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Comparing micellar electrokinetic chromatography and microemulsion electrokinetic chromatography for the analysis of preservatives in pharmaceutical and cosmetic products

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Abstract

In this study, separation and determination of nine preservatives ranging from hydrophilic to hydrophobic properties, which are commonly used as additives in various pharmaceutical and cosmetic products, by micellar electrokinetic chromatograpy (MEKC) and microemulsion electrokinetic chromatography (MEEKC) were compared. The effect of temperature, buffer pH, and concentration of surfactant on separation were examined. In MEKC, the separation resolution of preservatives improved markedly by changing the sodium dodecyl sulfate concentration. Temperature and pH of running buffers were used mainly to shorten the magnitude of separation time. However, in order to detect all preservatives in a single run in a MEEKC system, a microemulsion of higher pH was needed. The separation resolution was improved dramatically by changing temperature, and a higher concentration of SDS was necessary for maintaining a stable microemulsion solution, therefore the separation of the nine preservatives in MEEKC took longer than in MEKC. An optimum MEKC method for separation of the nine preservatives was obtained within 9.0 min with a running buffer of pH 9.0 containing 20 m*M* SDS at 25 °C. A separation with baseline resolution was also obtained within 16 min using a microemulsion of pH 9.5 which composed of SDS, 1-butanol, and octane, and a shorter capillary column at 34 °C. Finally, the developed MEKC and MEEKC methods determined successfully preservatives in various cosmetic and pharmaceutical products.
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Keywords: Pharmaceutical analysis; Cosmetics; Parabens; Benzoic acids; Sorbic acid; Imidurea; Triclosan; Dehydroacetic acid

1. Introduction

Preservatives are commonly added to various foods, pharmaceuticals and cosmetics in order to prolong products' shelf life. The addition of preservatives to these products, especially to those that have high water content, is essential for avoiding alteration and degradation by microorganisms during storage [1,2]. To date, most papers on preservative analyses have mainly focused on food products because levels of preservatives in food are strictly regulated. In contrast, few articles have reported on the analysis of preservatives in pharmaceutical and cosmetic products, especially those products that are solely for external use. The potential harm of pre-

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servatives to consumers increases especially when consume in large quantities, and elevated amounts of preservatives in topical products have been shown to induce allergic contact dermatitis [3–5]. Consequently, in order to protect consumer health, the development of analytical methods for detecting preservatives and their levels in cosmetics and pharmaceuticals has practical demand for ensuring compliance to existing government regulations.

In most literatures, analytical methods for preservatives have been developed for either hydrophilic (e.g. sorbic acid and benzoic acid) or hydrophobic (e.g. four parabens: methyl, ethyl, propyl, and butyl *p*-hydroxybenzoate) preservatives [6–9]. However, hydrophilic and hydrophobic artificial preservatives may be used in combination in real products, hence simultaneous determination of both types of preservatives by a single separation method would be more efficient. Presently, high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are employed as major separation tools for the analysis of preservatives with reliable results. Recently, CE has become a more attractive separation technique in preservative analysis due to its many advantages, which include high efficiency, low waste production, and fast separation [6–13]. To our knowledge, the usage of CE methods for simultaneously determining hydrophilic and hydrophobic preservatives has not been widely reported, except for Kuo and Hsieh [10] and Boyce [11], and in these cases only food samples were examined. In addition, reports for preservative analyses in cosmetics or pharmaceuticals have so far been focused mainly on four parabens (methyl, ethyl, propyl, and butyl parabens). Other preservatives, such as sorbic acid, benzoic acid, triclosan, dehydroacetic acid and imidurea, are also commonly used in commercial cosmetic or pharmaceutical products. Therefore, it is worth developing a new method that could simultaneously detect these common preservatives.

CE is ideal for analyzing charged analytes, but it can also be used to detect neutral or hydrophobic analytes if a surfactant or a modifier is added to the CE running buffer. In general, micelle can be formed in running buffer when the surfactant concentration is above its critical micelle concentration (CMC). These micelles are regarded as the pseudostationary phase, and influence markedly the separation behavior of hydrophobic analytes. Furthermore, this technique, which is known as micellar electrokinetic chromatography (MEKC), also provides good separation for hydrophilic analytes such as sorbic acid and benzoic acid [5,11]. MEKC technique has now been employed successfully in the analysis of various neutral and charged analytes ever since Terabe et al. first proposed the technique in 1984 [14–17]. Recently, a method known as microemulsion electrokinetic chromatography (MEEKC), which separates analytes in a similar fashion as MEKC, has been proposed [18]. An immiscible liquid, such as heptane, octane, or ethyl acetate, can form stable and dispersed nanometer-sized oil droplets in the aqueous running buffer by surfactant and co-surfactant to reduce surface tension between oil droplet and water, and results in a stable microemulsion solution. Unlike the micelles that are used as pseudostationary phase in MEKC, the surfactant coated oil droplets are used as pseudostationary phase in MEEKC. Since analytes are able to more easily penetrate the surface of oil droplet compared to micelles' more rigid surfaces, therefore, MEEKC is applicable to a wider range of analytes [19,20]. A MEEKC method had already been successfully applied for analyses of four parabens in a liquid pharmaceutical formulation [21,22].

In this study, analytical methods for the simultaneous separation of hydrophilic and hydrophobic preservatives commonly used in commercial drug products were developed based on MEKC and MEEKC. These methods were used for detecting preservatives in real cosmetic and pharmaceutical samples, such as liquid formulation, cream drug samples, oil-based and water-based lotion. The separation results and feasibility for the analysis of nine preservatives in real drug samples by MEKC and MEEKC were compared.

