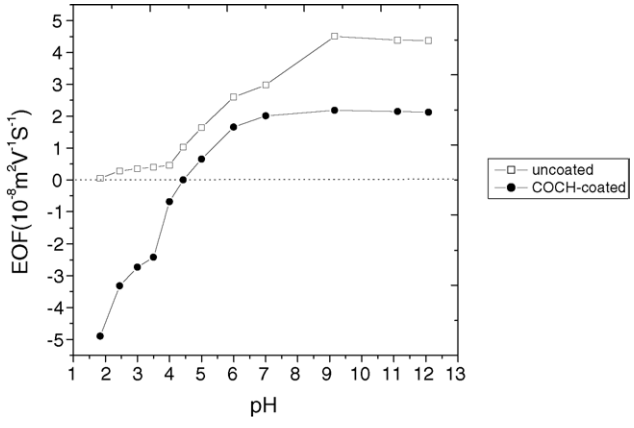

Fig. 3. EOF of uncoated and COCH-coated capillaries under different pH. Conditions: the concentration of chitosan, 0.2%; applied voltage, 8 kV for uncoated capillary, −8 kV for COCH-coated capillary at pH < 4.5 and 8 kV at pH > 4.5; buffers, 0.05 mol/L phosphate buffer at pH 1.8–3.5, 0.05 mol/L acetate buffer at pH 4–5, 0.05 mol/L phosphate buffer at pH 6–7 and 0.05 mol/L borate buffer at pH 9–12; capillary, 75 μm i.d. × 31.2 cm (21 cm effective length).

charges NH_2 groups decreased. When the pH value increased continuously, the number of charged silanol groups that have not been modified by coupled chitosan increased, while positive charges NH_2 groups decreased further. There was no EOF could be detected at pH 4.5 because the amount of charged silanol groups equal to the amount of charges NH_2 groups. EOF was normal from anode to cathode when the pH value was above 4.5 because ionization silanol groups predominate on the capillary wall, but it was lower than the EOF of uncoated capillary, which demonstrated that parts of the activity of silanol groups on the capillary wall was restrained by the coupled chitosan coating.

3.3. Stability of COCH-coated capillary

A comparison of stability test of the chitosan-coated capillary and the COCH-coated capillary against continuous analysis was performed, and the results were shown in Fig. 4. The stability was evaluated by measuring the EOF at pH 3.0 for chitosan-coated capillary (it could be used only pH 3–5.5 [9]), and pH 1.8 and 12.0 for COCH-coated capillary. If the EOF could not be detected within 60 min, the EOF was defined as 0 ($m^2\ V^{-1}\ s^{-1}$). It could be seen from the figure that the EOF of chitosan-coated capillary continuously decreased and endured only 14 runs at pH 3.0. On the other hand, the COCH-coated capillary endured at least 500 runs and 150 runs at pH 1.8 and 12, respectively. The severe disadvantage of the conventional chitosan-coated capillary was its low endurance. However, this problem was overcome simply by coupled chitosan coating, which described above.

3.4. Chemical stability

The chemical stability of the chitosan-coated and COCH-coated capillaries was investigated, and the results were shown in Table 1. The EOF was first measured when the coated capillary was prepared. Then the capillary was rinsed with the solvent listed in Table 1 for 15 min, and the EOF was measured again. The chemical stability was evaluated on the basis of the degradation ratio described in Table 1.

It could be seen that the chitosan-coated capillary was unstable after 0.1 moL/L HCl, 5 moL/L urea, CH_3OH, CH_3CN and 1% (v/v) HCOOH rinsing, the coating was severely detached after 0.1 moL/L NaOH rinsing. Although the degradation ratio for COCH-coated capillary was 12.25% after 0.1 moL/L NaOH rinsing, it was much more stable than chitosan-coated whose degradation ratio was 94.91%. After other solvent rinsing, the degradation ratio obtained from COCH-coated capillary was all below 4%, which demonstrated that the chemical stability was improved. The tolerance of COCH-coated capillary to both HCl and NaOH assured the relatively wider pH range to be used in analysis than that of chitosan-coated capillary.

