Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 475–480

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Separation and on-line preconcentration by stacking and sweeping of charged analytes in the plant by microemulsion electrokinetic chromatography with nonionic surfactants

Jun Cao[a,b], Lian-Wen Qi[a,b], E-Hu Liu[a], Wei-dong Zhang[a,b], Ping Li[a,b,*]

[a] Key Laboratory of Modern Chinese Medicines (China Pharmaceutical University), Ministry of Education, Nanjing 210009, PR China
[b] Department of Pharmacognosy, China Pharmaceutical University, Nanjing, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 25 August 2008
Received in revised form 7 October 2008
Accepted 9 October 2008
Available online 1 November 2008

Keywords:
Microemulsion electrokinetic chromatography
Nonionic surfactant
Phenolic acids
Stacking
Sweeping

ABSTRACT

A novel on-line technique for stacking and sweeping of long sample plugs with simultaneous determination of charged analytes in the plant (protocatechuic aldehyde, rosmarinic acid, danshensu, salvianolic acid B, and protocatechuic acid) by the nonionic microemulsion electrokinetic chromatography (MEEKC) is presented. The preconcentration efficiency provided about 9–28-fold for stacking and 7–14-fold for sweeping in the enhancements of LOD. The effects of oil phase, Brij-35 and buffer concentrations on stacking and sweeping efficiency were examined in order to optimize the two methods. In nonionic MEEKC, the effect of the type of oil and buffer contents on preconcentration mechanism is often sophisticated. This study had demonstrated that the oil type and buffer content in nonionic microemulsion indeed markedly altered the affinity of microemulsion with analytes. Finally, in comparison to the stacking method, the most apparent disadvantages of the sweeping method were the relatively high limits of detection and poor peak shapes.

1. Introduction

CE has matured over the past few years into a powerful and effective analytical tool especially for separations of charged analytes [1–4]. However, CE suffers from poor concentration sensitivity when accompanied with a short optical pathlength and a small sample volume injection. In response to the sensitivity problem, various stacking procedures have been developed to preconcentrate samples and to increase the amount of sample that can be loaded onto the column without degrading the separation [5–10].

The on-line concentration of charged analytes in CE is one of the most attractive topics in the contemporary practice of this technique as it enables one to increase the sensitivity of analyses by orders of magnitude. In order to address this issue, two different techniques for on-line sample concentration have been developed: sample stacking and sweeping. Sample stacking occurs as ions cross a boundary that separates regions of the high electric field sample zone and the low electric field background solution (BGS) zone. In sweeping, the analyte zones are narrowed due to partitioning mechanism as the sample molecules experience the pseudostationary phase zone. It should be mentioned that the conductivity of the sample zone is usually adjusted to be nearly equal to that of the running buffer solution but no micelle is added to the sample solution. The use of charged pseudostationary phases, like sodium dodecyl sulfate, is by far the most universal experimental form of CE, however, the use of uncharged pseudostationary phases like nonionic surfactants have been proved to be effective as well for the separation of some interesting charged molecules [11–14]. In recent years, there has been a discussion with regard to the mechanisms of sweeping with nonionic micelles as a preconcentration technique [15]. More specifically, there remains some uncertainty based on the difference in mobility of charged analytes in stacking and sweeping modes on the electrokinetic chromatography with uncharged pseudostationary phases.

Microemulsion electrokinetic chromatography (MEEKC) is a reliable separation mode of CE that shows the possibility of highly efficient separations of both charged and neutral solutes. The separation mechanism in MEEKC is very similar to what is known from micellar electrokinetic chromatography (MEKC), with the main difference that the microemulsion has a core of tiny droplets of oil inside the micelles. Recently, a series of reports had concluded MEEKC has been shown to be applicable to a wider range of

Abbreviations: MEEKC, microemulsion electrokinetic chromatography; MEKC, micellar electrokinetic chromatography.