2. Experimental

2.1. Preservatives standards

Methyl paraben (methyl *p*-hydrobenzoate), butyl paraben (butyl *p*-hydrobenzoate) and imidurea were purchased from Sigma (Steinheim, Germany). Sorbic acid and benzoic acid were obtained from TCI

(Tokyo, Japan). Ethyl *p*-hydrobenzoate, propyl *p*-hydrobenzoate and triclosan were obtained from Aldrich (Milwaukee, WI, USA). Dehydroacetic acid was purchased from ACROS (NJ, USA). These standards were individually dissolved in ethanol at a stock concentration of 2 mg/ml.

2.2. Chemicals and real samples

Disodium tetraborate, ethanol (absolute), boric acid, and phosphoric acid were bought from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) was obtained from TCI (Tokyo, Japan). Sodium hydroxide and hydrochloric acid were obtained from J.T. Baker (NJ, USA). Methanol was bought from Pharmco (CT, USA). Real pharmaceutical and cosmetic samples, such as liquid formulations, ointments, water-based lotions and oil-based liquid lotions, which are made by manufacturers in various countries, were obtained from supermarkets in Taiwan.

2.3. Real sample pretreatment

In order to be analyzed by CE, liquid formulations and water-based lotions were each diluted with running buffer to a suitable volume ratio. For ointment and oil-based lotions, 0.1 g of each sample was mixed with 6.0 ml of ethanol, and then was sonicated for 10 min. The mixture was centrifuged for 10 min at 6000 rev./min and the clear centrifuged liquid was ready to be analyzed by CE. The extracting recoveries of the nine preservatives by the above extraction condition were examined by spiking ointment and oil-based lotion samples with known amounts of the nine preservative standards. The recoveries, which were determined by triplicate measurements, were in the range of 84.3–120.6%, and thus indicated that the above extracting condition was acceptable.

2.4. Preparation of running buffer

Running buffer for MEKC was prepared by adding 0.1 *M* disodium tetraborate (borax) to 40 m*M* boric acid solution until the desired pH 9.0 was achieved, and then SDS of desired concentration (10–110 m*M*) was added into the running buffer.

Microemulsion buffer for MEEKC was prepared with 3.3% (w/w) SDS, 0.8% (w/w) octane, 6.6% (w/w) 1-butanol, and 89.3% (w/w) running buffer of pH 2.1 or 9.5. The mixture was then sonicated for 30 min in order to enhance dissociation. A running buffer of pH 2.1 was prepared by adding 1.0 *M* NaOH to 50 m*M* phosphoric acid solution until the desired pH was achieved, and running buffer of pH 9.5 was prepared by adding 0.1 *M* NaOH to 7.5 m*M* disodium tetraborate solution until the desired pH was achieved.

2.5. Apparatus and operating conditions for CE

All experiments were performed with a Beckman Coulter MDQ capillary electrophoresis system equipped with a photodiode-array detector (Fullerton, CA, USA). Beckman Coulter MDQ 32 Karat software was used for instrumental control and data analysis. Separations were performed in 50.2 cm (40 cm to detector)×50 μm I.D. or 31.2 cm (21 cm to detector)×50 μm I.D. uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The capillaries were conditioned prior to separation by washing with 1.0 *M* sodium hydroxide (3 min), deionized water (3 min), then with running buffer (5 min) for MEKC, alternatively washing with 1.0 *M* sodium hydroxide (5 min), then with running buffer (5 min) for MEEKC. Samples and standards were diluted with MEKC or MEEKC running buffer in the volume ratio of 1:9, and then were pressure-injected into the capillary column at 0.5 p.s.i. for 3 s (1 p.s.i.=6894.76 Pa). Separations were carried out using an electrical voltage of 25, 11, or −11 kV, and the temperature of the capillary was maintained at various values between 25 to 40 °C, while 200 nm was selected as the detection wavelength.

3. Result and discussion

Sorbic acid, benzoic acid, dehydroacetic acid, imidurea, four parabens (methyl, ethyl, propyl and butyl), and triclosan, which are nine commonly used preservatives in commercial products, were chosen as analytes in this study. SDS was used as a surfactant to form micelles in MEKC, and to stable oil droplet dispersion in the MEEKC system.

3.1. Preservatives separation by MEKC

A boric acid–borate buffer of pH 9.0 was used as running buffer in the experiment in which the influence of SDS on the separation of the nine preservatives was studied. When the concentration of SDS surfactant in a pH 9.0 running buffer was changed from 10 to 110 m*M*, migration behaviors for most analytes were markedly influenced (Fig. 1). As concentration of SDS increased in the running buffer, the migration velocities of four parabens and triclosan decreased. Alternatively, the migration velocities of sorbic acid, benzoic acid, dehydroacetic acid and imidurea, remained almost constant with different SDS concentrations. SDS micelles with hydrophobic interiors had stronger interactions with the four parabens and triclosan, which also had higher hydrophobic properties, therefore the five preservatives easily produced negatively charged complex with SDS micelles. In contrast, the other four analytes had higher water-soluble properties, therefore the interactions with SDS micelles were very weak. In addition, separation results improved markedly after the addition of SDS to running buffer. Baseline separations for all preservative standards were easily obtained by adjusting SDS content in running buffers. For example, all preservative standards separated well with running buffers containing 20 or 110 m*M* of SDS. Peak resolutions of 1.5 were achieved under these conditions, and the separation was completed within 9.0 min when 20 m*M* SDS was used; however, 12 min were needed for the condition of 110 m*M* SDS. This difference in time reflected that a change in SDS concentration was able to affect the retention factor for some preservatives with SDS micelles, thus the selectivity was also changed.