3.5. Reproducibility

The reproducibility of COCH-coated was investigated based on the detection of EOF, and the results were shown in Table 2. It could be seen from the table that good reproducibility were obtained. The run-to-run ($n = 6$), day-to-day ($n = 6$) and

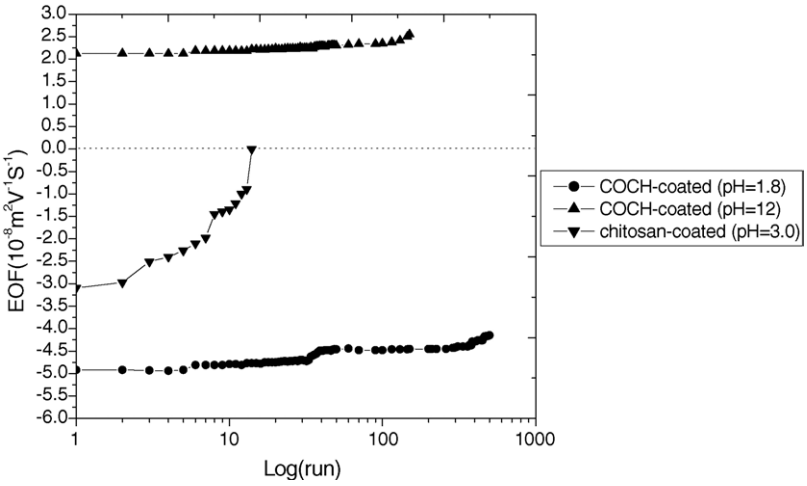

Fig. 4. Endurance of chitosan-coated and COCH-coated capillaries. Conditions: applied voltage, 8 kV for COCH-coated capillary (pH 12) and −8.0 kV for COCH-coated (pH 1.8) and chitosan-coated capillary (pH 3.0); other conditions were the same as in Fig. 3.

Table 1
Chemical stability of chitosan-coated and COCH-coated capillary

	Chitosan-coated			COCH-coated		
	EOF[a] (10^{-8} m^2 V^{-1} s^{-1})	EOF[b] (10^{-8} m^2 V^{-1} s^{-1})	Degradation ratio[c] (%)	EOF[a] (10^{-8} m^2 V^{-1} s^{-1})	EOF[b] (10^{-8} m^2 V^{-1} s^{-1})	Degradation ratio[c] (%)
0.1 M NaOH	−2.985	−0.152	94.91	−2.726	−2.392	12.25
0.1 M HCl	−2.995	−2.207	26.31	−2.722	−2.623	3.64
5 M urea	−2.984	−2.794	6.38	−2.729	−2.714	0.55
CH_3OH	−2.989	−2.722	8.91	−2.731	−2.682	1.79
CH_3CN	−2.990	−2.715	9.21	−2.735	−2.660	2.72
1% (v/v) HCOOH	−2.998	−2.849	4.96	−2.738	−2.696	1.53

[a] EOF was measured before rinsing with the solvent.
[b] EOF was measured after rinsing with the solvent.
[c] Degradation ratio = (EOF[b] − EOF[a])/EOF[a] × 100 (%). Conditions: applied voltage, −8 kV; buffer, 0.05 moL/L phosphate buffer at pH 3.0; capillary, 75 μm i.d. × 31.2 cm (21 cm effective length).

capillary-to-capillary ($n = 4$) R.S.D. were all below 2%. The good repeatability could be achieved easily just as other permanent coatings had done if the experiment was carefully carried out [5,37,38], which was one of the great advantages of COCH coating.

3.6. Protein analysis

The ability to perform the protein analysis of the COCH-coated capillary was evaluated by using acidic BSA and basic cytochrome *c* as test samples. The isoelectric points (p*I*) of BSA and cytochrome *c* are 4.4 and 10.2, respectively. Fig. 5a

Table 2
Reproducibility of COCH-coated capillary coating

	EOF average (10^{-8} m^2 V^{-1} s^{-1})	R.S.D. (%)
Run-to-run ($n = 6$)	−4.875	0.49
Day-to-day ($n = 6$)	−4.818	1.13
Capillary-to-capillary ($n = 4$)	−4.916	1.68

Conditions: applied voltage, −8 kV; buffers, 0.05 moL/L phosphate buffer at pH 1.8; capillary, 75 μm i.d. × 31.2 cm (21 cm effective length).