* Corresponding author at: Department of Pharmacognosy, China Pharmaceutical University, No. 24 Tongjia Lane, Nanjing 210009, PR China.
Tel.: +86 25 8539 1178x1244; fax: +86 25 8532 2747.
E-mail addresses: caojun91@163.com (J. Cao), fleude@126.com, lipingli@public1.ptt.js.cn, liping2004@126.com (P. Li).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.10.016

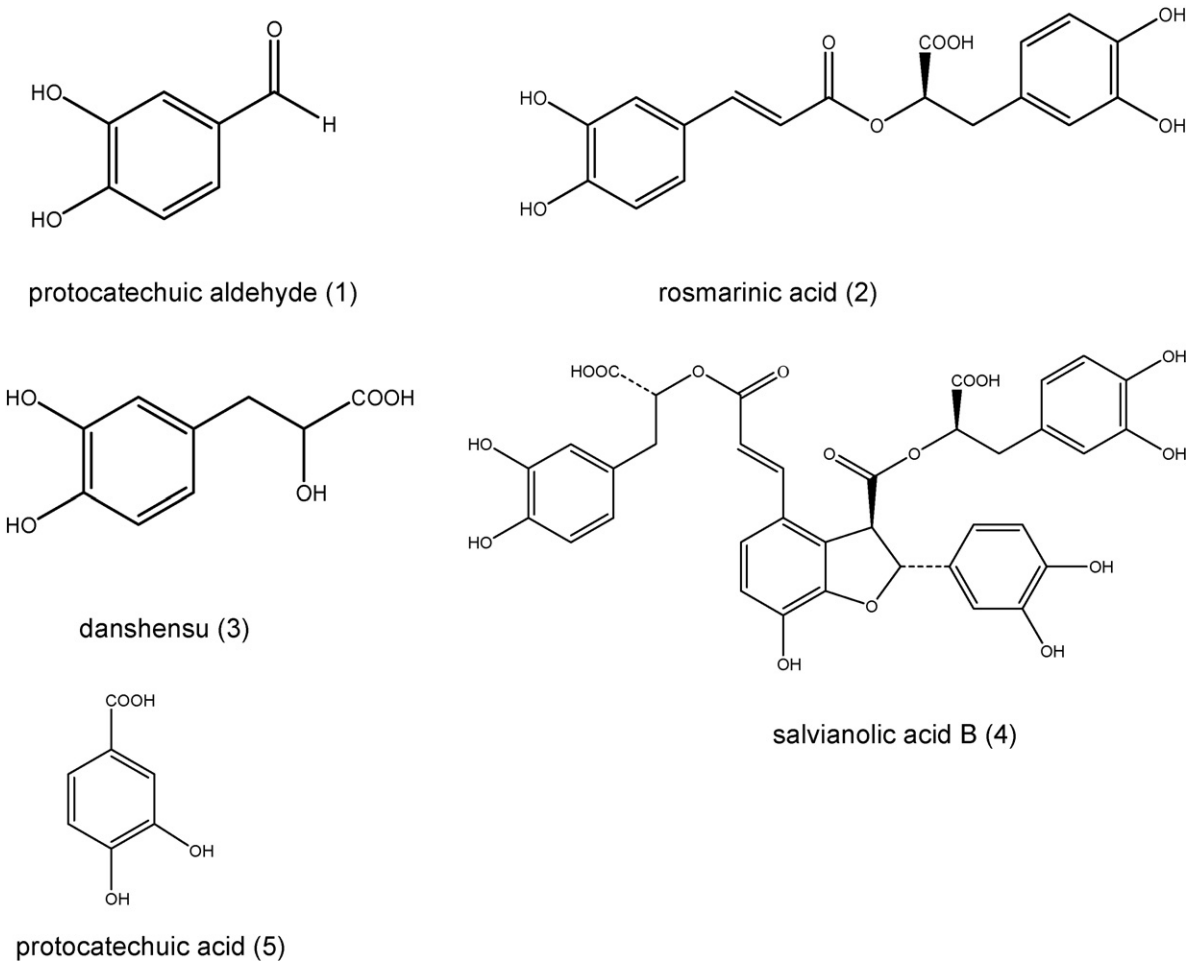

Fig. 1. Chemical structures of five phenolic acids examined in this study.

analytes and is able to provide higher separation efficiency than MEKC [16–18]. In MEEKC, a specific mixture of oil drops, surfactant, cosurfactant and aqueous buffer concentration along with an optimized condition in the capillary is adjusted to obtain and maintain a stable microemulsion phase when an on-line concentration method is applied. Based on this specificity, on-line stacking and sweeping method developed for MEEKC system would be more complicated than that of MEKC [19–22].