Separation by MEKC technique has been described to be easily influenced by temperature [15], therefore, the effect of temperature on the preservatives' migrations was also examined. As the temperature of running buffer increased from 20 to

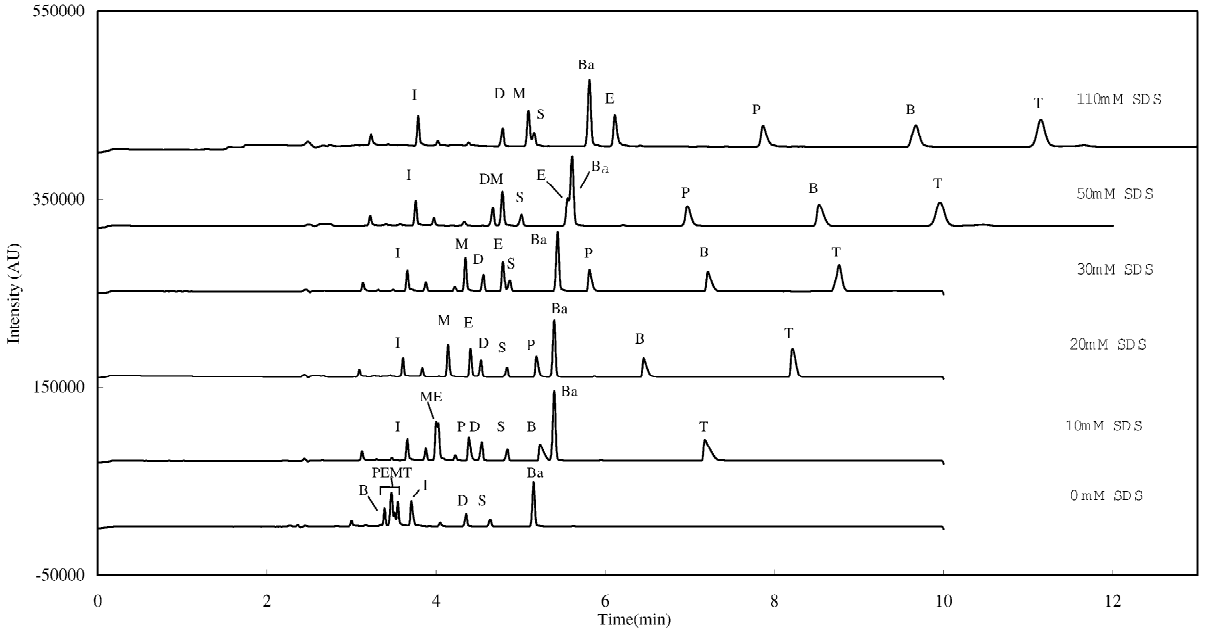

Fig. 1. The electropherogram of the nine preservatives with different SDS concentration produced by MEKC system. Separating conditions: a boric acid-borate running buffer (pH 9.0) containing 0 to 110 m*M* SDS; 25 kV voltage was applied to a fused-silica capillary tube with of 50.2 cm (40 cm from inlet end to the detection window). *M* (methyl paraben), E (ethyl paraben), P (propyl paraben), B (butyl paraben), S (sorbic acid), D (dehydroacetic acid), Ba (benzoic acid), I (imidurea), T (triclosan).

35 °C, the total separation time for the nine analytes was shortened from 10 to 6 min. The increase in migration velocities of analytes was probably due to an increase in magnitude of electroosmotic flow at the higher temperature since there was a lower viscosity for the running buffer (Fig. 2). In addition, the increased temperature also changed the capacity factor as the analytes were more soluble in a warmer buffer. The temperature also affected the mobility of the preservatives, which explained the changes in resolution between components with buffer's temperature. Although aggregation number of SDS micelles, and partition coefficient between analytes and SDS micelles were also dependent on temperature, column temperature did not influence the migration order of these analytes, thus selectivity was not changed with temperature in MEKC system.

The above results indicated that a suitable temperature control could effectively shortened separation time, but peak symmetry was affected when higher column temperature was used. An optimum baseline separation was achieved within 9.0 min when a running buffer, which contained 20 m*M* SDS, had its temperature controlled at 25 °C.

3.2. Preservatives separation by MEEKC methods

A common microemulsion solution, composed of 3.3% (w/w) SDS, 0.8% (w/w) octane, 6.6% (w/w) 1-butanol, and 89.3% (w/w) running buffer of a certain pH value, had been demonstrated to have an excellent ability for separating a wide range of analytes [18]. Therefore, this stable microemulsion preparation was used in the following experiment for improving separation for the nine preservatives. In an earlier study, a MEEKC method, which was used to separate parabens in pharmaceutical products, determined that a microemulsion solution with a low pH produced a better separation than one with a high pH because the former ensured that all parabens and their acidic hydrolysis impurity were neutral and provided improved peak shape for the acidic hydrolysis impurity [21]. Higher temperature in MEEKC reduced analysis times because solution's