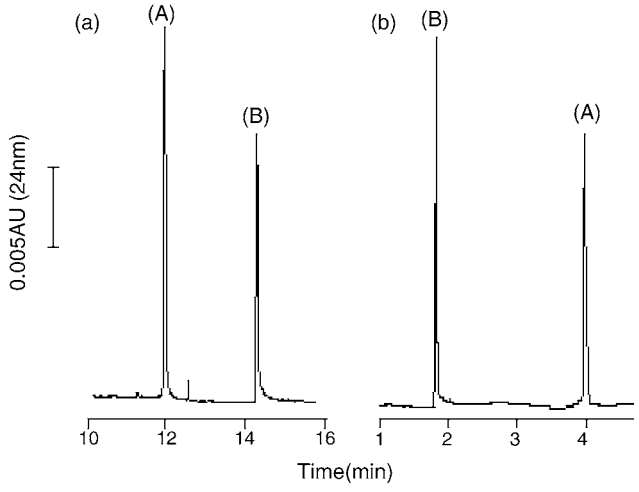

Fig. 5. Electropherogram of proteins obtained from COCH-coated capillary at pH 4.0 (a) and pH 7.5 (b). Conditions: applied voltage, −8 kV for pH 4.0 and 8.0 kV for pH 7.5; the others were the same as described in Fig. 3. Symbols: (A) BSA and (B) cytochrome *c*.

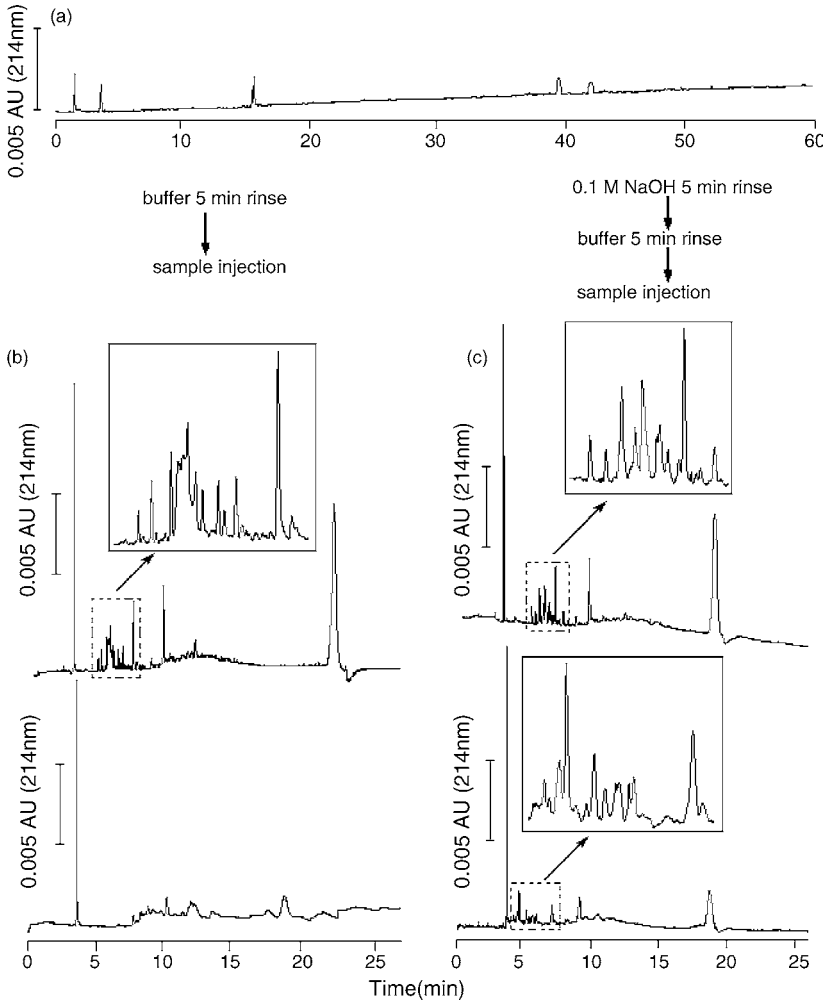

Fig. 6. Comparison of the electropherograms of the extract from *Elaphglossum yoshinagae* on uncoated capillary (a), chitosan-coated capillary (b) and COCH-coated capillary (c). Conditions: applied voltage, (a) 15 kV, (b) and (c) −15 kV; buffer: 0.05 mol/L acetate buffer at pH 4.0; capillary, 75 μm i.d. × 50.2 cm (40 cm effective length); the other conditions were the same as in Fig. 3.