In this study, two on-line concentration methods, stacking and sweeping technology on charged analytes based on nonionic surfactants, which was coupled with MEEKC, were used to detect five phenolic acids (protocatechuic aldehyde, rosmarinic acid, danshensu, salvianolic acid B, and protocatechuic acid), which are commonly found in various plant products. The goal of this investigation is to develop a better understanding of microemulsion stacking and sweeping containing nonionic surfactants and the extent to which it is influenced by the sample matrix concentration, oil type, Brij-35 and buffer contents. In addition, the limits of detection of microemulsion stacking and sweeping on the enrichment of five analytes during MEEKC separations were explored.

2. Experimental

2.1. Apparatus

All experiments were performed with a Hewlett Packard 3D capillary electrophoresis system equipped with a 3D UV–vis detector (Waldbronn, Germany). Agilent ChemStation software was used for instrumental control and data analysis. Separations were performed in a 55.0 cm total length (46.5 cm to the detector) and 50 μm id uncoated fused-silica capillary (Ruifeng Inc., Heibei, China). Conductivities were measured with a DSJ-308 A conductivity meter (Shanghai, China).

2.2. Chemicals and reagents

Five phenolic acids compounds: protocatechuic aldehyde (1), rosmarinic acid (2), danshensu (3), salvianolic acid B (4), and protocatechuic acid (5) were isolated from the dried root or rhizome of Salvia miltiorrhiza Bge by repeated Silica Gel, Sephadex LH-20 and Rp-18 silica gel column chromatography in our laboratory. Their structures were elucidated by comparison of their spectral data (UV, IR, MS, ^{1}H NMR and ^{13}C NMR) [23,24]. The purity of each compound was determined to be higher than 98% by HPLC. The mobile phase was mixtures of acetonitrile and formic acid, methanol and formic acid.

The structures of these compounds are shown in Fig. 1. The standards were individually dissolved in 70% methanol at a stock concentration of 1 mg/mL. Standards were stored at 4 °C when not in use. Analyte concentrations were 5 μg/mL for all experiments except where noted. All other chemicals were of reagent-grade.

2.3. Real samples and pretreatment

Danshen sample was gathered from Shandong province of China. Danshen sample was treated as follows: 0.5 g of the sample