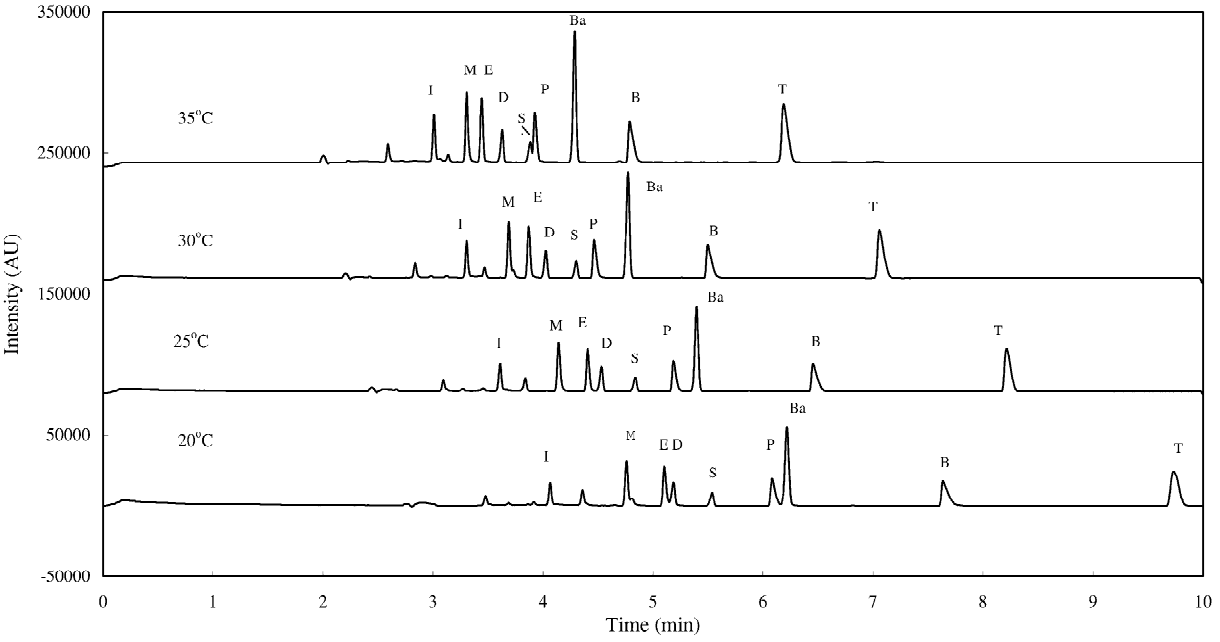

Fig. 2. The electropherograms of the nine preservatives with different temperatures resulted by MEKC system. Separating conditions: a boric acid-borate running buffer (pH 9.0) containing 20 m*M* SDS, temperature ranged from 20 to 35 °C. All other separating conditions were the same as in Fig. 1.

viscosity was lower which led to a faster electroosmotic flow (EOF) rate [19]. Thus, a pH 2.1 microemulsion solution and 40 °C of column temperature were first tested.

Fig. 3a was the electropherograms of the nine preservative standards with a microemulsion solution of pH 2.1, which was applied with a −11 kV negative voltage. All preservatives had relatively good resolution (>1.5), except for imidurea, which was not detected under this condition. Alternatively, only imidurea was detected after positive voltage was applied (Fig. 3b). The magnitude of electroosmotic flow approached zero when a pH 2.1 microemulsion buffer was used, because silanol rarely dissociated at this pH. In this situation, only charged analytes could migrate by electrical field attraction. As negative voltage was applied, the four parabens, benzoic acid, sorbic acid, dehydroacetic acid and triclosan migrated toward the detector that is located near the positive electrode, and were detected because they carried negative charges either by ionization themselves or by complex formation with oil droplet coated with negatively charged SDS. In fact, these analytes barely dissociated as anions at pH 2.1, therefore they were able to migrate under pH 2.1 condition mainly by forming negative charged complex.

In contrast, imidurea carries a positive charge in pH 2.1 buffer, and in theory it should have both partitioning and ion-pair interactions with negatively charged oil droplets. In this case, however, imidurea only migrated toward the capillary inlet end (i.e. negative electrode). This result indicated that imidurea had no interaction with the negatively charged oil droplets under this condition, thus its migration was not affected by these oil droplets.

As noted in the previous section, SDS micelles had little influence on the migration time of imidurea, benzoic acid, sorbic acid and dehydroacetic acid. However, in microemulsion system, most analytes, except for imidurea, formed charged complex with oil droplets either by electrostatic (ion pair)

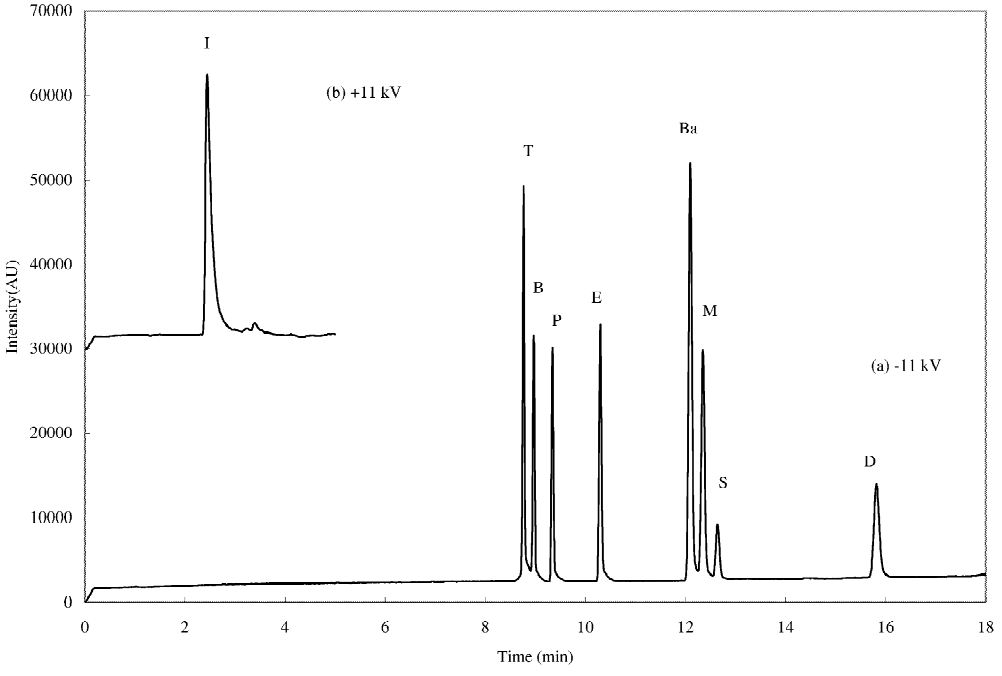

Fig. 3. The electropherograms of the nine preservatives with a pH 2.1 microemulsion solution in MEEKC system, (a) −11 kV, (b) 11 kV applied voltage. Separating conditions: a microemulsion solution composed of 3.3% (w/w) SDS, 0.8% (w/w) octane, 6.6% (w/w) 1-butanol, and 89.3% (w/w) phosphate buffer of a pH 2.1 was used as running buffer, and the temperature was controlled at 40 °C, either −11 kV or 11 kV was applied to a fused-silica capillary tube with 31.2 cm (21 cm from inlet end to the detection window).

attraction or by hydrophobic attraction to include analytes in the interior of oil droplet, thus allowed them to migrate under pH 2.1 condition. We concluded that the MEEKC condition had lower hydrophobic property than SDS micelles, therefore could interact with a wider polar range of analytes.