showed the electropherogram of the two proteins at pH 4.0. Good separation result was obtained. The plate numbers of BSA and cytochrome *c* were 5.55×10^5 and 6.01×10^5 plates/m, respectively. COCH-coated capillary had an advantage of analyzing protein under alkaline conditions because its amphoteric character on the capillary wall. Fig. 5b depicted the separation result of bovine and cytochrome *c* at pH 7.5. The plate numbers of cytochrome *c* and BSA were 2.01×10^5 and 2.15×10^5plates/m, respectively. The separations of BSA and cytochrome *c* on uncoated capillary and chitosan-coated capillary were also investigated (data not shown). No peak could be detected on uncoated capillary after running 1 h at pH 4.0 and 7.5 because the proteins were strongly adsorbed to the capillary wall. Good separation results of BSA and cytochrome *c* could be obtained on chitosan-coated capillary at pH 4.0. However, the chitosan-coated capillary could not be used to separate protein at alkaline conditions because it could be used only under the pH range of 3.0–5.5 [9].

The separation ability to real sample analysis is an important evaluation criterion for a new-coated capillary. The electropherograms of the water extract from *Elaphglossum yoshinagae* on uncoated capillary, chitosan-coated capillary and COCH-coated capillary were shown in Fig. 6. Only several small peaks were detected on the uncoated capillary at pH 4.0 after running 1 h (Fig. 6a). No more peaks could be detected at pH 3.0, 6.0 and 10.0 on uncoated capillary (data not shown), which demonstrated that the extract strongly adsorbed to the uncoated capillary wall. On the other hand, good separation results could be obtained on chitosan-coated capillary (Fig. 6b) and COCH-coated capillary (Fig. 6c). These might be owing to the coulombic repulsion between ionic-polymer layers that could avoid the interaction between the proteins and the capillary wall besides the activity of silanol groups were restrained by polymer layer. In Fig. 6b, the chitosan-coated capillary was flushed with buffer for only 5 min prior to sample injection. The peaks were detected quite well during the first run. However, the separation efficiency decreased and most of the peaks could not be detected when four replicate analyses were performed. It may be that the analytes were adsorbed on capillary wall and impossible to be removed simply by rinsing with the buffer prior to analysis. To overcome this problem, rinsing the capillary with NaOH to flush out the adsorbed sample before analysis should be preformed. The

optimum conditions were first rinsed the COCH-coated capillary with 0.1 moL/L NaOH for 5 min and next with the buffer for 5 min prior to the sample injection. The adsorbed sample was flushed out to the capillary by 0.1 moL/L NaOH, and hence reproducible peaks were obtained during four runs (Fig. 6c). It was impossible to perform a NaOH rinse when the conventional chitosan-coated capillary was used. The strong stability of the COCH-coated capillary against alkaline conditions assured the NaOH rinsing.

4. Conclusions

A stable and amphoteric coating for capillary electrophoresis was developed by coupled chitosan coating procedure. COCH coating could be achieved by a simple and automated procedure with good reproducibility. In comparison with conventional chitosan-coated capillary, the COCH-coated capillary exhibited strong endurance, chemical stability and could be used in the range of pH 1.8–12.0. Highly separation column efficiency of protein could be obtained on the new COCH-coated capillary. The comparison between unmodified capillary, chitosan-modified and COCH-modified capillary for the separation of the real sample, extracted from *E. yoshinagae* with water showed that good reproducible result could be obtained on COCH-coated capillary than the others because the strong chemical stability of COCH-coated capillary assured that it could be rinsed with 0.1 moL/L NaOH to flushed out the adsorbed sample. However, chitosan-coated capillary could not be rinsed with basic solution. In the future, we will apply the COCH-coated capillary to micro- or nanospray CE/MS for the detecting the proteins in the extract of *E. yoshinagae*.

Acknowledgements

This work was financially supported by Specialized Research Fund for the Doctoral Program of Higher Education (20040384002), China Postdoctoral Science Foundation (2003034438), Pre-research Project of Xiamen University (2003sdyy08) and Open Foundation of the MOE Key Laboratory of Analytical Sciences (2004K01).