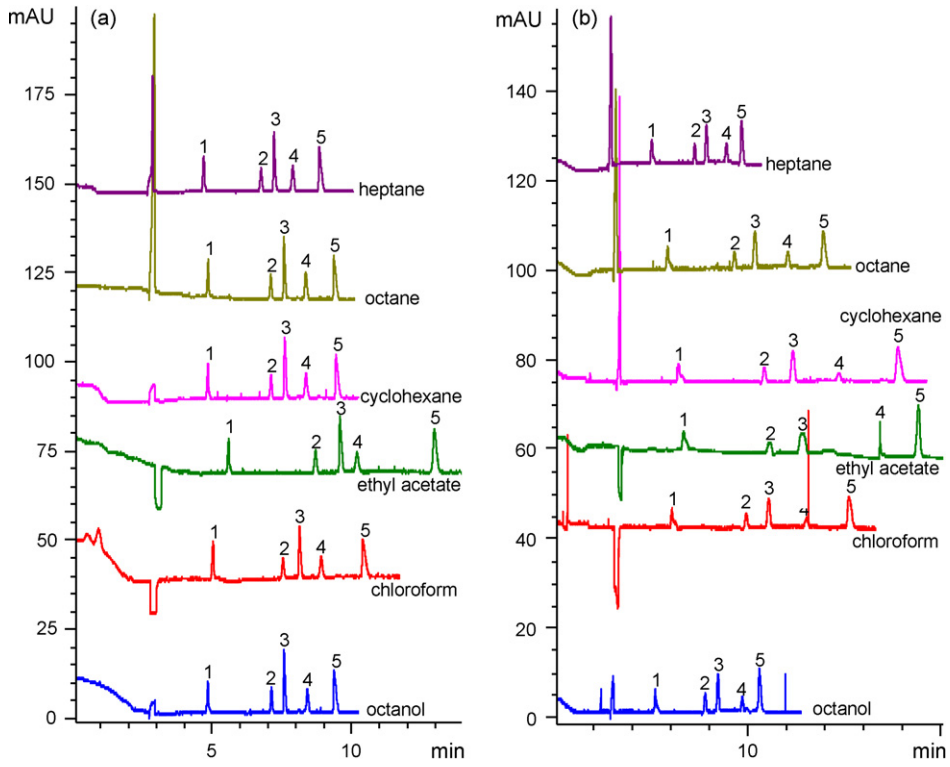

Fig. 2. Effect of oil types on the preconcentration abilities of phenolic acids. Solution composition: 0.6% (w/v) oils (heptane, octane, cyclohexane, ethyl acetate, chloroform and octanol), 3.0% (w/v) Brij-35, 6.0% (w/v) 1-butanol, and 90.4% (v/v) 10 mM sodium tetraborate buffer of pH 9.0. Standards concentrations: 5 μg/mL of each analyte. Applied voltage, 30 kV; a bare fused-silica capillary, 46.5 cm (effective length) × 50 μm id; wavelength, 200 nm; pressure injection: 50 mbar, 50 s. Peaks: 1, protocatechuic aldehyde; 2, rosmarinic acid; 3, danshensu; 4, salvianolic acid B; 5, protocatechuic acid: (a) stacking and (b) sweeping.

was milled and sonicated with 10 mL of 70% methanol for 60 min. The mixture was diluted with deionized water in the ratio of 1:40 and centrifuged for 5 min at 6000 rpm, then the clear liquid was ready for MEEKC analysis.

2.4. Preparation of solution for MEEKC

All microemulsions were prepared on a (w/v) basis in 10–40 mM sodium tetraborate buffer solution of pH 9.0. After the addition of oils (heptane, octane, cyclohexane, ethyl acetate, chloroform and octanol, 0.6%), various ratios of surfactant (Brij-35, 1.0–4.0%), and cosurfactant (1-butanol, 6.0%), the mixture was sonicated for 30 min until it became homogenous. The microemulsions were filtered prior to use through a 0.22 μm filter. The running buffer of pH was prepared by adding 0.1 M NaOH and 0.1 M HCl solution until the desired pH was achieved.

2.5. Operating conditions for CE

The capillaries were conditioned prior to separation by washing with 0.1 M sodium hydroxide (2 min), deionized water (4 min), and then with microemulsion solution (5 min) under a pressure of 935 mbar. After the last run of each day, capillaries were washed with 0.1 M sodium hydroxide (10 min), and then with deionized water (10 min). Separations were carried out using electrical voltage at 30 kV, and the temperature of the capillary was maintained at 25 °C, while 200 nm was selected as the detection wavelength.

Stacking and sweeping procedures: 5 μg/mL of each analyte was diluted with deionized water in stacking, and adjusted by sodium tetraborate solution to the same conductivity as the BGS in sample matrix in sweeping. The buffer was loaded at a pressure of 935 mbar for 5 min into the electrophoretic system in order to avoid the phase separation during MEEKC separation. Subsequently, the phenolic analytes prepared in the suitable sample matrices were injected by pressure injections for 50 mbar, 50 s. After the sample was injected, a separation voltage of 30 kV was applied with the microemulsion solution in the inlet vial the separation preceded by MEEKC.