In order to detect all analytes, the pH of microemulsion buffer was adjusted to 9.5, and positive voltage was applied. All preservatives including imidurea were detected within 11.0 min because a relatively fast EOF rate was produced at pH 9.5, and forced all analytes to migrate toward the column outlet (negative electrode). The migration order for all preservatives was almost reversed to that in pH 2.1; most analytes had relatively good resolution, but both sorbic acid and ethyl paraben had the same migration rate (Fig. 4). In order to further improve separation of the nine preservatives, the composition of microemulsion solution, such as the oil type, co-surfactant type, surfactant concentration, buffer type and pH were examined. However, no significant improvement was observed, and the original microemulsion composition (3.3% (w/w) SDS, 0.8% (w/w) octane, 6.6% (w/w) 1-butanol, and 89.3% (w/w) running buffer of a pH 9.5), still had a better resolution. According to a previous report, temperature probably affected the selectivity of oil droplet for charged and neutral solutes [18], thus temperature effect of microemulsion solution on the separation was examined in the following experiments. When the temperature of microemulsion solution was increased from 25 to 40 °C, migration velocities of all analytes markedly increased, especially for paraben and tricolsan (Fig. 4), and the reason for this was similar to that described for MEKC system, in which a higher temperature provided a faster EOF velocity due to a lower viscosity of microemusion solution, and a higher temperature also affected the mobilities of the analytes. This trend indicated that the solubility and partition coefficient of analytes with the oil droplet pseudostationary phase were influenced by temperature, thus the selectivity of oil droplet for these analytes was altered and caused the migration orders of these analytes to change. Peak

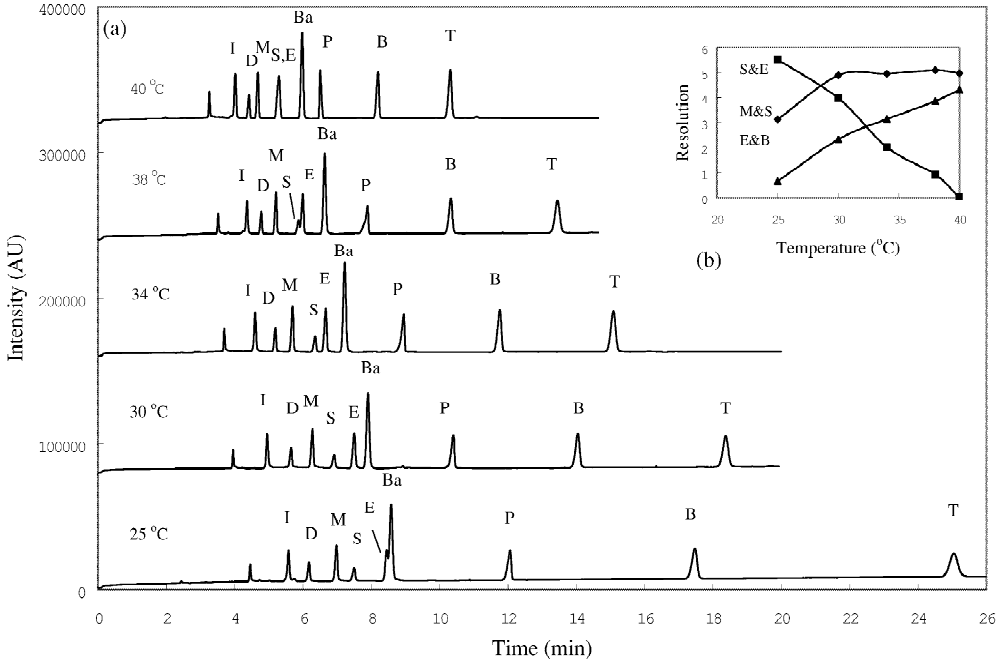

Fig. 4. The electropherograms of the nine preservatives with a pH 9.5 microemulsion solution in MEEKC system. Separating conditions: a microemulsion solution composed of 3.3% (w/w) SDS, 0.8% (w/w) octane, 6.6% (w/w) 1-butanol, and 89.3% (w/w) borate buffer of a pH 9.5 was used as running buffer, temperature ranged from 20 to 40 °C, 11 kV voltage was applied to a fused-silica capillary tube with 31.2 cm (21 cm from inlet end to the detection window). ■ (S and E), ▲ (E and Ba), ♦ (M and S).

resolutions were markedly changed by temperature (shown in the upper right corner of Fig. 4), and the peak resolution for sorbic acid and ethyl paraben was effectively increased with a decrease in temperature. However, the resolution for ethyl paraben and benzoic acid was not enough (~0.7) at 25 °C. A baseline separation for all analytes was obtained at 30 or 34 °C, but the latter provided a complete separation within 16 min. Therefore, the condition of 34 °C was chosen for the analysis of real pharmaceutical and cosmetic samples.