References

[1] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298–1302.
[2] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266–272.
[3] A. Bernal, U. Ear, N. Kyrpides, Nucleic Acids Res. 29 (2001) 126–127.
[4] J.K. Towns, F.E. Regnier, Anal. Chem. 64 (1992) 2473–2478.
[5] M.N. Albarghouthi, T.M. Stein, A.E. Barron, Electrophoresis 24 (2003) 1166–1175.
[6] R.M. McCormick, Anal. Chem. 60 (1988) 2322–2328.
[7] H.H. Lauer, D. McManigill, Anal. Chem. 58 (1986) 166–170.
[8] M.J. Gordon, K.J. Lee, A.A. Arias, R.N. Zare, Anal. Chem. 63 (1991) 69–73.
[9] Y.J. Yao, S.F.Y. Li, J. Chromatogr. A 663 (1994) 97–104.
[10] M. Chiari, M. Nesi, J.E. Sancoval, J.J. Pesek, J. Chromatogr. A 717 (1995) 1–13.
[11] C. Gelfi, M. Curcio, P.G. Righetti, R. Sebastiano, A. Citterio, H. Ahmadzadeh, N.J. Dovichi, Electrophoresis 19 (1998) 1677–1682.
[12] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 5272–5277.
[13] Y. Mechref, Z. Elrassi, Electrophoresis 16 (1995) 617–624.
[14] M. Chiari, N. Dellorto, A. Gelain, Anal. Chem. 68 (1996) 2731–2736.
[15] B.G. Belen'kii, G.E. Kasalainen, D.G. Nasledov, Y.V. Belov, M.I. Medvedeva, S.T. Khor, J. Anal. Chem. 53 (1998) 940–947.
[16] M. Du, J.H. Flanagan, B.C. Lin, Y.F. Ma, Electrophoresis 24 (2003) 3147–3153.
[17] F.C. Leinweber, J. Stein, M. Otto, Fresenius J. Anal. Chem. 370 (2001) 781–788.
[18] X.L. Ren, Y.F. Shen, M.L. Lee, J. Chromatogr. A 741 (1996) 115–122.
[19] H. Wan, M. Ohman, L.G. Blomberg, J. Chromatogr. A 924 (2001) 59–70.
[20] S. Konig, T. Welsch, J. Chromatogr. A 894 (2000) 79–88.
[21] H.J. Fu, W.H. Jin, H. Xiao, C.H. Xie, B.C. Guo, H.F. Zou, Electrophoresis 25 (2004) 600–606.
[22] Y. Jin, J.Q. Mi, H.T. He, Z.Q. Guo, M.P. Zhao, W.B. Chang, Talanta 66 (2005) 1005–1011.
[23] S. Hjerten, J. Chromatogr. 347 (1985) 191–198.
[24] M.X. Huang, W.P. Vorkink, M.L. Lee, J. Microcol. Sep. 4 (1992) 233–238.
[25] M. Gilges, M.H. Kleemiss, G. Schomburg, Anal. Chem. 66 (1994) 2038–2046.
[26] D. Belder, A. Deege, H. Husmann, F. Kohler, M. Ludwig, Electrophoresis 22 (2001) 3813–3818.
[27] J. Peuravuori, T. Lehtonen, V. Lepane, K. Pihlaja, Talanta 67 (2005) 103–111.
[28] G.J.M. Bruin, R. Huisden, J.C. Kraak, H. Poppe, J. Chromatogr. 480 (1989) 339–349.
[29] W. Nashabeh, J.T. Smith, Z. El Rassi, Electrophoresis 14 (1993) 407–416.
[30] M. Chiari, A. Gelain, in: C. Heller (Ed.), Analysis of Nucleic Acids by Capillary Electrophoresis, Vieweg Press, Braunschweig, 1997, pp. 135–173.
[31] M. Chiari, M. Cretich, F. Damm, L. Ceriotti, Electrophoresis 21 (2000) 909–916.
[32] J. Horvath, V. Dolnik, Electrophoresis 22 (2001) 644–655.
[33] E.A.S. Doherty, R.J. Meagher, M.N. Albarghouthi, A.E. Barron, Electrophoresis 24 (2003) 34–54.
[34] I. Rodriguez, S.F.Y. Li, Anal. Chim. Acta 383 (1999) 1–26.
[35] X.Y. He, Y.S. Ding, D.Z. Li, B.C. Lin, Electrophoresis 25 (2004) 697–711.
[36] R.A.A. Muzzarelli (Ed.), Natural Chelating Polymers, Pergamon Press, New York, 1973.
[37] Y. Liu, R.N. Fu, J.L. Gu, J. Chromatogr. A 694 (1995) 498–506.
[38] C.Q. Shou, C.L. Zhou, C.B. Zhao, Z.L. Zhang, G.B. Li, L.R. Chen, Talanta 63 (2004) 887–891.