3. Results and discussion

3.1. Effect of oil phase on stacking and sweeping efficiency

Many reports have demonstrated that the composition of microemulsion significantly influenced the selectivity and resolution on MEEKC separation [25,26]. However, the influence of composition of microemulsion buffer on preconcentration ability is not definite, thus it is worthwhile to explore the relationship. Typically, organic solvents with high hydrophobicity, such as long chain alkanes, alcohols and esters, were used as oil phase in microemulsion. First, six common organic solvents (heptane, octane, cyclohexane, ethyl acetate, chloroform and octanol) were employed as oil phase in normal MEEKC (i.e. pressure injection with 50 mbar, 3 s) for phenolic acids separation. When chloroform and octanol are used in nonionic MEEKC, they lead to unstabilized microemulsions. Moreover, the results indicated that five analytes had similar separation resolutions in all oil phases (data not shown). Hence, oil type did not cause a marked change in the separation of these analytes, and this result was consistent with most other MEEKC studies. Fig. 2 shows the electropherograms of phenolic acids by the stacking and sweeping MEEKC method (sample injection, 50 mbar, 50 s) in which the six organic solvents (heptane, octane, cyclohexane, ethyl acetate, chloroform and octanol) were also used as oil phases of microemulsion solution. Table 1 indicates that the peak high stacking ability of phenolic acids did not

Table 1
Effect of oil phase on stacking and sweeping modes[a].

Oil phase	Peak high in stacking					Peak high in sweeping				
	1	2	3	4	5	1	2	3	4	5
Heptane	9.5	6.6	16.5	7.1	12.5	5.4	3.6	6.9	3	7.4
Octane	9.6	6.8	16.8	7.2	11.9	5.3	3.6	6.6	3.5	7.0
Cyclohexane	10.0	6.7	16.8	6.9	12.2	4.7	3.8	6.2	2.1	6.9
Ethyl acetate	9.6	6.5	15.6	5.8	12.2	5.0	3.3	5.8	1.1	8.1
Chloroform	9.4	5.9	14.6	5.8	10.8	4.3	3.3	6.3	1.0	6.9
Octanol	8.8	7.2	17.3	6.5	11.9	5.1	3.6	6.8	1.3	7.2

[a] Conditions as in Fig. 2. Analytes 1–5: protocatechuic aldehyde (1), rosmarinic acid (2), danshensu (3), salvianolic acid B (4), and protocatechuic acid (5).

significant changes for oil phases except for chloroform. However, Table 1 shows the peak high sweeping ability of compounds was different in each oil phase. All phenolic acids had a lower sensitivity enhancement when cyclohexane, ethyl acetate, chloroform and octanol were used as oil phases, whereas heptane, and octane had a higher preconcentration effect on all analytes for sweeping methods. Furthermore, the profile in Fig. 2 also reflected the same type of oil phase was able to markedly impact preconcentration ability for two modes. In comparison to the stacking method (Fig. 2a), sweeping had lower preconcentration ability in the same injection condition (Fig. 2b). Since the concentration ability of the stacking method was highly dependent on the affinity of the pseudostationary phase with the analytes, thus the negative charged oil droplet should have stronger affinity with the stacking than sweeping modes. The above result has definitely demonstrated that there is a correlation between the type of oil phase and the affinity of the oil droplet with analytes in the on-line MEEKC method, whereas it was not evident in the normal MEEKC. This is likely to contribute to the stacking step in oil phases.