3.3. Methods validation

So far, the optimum separation condition, where all nine preservative standards were completely separated and the resolutions were more than 1.5, was achieved with either a boric acid–borax buffer containing 20 m*M* SDS and 25 °C for MEKC, or a microemulsion solution containing 3.3% (w/w) SDS, 0.8% (w/w) octane, 6.6% (w/w) 1-butanol, and 89.3% (w/w) borate buffer with a pH 9.5 for MEEKC. However, further comparison of the performances of both MEKC and MEEKC on both qualitative and quantitative was also needed. The relative standard deviations (RSDs) of migration time were in the range of 0.28 to 0.62% for MEKC method, and 0.24 to 2.31% for MEEKC method. The correlation coefficients (r) of the calibration curves were greater than 0.999 for MEKC method, and greater than 0.980 for MEEKC method (Table 1). The reproducibility of sample injection was examined based on five replicated injections of 3 s (0.5 p.s.i.) for 100 μg/ml standards, and the RSDs of peak area for the nine analytes were in the range of 0.16 to 3.89% for MEKC and 3.29 to 6.22% for MEEKC. The detection limits for the methods were in the range of 0.04–0.77 μg/ml based on a S/N ratio of 3 for MEKC, and 0.13–1.49 μg/ml for MEEKC. The efficiency for all analytes were in the range of 101 000–235 000 plates/m for MEKC and 72 000–148 000 plates/m for MEEKC. The above results indicated that MEKC had a higher efficiency, higher linear calibration curves and higher reproducible sample injection than MEEKC.

3.4. Method application

Real cosmetic and pharmaceutical samples were examined in the following experiments for assessing the feasibility of MEKC and MEEKC methods. Several pharmaceutical liquid formulations were first

Table 1
Average migration times, theoretical plate numbers, and correlation coefficients of calibration curves of nine preservatives standards in MEKC and MEEKC method

Preservatives	Migration time (min) (%)[a]		Reproducibility of sample injection (%)[d]		Theoretical plate numbers (N/m)		Correlation coefficients of calibration curves (r)[e]	
	MEKC[b]	MEEKC[c]	MEKC	MEEKC	MEKC	MEEKC	MEKC	MEEKC
Imidurea	3.63 (0.28)	4.57 (1.23)	0.167	5.79	157000	83000	1	0.9930
Methyl paraben	4.15 (0.31)	5.81 (0.26)	0.183	3.67	165000	86000	0.9999	0.9965
Ethyl paraben	4.41 (0.29)	6.91 (0.87)	2.56	5.31	165000	124000	1	0.9982
Dehydroacetic acid	4.54 (0.44)	5.29 (0.24)	2.45	4.30	221000	129000	0.9999	0.9850
Sorbic acid	4.85 (0.32)	6.51 (0.24)	3.89	4.89	235000	135000	0.9998	0.9983
Propyl paraben	5.28 (0.46)	9.55 (2.31)	2.14	6.01	140000	104000	1	0.9921
Benzoic acid	5.41 (0.50)	7.43 (0.29)	2.59	5.23	126000	72000	1	0.9826
Butyl paraben	6.46 (0.54)	12.50 (2.03)	1.66	6.22	101000	137000	1	0.9938
Triclosan	8.25 (0.62)	15.92 (0.56)	1.38	3.29	204000	148000	1	0.9808

[a] Values are means of 5 intra-day replicates. The value in parenthesis indicates the RSD of migration time in percentage.
[b] MEKC conditions: a borax–boric acid running buffer (pH 9.0) was containing 20 m*M* SDS, 25 kV voltage was applied to a capillary tube with 40 cm of effective length, and temperature was fixed at 25 °C.
[c] MEEKC conditions: a microemulsion solution of a pH 9.5 was used running buffer, temperature was fixed at 34 °C, and 25 kV voltage was applied to a capillary tube with 21 cm of effective length.
[d] The RSD of each peak area for five intra-day replicated injections was represent as reproducibility of sample injection.
[e] The calibration curves constructed from triplicate measurements at each concentration in the region of 5 to 100 μg/ml.

diluted with the appropriate running buffer and ethanol, and then were analyzed directly by the two methods. Results indicated that both methods allowed clear detection of preservatives in these cold syrup samples without any interference (Fig. 5). For solid forms of pharmaceutical samples, ointments were extracted by steps described in the experimental section prior to analysis by MEKC and MEEKC methods. Fig. 5 showed that several preservatives in the ointment samples, such as methyl, propyl and butyl paraben, sorbic acid and triclosan, were able to be analyzed and determined simultaneously by either MEKC or MEEKC method.

For real cosmetic samples, several commercially available water-based lotions were analyzed by MEKC and MEEKC methods after a suitable dilution with the appropriate running buffer and ethanol. Several preservatives, including ethyl and propyl parabens, and imidurea, were clearly detected in water-based lotion samples (Fig. 6). Oil-based lotions were also analyzed after a suitable sample pretreatment described in the experimental section, and the analytical results are shown in Fig. 6. Similar to water-based lotion samples, both MEKC and MEEKC were also able to detect the preservatives in these oil-based cosmetic samples. Upon further examination of Figs. 5 and 6, it appeared that MEEKC had a better resolution of components from the solvent front than MEKC, therefore MEEKC may be more useful for the simultaneous analysis of

