Enhanced separation of Compound Xueshuantong capsule using functionalized carbon nanotubes with cationic surfactant solutions in MEEKC

A novel additive of multi-walled carbon nanotubes (MWNTs) dispersed with cationic surfactants or mixed cationic/anionic surfactants was used for MEEKC separation of eight phenolic compounds, four glycosides, and one phenanthraquinone. In this context, several parameters affecting MEEKC separation were studied, including the dispersion agents of MWNTs, MWNTs content, oil type, SDS concentration, and the type and concentration of cosurfactant. Compared with conventional MEEKC, the addition of all types of MWNTs dispersions using single or mixed cationic surfactant solutions in running buffers was especially useful for improving the separation of solutes tested, as they influenced the partitioning between the oil droplets and aqueous phase due to the exceptional electrical properties and large surface areas of MWNTs. Use of cationic surfactant-coated MWNTs (6.4 μg/mL) as the additive in a microemulsion buffer (0.5% octanol, 2.8% SDS, 5.8% isopropanol, and 5 mM borate buffer) yielded complete resolution of 13 analytes. The proposed method has been successfully applied for the detection and quantification of the studied compounds in a complex matrix sample (Compound Xueshuantong capsule).

Keywords:
Carbon nanotubes / Cationic surfactant-coated multi-walled carbon nanotubes / Compound Xueshuantong capsule / MEEKC / Multi-walled carbon nanotubes

1 Introduction

Ever since the pioneering work of Sumio Iijima in 1991, carbon nanotubes (CNTs) have received much attention from researchers in a number of fields owing to their unique properties such as high electrical conductivity, large surface area, and chemical stability [1, 2]. Their actual applications were, however, greatly limited because of the insolubility of CNTs in either water or organic solvents due to the inherent bundling of the tubes caused by strong intertube van der Waals interactions. Recognizing this problem, researchers have invested substantial efforts toward the development of strategic approaches for the dispersion and solubilization of CNTs based on both mechanical and chemical approaches [3]. Recently, it was demonstrated that the approaches with noncovalent bonding or physical adsorption have been used to exfoliate the bundles and stabilize individual tubes while maintaining the integrity and intrinsic properties of CNTs [4]. In particular, a wide variety of surfactants have been extensively investigated to date for physical dispersion of CNTs such as SDS, Triton X-100, dodecyltrimethylammonium chloride (DTAC), and N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (DAPS) [5, 6].

In recent years, CE became a powerful technique capable of rapidly separating a variety of compounds ranging from small ions to large biological molecules while requiring only very small sample volume and low running cost. Nowadays, various types of pseudostationary phases (PSPs), including micelles [7, 8], ionic liquids [9, 10], liposomes [11], microemulsions [12, 13], molecularly imprinted polymers [14, 15], nanoparticles [16, 17], and vesicles [18, 19] are used to tune selectivity and resolution of analytes with different solute properties according to their interaction with the electrophoretic system. Recently, the use of CNTs was an interesting alternative to conventional SDS micelles.
2 Materials and methods

2.1 Reagents and samples

DTAC, tetradecyl trimethyl ammonium chloride (TTAC), hexadecyl trimethyl ammonium chloride (CTAC), and SDS were purchased from Sigma-Aldrich Shanghai Trading Co. (Shanghai, China). All the other chemicals, including 1-octanol, isopropanol, propanol, 1-butanol, 1-pentanol, 1-hexanol, 2-butanol, heptane, cyclohexane, ethyl acetate, methylene chloride, and borate were analytical-reagent grade and possessed the capacity to adsorb organic compounds due to high surface area, and therefore it could greatly facilitate CE resolution [20]. Among these investigations, carboxylic single-walled CNTs (C-SWNTs) and carboxylic multi-walled CNTs (C-MWNTs) have been used as additives in running buffers to enhance the separation performance of the homologs of caffeine and theobromine, seven purine and pyrimidine bases [21, 22], respectively. On the other hand, the use of surfactant-coated CNTs in the BGE was a more effective choice for increasing the resolution and selectivity of chlorophenols, nonsteroidal anti-inflammatory drugs, triazines and nitroimidazole derivatives, lactams antibiotics, amphenicols, flavonoids, phenolic acids, saponins, and enantioseparation of ephedrines and clenbuterol compared to carboxylic ones, due to the fact that they often preserved the initial structure of the CNTs [23–28]. Specifically, SDS was, perhaps, the most widely used surfactant for dispersion of CNTs, sodium dodecylbenzenesulfonate, sodium cholate, DAPS, Brij-35, Triton X-100, and 1-dodecyl-3-methylimidazolium chloride ([C12 mim][Cl]) were among the most efficient surfactants [26, 29, 30]. Unfortunately, however, the use of CNTs dispersions using cationic surfactants or mixed surfactant has not been investigated in CE system.