3.2. Effect of Brij-35 concentration on stacking and sweeping efficiency

Surfactant affects oil droplet charge and size, the level and direction of the EOF, and the level of any ion-pairing charged solutes. Brij-35 is the most widely used nonionic surfactant in MEEKC. The problems with excessive conductivity within the capillary and subsequent deleterious joule heating at increasing concentrations of ionic surfactants can be avoided by using nonionic surfactants, which can be added to the buffer at higher concentrations. Hence, the effect of contents of the Brij-35 in the microemulsion buffer on the preconcentration ability in two modes (sample injection, 50 mbar, 50 s) was examined over the 1.0–4.0% (w/v) concentration range (Table 2). With the 1.0% Brij-35 buffer, analytes migrated with the sample solvent zone, instable with the use of a subcritical microemulsion concentration in the separation buffer. An increase in Brij-35 concentration in the separation buffer affected EOF velocity (S.D. = 3), which decreased from 5.180 to 4.746 $cm^2\ S^{-1}$ for 1.0–4.0% Brij-35 separation buffers in stacking, and insignificantly changed in sweeping. It is apparent the migration times of analytes can be slightly increased by increasing the concentration of the Brij-35 from 1.0% to 4.0% in the stacking and sweeping modes. Furthermore, the results indicated that the preconcentration efficiency of all analytes was not obviously altered by the changes of Brij-35 concentration in microemulsions. All analytes had higher enhanced ability when Brij-35 content was maintained at 3.0% (w/v) in stacking. Alternatively, with the 2.0% Brij-35 content, sweeping efficiency is reduced. As can be seen from the data in Table 2, enhancement factor of protocatechuic acid had been insignificantly improved in the stacking mode when the Brij-35 content was increased from 1.0% to 4.0%. Compared to that of the stacking, preconcentration ability of protocatechuic acid was obviously raised in the sweeping mode. In this case, the on-line concentration ability of phenolic acids was highly dependent on the type of sample preconcentration, and it further demonstrated that the Brij-35 contents indeed play a minor role in determining the affinity behavior of analytes with microemulsion for two on-line methods.

3.3. Effect of buffer concentration on stacking and sweeping efficiency

Typically, low-ionic-strength (5–10 mM) borate or phosphate buffers are used as the microemulsion aqueous phase, giving a swift EOF while generating low currents. Nonionic surfactants have the distinct advantage of not contributing significantly to Joule heating. Hence, the effect of contents of the borate buffer in the microemulsion buffer on the on-line concentration ability in the stacking and sweeping (sample injection, 50 mbar, 50 s) was examined over the 10–40 mM (w/v) concentration range (Table 3). There was an effect of borate concentration on the EOF velocity (S.D. = 3), which decreased from 4.972 to 3.832 $cm^2\ S^{-1}$ for stacking, and from 4.939 to 4.136 $cm^2\ S^{-1}$ for sweeping. The results also indicated that the migration times of all analytes were obviously increased by the changes in buffer concentration in the stacking and sweeping methods. In addition, there was a striking improvement in enhancement factors with borate buffer concentrations from 10 to 40 mM. Increasing the borate buffer concentration to 40 mM

Table 2
Effect of Brij-35 concentrations (1–4%) on migration times and enhancement factors[a] in stacking and sweeping modes[b].

Micelle contents	Stacking										Sweeping									
	Migration times					Enhancement factors					Migration time					Enhancement factors				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1%	4.4	6.1	6.5	7.1	7.7	16	13	15	9	15	5.6	8.8	9.6	11.4	12.6	18	18	21	6	25
2%	4.7	6.6	6.9	7.8	8.2	18	16	18	12	19	5.6	8.4	9.3	10.2	12.0	15	16	16	6	19
3%	4.7	6.7	7.2	7.9	8.8	19	16	20	17	19	5.0	7.2	7.8	8.9	9.6	18	16	17	13	20
4%	4.7	6.4	6.8	7.3	8.0	14	11	14	9	13	5.8	9.7	10.4	13.2	14.2	15	22	26	8	28

[a] $S.E._{area} = A_{stack}/A$, where the numerator is the peak area obtained with preconcentration and the denominator is the peak area obtained from conventional injection (50 mbar, 3 s).
[b] Conditions as in Fig. 2. Analytes 1–5: protocatechuic aldehyde (1), rosmarinic acid (2), danshensu (3), salvianolic acid B (4), and protocatechuic acid (5).

Table 3
Effect of borate buffer concentrations (10–40 mM) on migration times and enhancement factors in stacking and sweeping modes[a].