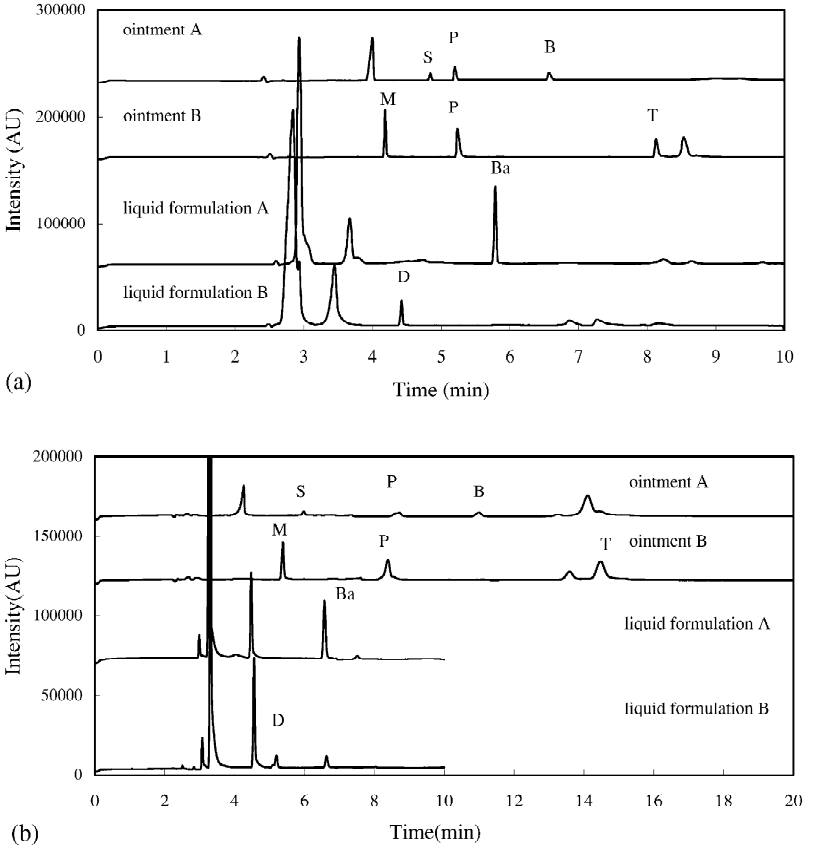

Fig. 5. The electropherograms of commerical pharmaceutical liquid formulation and ointment products determined by MEKC and MEEKC method. (a) MEKC, (b) MEEKC. Methyl, propyl and butyl paraben, sorbic acid and triclosan were found in the products. For MEKC method, the concentration of SDS in running buffer was 20 m*M*, and temperature was fixed at 25 °C, other conditions were same as in Fig. 1. For MEEKC method, a microemulsion solution of a pH 9.5 was used as running buffer, temperature was fixed at 34 °C, and other conditions were the same as in Fig. 4.

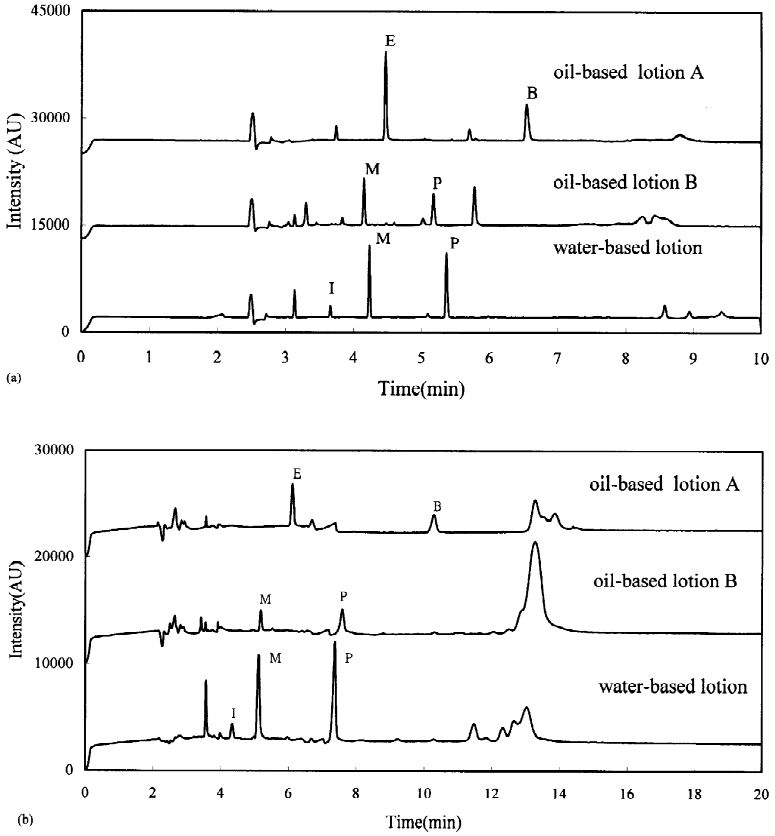

Fig. 6. The electropherograms of commercially available cosmetic lotions separated by MEKC and MEEKC methods. (a) MEKC, (b) MEEKC. Methyl, propyl paraben, and imidurea were found in these products. Other separation conditions as in Fig. 5.

these preservatives and other active ingredients in pharmaceutical and cosmetic samples. In addition, MEEKC, which in contrast to MEKC, seemed to allow the determination of very hydrophobic solutes which migrated after the preservatives peaks.

The above results demonstrated that both MEKC and MEEKC methods possess enough separation ability to analyze these preservatives in four different types of pharmaceutical and cosmetic samples. Table 2 summarized the content of preservatives in several pharmaceutical and cosmetic samples discussed above by MEKC. The RSDs of the quantitative results were in the range of 0.21–4.55% with triplicate measurements. When MEEKC was used to determine the contents of the same four samples, similar components compared to MEKC were found (data not shown) and the RSDs were in the range of 0.76–6.91%), which implied that both methods were close in their abilities in quantitative analysis. In order to improve the RSDs of the quantitative results in the future in real samples, it has been suggested that an internal standard be spiked in the sample, which can effectively reduce the variation in injected sample amount between repeated injects or in real samples with different viscosities.