In this work, functionalized MWNTs, with cationic surfactants or mixed cationic/anionic surfactants were used as microemulsion additives for the detection of glycosides, phenanthraquinone, and phenolic compounds. In addition, the results obtained from the new developed method were compared with existing MEEKC method in terms of separation efficiency. To our knowledge, this work was the first report of the use of cationic surfactant-coated MWNTs (CSMWNTs) in the MEEKC separation of a complex plant preparation (Compound Xueshuantong capsule).

2.2 Apparatus

All CE separations were performed in an Agilent 3D CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a photodiode array UV detector (wavelength range from 190 to 600 nm). Fused-silica capillaries were obtained from Yongnian Optical Fiber Factory (Hebei province, China) and were 50 cm in total length, 41.5 cm effective length, 50 μm id, and 365 μm od.

2.3 Electrophoretic conditions

Initially, a new capillary was sequentially conditioned by flushing 1 M NaOH, 0.1 M NaOH, and ultrapure water for 10 min, and finally with the running buffer for 10 min. Between consecutive injections, the capillary was rinsed with 0.1 M NaOH, water, and running buffer for 3 min each. Sample solutions were introduced into the capillary by applying a pressure of 50 mbar for 4 s. In all cases, the temperature of the capillary was set at 30° C and the applied voltage for the MEEKC separation was 28 kV. The detection wavelength was fixed at 254 nm for all the analytes.

Buffer solutions were composed of 0.5% 1-octanol (heptane, cyclohexane, ethyl acetate, or methylene chloride), 2.2–3.1% SDS, 5.2–6.1% isopropanol (propanol, 1-butanol, 1-pentanol, or 1-hexanol), 5 mM borate buffer (pH 9.0), and the addition of MWNTs suspensions was ultrasonicated for 20 min before usage. All the buffers were filtered through 0.22 μm filters before being introduced to the CE system.

A total of 1 mg MWNTs were dispersed in 0.5% w/v surfactant aqueous solution containing 10% v/v of 2-butanol by ultrasonication for 20 min to obtain uniform black dispersions.

2.4 Sample preparation

About 1.0 g Compound Xueshuantong capsule was ultrasonically extracted with 15 mL (70:30, v/v) methanol–water for 40 min. The sample solution was cooled to room temperature, vortexed for 3 min, and then centrifuged at 13 000 rpm for 5 min to remove particles.

3 Results and discussion

3.1 Effect of functionalized MWNTs on MEEKC separation

Initially, the separation of the studied compounds was evaluated using the electrolyte composed of 0.5% octanol, 2.8% Xueshuantong capsule were purchased from local pharmacy (Nanjing, China).
Figure 1. The chemical structures of solutes tested in the study.