Buffer contents	Stacking										Sweeping									
	Migration time					Enhancement factors					Migration time					Enhancement factors				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
10 mM	4.7	6.7	7.2	7.9	8.8	19	16	20	17	19	5.0	7.2	7.8	8.9	9.6	18	16	17	13	20
20 mM	6.6	10.3	11.5	12.6	16.0	21	18	25	13	28	5.8	8.9	9.9	11.0	13.2	21	22	22	8	26
30 mM	6.8	10.5	11.9	12.9	16.7	23	19	24	11	27	6.5	10.3	11.8	13.0	17.2	24	22	25	8	33
40 mM	7.0	10.9	12.5	13.5	18.2	21	18	25	11	29	6.8	10.9	12.7	13.8	19.7	27	23	27	8	38

[a] Conditions as in Fig. 2. Analytes 1–5: protocatechuic aldehyde (1), rosmarinic acid (2), danshensu (3), salvianolic acid B (4), and protocatechuic acid (5).

yields a highest sample stacking for protocatechuic acid, while the smallest analyte stacking is observed for salvianolic acid B in two methods. Moreover, the on-line concentration abilities for the enhancement factors are higher with sweeping than stacking. In general, using a low borate buffer concentration in the microemulsion gives a faster separation and relatively lower preconcentration ability because of the higher EOF generated at low ionic strengths. On the other hand, high buffer concentrations suppress the EOF, generate slightly higher currents and improve enhancement efficiency, which may not limit the level of voltage that can be applied.

3.4. Limits of detection and real sample analysis

A LOD comparison of the conventional MEEKC, the proposed stacking and sweeping MEEKC method is shown in Table 4. Separation buffer was 0.6% (w/v) heptane, 3.0% (w/v) Brij-35, 6.0% (w/v) 1-butanol, and 90.4% (v/v) sodium tetraborate buffer. With respect to limits of detection, stacking (sample injection, 125 s) and sweeping separations (sample injection, 250 s) of five phenolic acids at a concentration of 1.25 and 2.5 μg/mL, respectively showed well-resolved peaks with a signal-to-noise ratio of 3. These compounds were identifiable and baseline-resolved. Compared to the peak intensity obtained with conventional hydrodynamic sample injection (50 mbar, 3 s) at a concentration of 50 μg/mL, the preconcentration efficiency provided about 9–28-fold for stacking and 7–14-fold for sweeping in the enhancements of LOD without loss in separation resolution (Table 4.). In addition, the proposed MEEKC method was used to determine phenolic acids contents in Danshen. The electropherogram of Danshen sample that was separated by conventional MEEKC with hydrodynamic injection (50 mbar, 3 s) is shown in Fig. 3a, in which one phenolic acid was detected in the sample. On the other hand, the sample was separated by the stacking MEEKC method (50 mbar, 80 s), and four compounds were determined without any interference (Fig. 3b).

Table 4
Limits of detection with conventional MEEKC, stacking and sweeping modes MEEKC[a].

Analytes	LOD (ug/mL)[b]		
	Conventional MEEKC	Stacking MEEKC	Sweeping MEEKC
1	17.9	0.9	1.4
2	24.8	2.7	3.3
3	10.4	0.8	1.6
4	20.5	1.3	1.5
5	14.9	0.5	1.1

[a] Conditions as in Fig. 2.
[b] Values were obtained by extrapolation to S/N = 3. Analytes 1–5: protocatechuic aldehyde (1), rosmarinic acid (2), danshensu (3), salvianolic acid B (4), and protocatechuic acid (5).

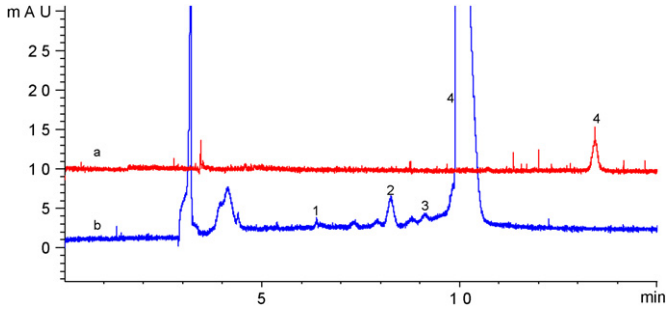

Fig. 3. Electropherogram of Danshen sample determined by the conventional and stacking MEEKC method. Separation conditions were the same as in Fig. 2.

The results infer that varied conductivity in different injection mode is a key factor; thus, stacking mode allows for more efficient sample on-line concentration for charged compounds.