4. Conclusion

In this paper, two methods for analyzing nine preservatives commonly used in pharmaceutical and cosmetic samples were developed: MEKC and MEEKC, separately. The usage of a microemulsion system was found to have different selectivity from SDS micelles for the nine preservatives, and the selectivity in MEEKC was altered by temperature,

Table 2
Contents of preservatives determined in commercial pharmaceutical and cosmetic samples

	Pharmaceutical samples				Cosmetic samples		
	Preservatives	Concentration[a]	RSD (%)		Preservatives	Concentration[a]	RSD (%)
Liquid formulation A	Benzoic acid	292 μg/ml	1.47	Water-based lotion A	Imidurea	1285 μg/ml	3.61
					Methyl paraben	795 μg/ml	2.56
					Propyl paraben	981 μg/ml	4.55
Liquid formulation B	Dehydroacetic acid	465 μg/ml	1.44	Water-based lotion B	Methyl paraben	895 μg/ml	2.84
Liquid formulation C	Dehydroacetic acid	275 μg/ml	3.67	Water-based lotion C	Methyl paraben	1227 μg/ml	3.31
Liquid formulation D	Propyl paraben	74 μg/ml	1.92	Water-based lotion D	Methyl paraben	620 μg/ml	1.86
Ointment A	Sorbic acid	3.31 mg/g	1.43	Oil-based lotion A	Ethyl paraben	1.53 mg/g	0.53
	Propyl paraben	2.64 mg/g	1.16		Butyl parabne	0.91 mg/g	2.30
	Butyl paraben	1.69 mg/g	1.10				
Ointment B	Methyl paraben	6.05 mg/g	0.21	Oil-based lotion B	Methyl paraben	0.636 mg/g	1.48
	Propyl paraben	7.21 mg/g	1.61		Propyl paraben	0.64 mg/g	3.10
	Triclosan	2.33 mg/g	2.08				
Ointment C	Methyl paraben	0.94 mg/g	0.68	Oil-based lotion C	Methyl paraben	0.90 mg/g	1.59
Ointment D	Methyl paraben	1.13 mg/g	2.88	Oil-based lotion D	Methyl paraben	1.39 mg/g	0.38
					Propyl paraben	0.92 mg/g	1.20

[a] Values are means of triplicate determination by MEKC, and the MEKC condition was as the same as the description in Table 1.

however, SDS concentration determined the selectivity in MEKC. For MEEKC, pseudostationary phase of oil droplet formed complexes with most analytes, and influenced the migration time for hydrophilic and hydrophobic preservatives. SDS micelles only interacted with hydrophobic preservatives in MEKC, but were able to provide a shorter separation time for all preservatives. With an increase in temperature for the running buffer, migration velocities for all analytes were increased in MEKC and MEEKC. A higher temperature was avoided in order to obtain better peak symmetry, thus 25 °C was suggested in the MEKC system. However, some preservatives were unable to be separated at 25 °C (ethyl paraben and benzoic acid) or above 35 °C (ethyl paraben and sorbic acid) in MEEKC system, thus 34 °C was suggested for obtaining enough peak resolution and a reasonable separation time. These two optimum conditions developed for MEKC and MEEKC were able to successfully separate these preservatives in several pharmaceutical and cosmetic samples after a simple sample pretreatment.

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References

[1] M.C. Boyce, Electrophoresis 22 (2001) 1447.
[2] M.D. Paciolla, S.A. Jansen, S.A. Martellucci, A.A. Osei, J. Pharm. Biomed. Anal. 26 (2001) 143.
[3] E. Mikami, T. Goto, T. Ohno, H. Matsumoto, M. Nishida, J. Pharm. Biomed. Anal. 28 (2002) 261.
[4] L. Labat, E. Kummer, P. Dallet, J.P. Dubost, J. Pharm. Biomed. Anal. 23 (2000) 763.
[5] I. Pant, V. Trenerry, Food Chem. 53 (1995) 219.
[6] J.E. Koundourellis, E.T. Malliou, T.A. Broussali, J. Pharm. Biomed. Anal. 23 (2000) 469.
[7] M. Spangler, E. Mularz, Chromatographia 54 (2001) 329.
[8] A.B. Prevot, E. Pramauro, M. Gallarate, M.E. Carlotti, G. Orio, Anal. Chim. Acta 412 (2000) 141.
[9] K. Waldron, J. Li, J. Chromatogr. B 683 (1996) 47.
[10] K.-L. Kuo, Y.-Z. Hsieh, J. Chromatogr. A 768 (1997) 334.
[11] M.C. Boyce, J. Chromatogr. A 847 (1999) 369.
[12] R.A. Frazier, E.L. Inns, N. Dossi, J.M. Ames, H.E. Nursten, J. Chromatogr. A 876 (2000) 213.
[13] Q.-C. Chen, J. Wang, J. Chromatogr. A 937 (2001) 57.
[14] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[15] T. Watanabe, S. Terabe, J. Chromatogr. A 880 (2000) 295.
[16] J.P. Quirino, S. Terabe, Anal. Chem. 71 (1999) 1638.
[17] K. Isoo, K. Otsuka, S. Terabe, Electrophoresis 22 (2001) 3426.
[18] K.D. Altria, J. Chromatogr. A 892 (2000) 171.

[19] C. Gabel-Jensen, S.H. Hansen, S. Pedersen-Bjergaard, Electrophoresis 22 (2001) 1330.
[20] S. Pedersen-Bjergaard, C. Gabel-Jensen, S.H. Hansen, J. Chromatogr. A 897 (2000) 375.
[21] P.-E. Mahuzier, K.D. Altria, B.J. Clark, J. Chromatogr. A 924 (2001) 465.
[22] P.-E. Mahuzier, B.J. Clark, S.M. Bryant, K.D. Altria, Electrophoresis 22 (2001) 3819.