SDS, 5.8% isopropanol, and 5 mM borate solution at pH 9.0 in the absence of CS-MWNTs. With the pure microemulsion system, only three compounds (analytes 1, 12, and 13) were eluted as the single component and the others were coeluted as shown in Fig. 2A. In accordance with $pK_a$ (around 3–7) of the tested compounds (Fig. 1), they were neutral or negatively charged under these conditions and their separations were achieved by the different partitioning behavior of solutes between the aqueous phase and moving oil droplets. Recently, researchers reported that the presence of dispersed nanostuctures had a dramatic effect on the electrophoretic behavior of CE system [4]. In the study, the amount of DTAC-coated MWNTs (DTAC-MWNTs) added was examined in MEEKC in order to achieve most favorable separation conditions without losing the resolution. At 1.6 $\mu$g/mL DTAC-MWNTs, the separation of all 13 analytes was not improved as expected and just when it was higher than 1.6 $\mu$g/mL it was possible to obtain a considerable improvement in the resolution for the studied compounds (Fig. 2). When both 3.2 and 4.8 $\mu$g/mL DTAC-MWNTs were employed in the MEEKC system, it can be seen from Fig. 2C and D that the resolution of analytes 3, 6, 7, and 8 increased apparently; however, with regard to analytes 4 and 5, 10 and 11, no baseline separation was observed during the process of this study. As the concentration of DTAC-MWNTs increased to 6.4 $\mu$g/mL, all compounds used were fully resolved with higher efficiency, and at 8.0 $\mu$g/mL DTAC-MWNTs, the resolution of analytes 9 and 10 tended to decrease (Fig. 2E and F). Additionally, it should be noted that the migration order of analyte 2 differed somewhat between two microemulsion systems as shown in Fig. 2. One possible explanation for this phenomenon may be that the change in the retention mechanisms governing the MEEKC separation and this process affected separation selectivity to a certain degree even when a small amount of CNTs was added to the buffer. As it can be seen from Fig. 2, the EOF slightly decreased from $4.45 \times 10^{-4}$ to $3.76 \times 10^{-4}$ cm$^2$V$^{-1}$s$^{-1}$ with increasing CS-MWNTs additives, and resulted in slightly longer retention times. This result could be explained since DTAC-MWNTs had positive charge partially as a result of the cationic nature of DTAC so the dispersant probably adsorbed to the capillary wall and changed the EOF. It was obvious that the presence of DTAC-MWNTs in running buffers significantly enhanced resolution when compared to common MEEKC. In the case, the additional interactions induced by the functional groups of MWNTs and the chemical nature of the analytes could alter selectivity in MEEKC separations in addition to the
Figure 2. Electrokinetic separation of 13 analytes by varying DTAC-MWNTs concentrations in MEEKC. (A) without DTAC-MWNTs; (B) 1.6 μg/mL DTAC-MWNTs; (C) 3.2 μg/mL DTAC-MWNTs; (D) 4.8 μg/mL DTAC-MWNTs; (E) 6.4 μg/mL DTAC-MWNTs; and (F) 8.0 μg/mL DTAC-MWNTs. BGE: 0.5% 1-octanol, 2.8% SDS, 5.8% isopropanol, 5 mM borate buffer and 0–8.0 μg/mL DTAC-MWNTs additives. Other CE conditions: analyte concentration: 0.06 mg/mL; capillary length: 50 cm total (41.5 cm effective length) × 50 μm id; temperature: 28°C; voltage: 30 kV, injection: 50 mbar, 4 s; detection: 205 nm; 13 analytes: (1) calycosin-7-O-β-D-glucoside, (2) calycosin, (3) formononetin, (4) kaempferol, (5) ononin, (6) rosmarinic acid, (7) Danshen su, (8) caffeic acid, (9) notoginsenoside R1, (10) salvianolic acid B, (11) protocatechuic acid, (12) ginsenoside Rb1, (13) tanshinone IIA.

3.2 Effect of composition of microemulsion solution on MEEKC separation

Previous studies have demonstrated that oil phase of microemulsion provided an enlarged hydrophobic surface area and had a striking affect on the selectivity and the resolution of MEEKC separation [31]. In our study, the five oils employed, including 1-octanol, heptane, cyclohexane, ethyl acetate, and methylene chloride, all at concentration of 0.5%, were tested for MEEKC separations when CS-MWNTs were added in microemulsion system. Figures 2E and 3 show the effects of the oil phase on the separations. It was obvious that among the five oils, 1-octanol allowed baseline resolution for all 13 compounds with proper migration times (Fig. 2E), whereas heptane gave relatively worse resolution and peak shapes especially for analyte 7 (Fig. 3A). By contrast, at the same MEEKC conditions, cyclohexane and methylene chloride gave coluted peaks of analytes 1 and 2, as well as partial separations of analytes 9 and 10 (Fig. 3B and D), whereas ethyl acetate showed no complete separation of analytes 9 and 10 (Fig. 3C). Additionally, we also found that the migration window became very wider and the analysis time increased from 14.5 to 23 min than other four oils when methylene chloride was added. It was also shown that the presence of 1-octanol in microemulsion solution increased the affinity between the microemulsion droplets with analytes, resulting in larger efficiency and resolution for target compounds. Therefore, we chose 1-octanol as the oil phase for the separations of the tested analytes.
It has been reported that the surfactant used in MEEKC could affect the stability of microemulsion system, lower the surface tension between the oil and aqueous phase, and change the separation selectivity [32]. The effect of SDS was studied over the range of 2.2–3.1% in 5 mM borate buffer containing 0.5% 1-octanol, 5.8% isopropanol, and 6.4 μg/mL DTAC-MWNTs. Figure 4 shows the profiles of SDS concentrations on the apparent mobility ($\mu_{\text{app}}$) of 13 analytes. As a result, it was found that the $\mu_{\text{app}}$ evidently decreased as SDS concentrations were increased from 2.2 to 3.1%. During the optimization of surfactant concentrations in MEEKC, it was observed that, as the SDS concentration was increased, the resolution of analytes 4 and 5 increased, whereas the resolution of analytes 9 and 10 decreased. Hence, 2.8% SDS was chosen as optimal.