4. Conclusion

In this paper, a high-sensitivity stacking and sweeping technique was first developed in the nonionic MEEKC system for analyzing charged analytes. It is postulated that maintaining different sample matrix conductivity and that of the separation buffer is a fundamental aspect of maximizing peak efficiency and detection limits for two preconcentration modes. This study demonstrated that the type of oil and buffer content did strongly influence the affinity ability of on-line concentration in nonionic microemulsion. In addition, stacking has the potential to provide an efficient mode versus sweeping. This paper has demonstrated that the usage of the stacking and sweeping technique in the nonionic MEEKC system is feasible, and the analytical results by the proposed method for five phenolic acids were also significant and eventually facilitate the more widespread use of nonionic MEEKC to applications that require greater column efficiency and lower limits of detection.

Acknowledgement

This study was supported by Grant no. 530870 from the key program of National Science Foundation of China.

References

[1] C. Tu, H.K. Lee, J. Chromatogr. A 966 (2002) 205–212.
[2] S. Furlanetto, S. Lanteri, S. Orlandini, R. Gotti, I. Giannini, S. Pinzauti, J. Pharm. Biomed. Anal. 43 (2007) 1402–1408.
[3] M. Silva, Electrophoresis 28 (2007) 174–192.
[4] J.P. Foley, M. Hong, M.A. Polinko, R.J. Pascoe, E.S. Ahuja, Electrophoresis 29 (2008) 1180–1188.
[5] R.-L. Chien, Electrophoresis 24 (2003) 486–497.
[6] J.P. Quirino, J.B. Kim, S. Terabe, J. Chromatogr. A 965 (2002) 357–373.
[7] C. Basheer, H.J. Wang, A. Jayaraman, S. Valiyaveettil, H.K. Lee, J. Chromatogr. A 1128 (2006) 267–272.
[8] S.D. Arnett, C.E. Lunte, Electrophoresis 28 (2007) 3786–3793.
[9] S.L. Simpson Jr., J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504–541.
[10] M.C. Breadmore, Electrophoresis 28 (2007) 254–281.
[11] M.X. Zhou, J.P. Foley, Electrophoresis 25 (2004) 653–663.
[12] N. Matsubara, S. Terabe, J. Chromatogr. A 680 (1994) 311–315.
[13] M. Molina, M. Silva, Electrophoresis 22 (2001) 1175–1181.

[14] C.W. Harrell, J. Dey, S.A. Shamsi, J.P. Foley, I.M. Warner, Electrophoresis 19 (1998) 712–718.
[15] M.R.N. Monton, J.P. Quirino, K. Otsuka, S. Terabe, J. Chromatogr. A 939 (2001) 99–108.
[16] H.Y. Huang, C.W. Chiu, Y.C. Chen, J.M. Yeh, Electrophoresis 26 (2005) 895–902.
[17] M.D. Mertzman, J.P. Foley, Electrophoresis 26 (2005) 4153–4163.
[18] S.H. Hansen, Electrophoresis 24 (2003) 3900–3907.
[19] H.Y. Huang, W.C. Lien, I.Y. Huang, Electrophoresis 27 (2006) 3202–3209.
[20] H.Y. Huang, I.Y. Huang, H.H. Liang, S. Lee, Electrophoresis 28 (2007) 1735–1743.
[21] P. Puig, F. Borrull, C. Aguilar, M. Calull, J. Chromatogr. B 831 (2006) 196–204.
[22] J.P. Quirino, S. Terabe, K. Otsuka, J.B. Vincent, G. Vigh, J. Chromatogr. A 838 (1999) 3–10.
[23] M. Gu, G.F. Zhang, Z.G. Su, F. Ouyang, J. Chromatogr. A 1041 (2004) 239–243.
[24] Y.R. Lu, L.Y. Foo, Phytochemistry 2 (2002) 117–140.
[25] J. Cao, J. Chen, L. Yi, P. Li, L.W. Qi, Electrophoresis 29 (2008) 2310–2320.
[26] E. McEvoy, A. Marsh, K. Altria, S. Donegan, J. Power Electrophor. 28 (2007) 193–207.