Manipulation of cosurfactant was still an important strategy to change solutes migration and separation selectivity in MEEKC [33]. Accordingly, a series of lower alcohols was tested, including propanol, isopropanol, 1-butanol, 1-pentanol, and 1-hexanol. Among them, it was found that 1-pentanol and 1-hexanol were not suitable as cosurfactants since they resulted in turbid microemulsions and subsequent phase separation, most likely caused by insufficient emulsifying power. Using isopropanol as cosurfactant, all test solutes were resolved with excellent resolution (data not shown). In addition, the effect of isopropanol concentrations on $\mu_{\text{app}}$ of analytes was studied over the range of 5.2–6.1%. As shown in Fig. 5, the increase of isopropanol concentrations brought a decrease in the $\mu_{\text{app}}$ of analytes studied probably due to the changes of microemulsion buffer viscosity. On the other
hand, it was also observed that the analysis times increased by increasing cosurfactant concentrations without making significant changes in separation efficiency. Therefore, 5.8% isopropanol was demonstrated to be appropriate for the separations of target compounds.

### 3.3 Method validation and application

The developed MEEKC method was subsequently validated under the optimized separation conditions in terms of linearity, LODs, and precision. The linearity was studied between the concentration of the analyte as abscissa (x) and the corresponding peak area as ordinate (y). The results obtained showed excellent linear relationship was obtainable for the concentration range studied, each giving correlation coefficients ($R^2 > 0.9980$ ($n = 6$). The LODs of the method were in the range of 0.31–3.73 μg/mL for analytes 1–8, 10, 11, and 13 and 70.55–73.76 μg/mL for analytes 9 and 12, respectively. The LOQs under the chromatographic conditions were 0.90–11.35 μg/mL for analytes 1–8, 10, 11, and 13 and 210.22–222.19 μg/mL for analytes 9 and 12, respectively. The precision of the method was evaluated from five replicate injections on the same day and interday precision was determined for three consecutive days. The results showed that the intraday and interday variations (the RSD values of the migration times) were less than 1.95 and 3.88%, respectively. Stability of sample solution was analyzed at 0, 4, 8, 12, 16, and 24 h, within two days. The results of the stability test showed that the sample solution was stable over two days (RSDs < 3.78%).

In order to demonstrate the practical application of proposed MEEKC method, the determination of glycosides, phenanthraquinone, and phenolic compounds in real samples of Compound Xueshuantong capsule was conducted under the selected optimum conditions. Compound Xueshuantong capsule is refined from four crude herbs, i.e., *Panax notoginseng*, *Astragalus membranaceus*, *Salviae miltiorrhizae*, and *Radix scrophulariae*. It is often used to treat acute coronary heart disease and atherosclerosis in clinic. Figure 6A displays sample electropherogram obtained in the common microemulsion buffer with 0.5% 1-octanol, 2.8% SDS, 5.8% isopropanol, and 5 mM borate buffer. It was observed that analytes 1, 6, and 7 could not be resolved at all, and analytes 9, 10, 11, 12, and 13 were not perfectly separated, although provided shorter migration times due to the increased EOF velocity compared to the use of CS-MWNTs. With the functionalized MWNTs (6.4 μg/mL DTAC-MWNTs) in MEEKC, all ten analytes tested were completely resolved within 18 min (Fig. 6B). In addition, no significant matrix interference in complex sample was obtained with the developed MEEKC method. Therefore, the above results indicated the MWNTs-based MEEKC method provided better separation efficiency and selectivity for the studied bioactive compounds than the classical microemulsion system.

### 4 Concluding remarks

This work demonstrated that the presence of suspensions of MWNTs using single or mixed cationic surfactant solutions in MEEKC showed excellent separation performance for...
the detection of glycosides, phenanthraquinone, and phenolic compounds in comparison with a classical PSP. In addition, some factors, including CS-MWNTs concentration, cationic surfactant type and concentration, oil phase, and surfactant and cosurfactant of microemulsion BGE have been observed to influence the separation of solutes tested. Compared with other separation modes of CE, the use of CNTs in microemulsion system was more favorable for the separation of neutral as well as charged solutes, probably due to the novel separation mechanism between the microemulsion droplet and the aqueous phase. These results proved that the proposed method was a valuable tool for the rapid determination of bioactive constituents in complex herbal preparations.

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5 References