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A novel method of reversed migration capillary electrophoresis for determination of linear alkylbenzene sulfonates

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Abstract

A reversed migration capillary electrophoresis (RMCE) has been developed to determine linear alkylbenzene sulfonates (LAS). The sample stacking and separation conditions have been systematically investigated and optimized under reversed separation voltage at a low pH value. The separation effect of LAS homologs has been greatly improved based on the relative motion of electrophoresis and electroosmotic flow. RMCE demonstrates a good linear range of 0.1 mg/l to 10.0 mg/l, and the detection limit of LAS homologs reaches 0.001–0.004 mg/l. The relative standard deviations (*n*=6) of peak area and migration time were 2.25–4.40% and 0.67–0.75%, respectively. RMCE has also been applied for LAS detection in practical wastewater. The results show RMCE exhibits easy pretreatment, fast detection, high sensitivity, good peak shapes and resolution, and less solvent consumption, compared with the established high-performance liquid chromatography method.

Keywords: Reversed migration capillary electrophoresis, Field-amplified sample stacking, Reversed polarity, Linear alkylbenzene sulfonates

Introduction

Surfactants are a kind of extremely important fine chemical product that have been widely used in various fields of the national economic development, especially anionic surfactants. However, discharged surfactant-contained wastewater has induced serious environmental pollution (Chen et~al. 2006; Zhu et~al. 2016; Sakai et~al. 2016). At present, the most commonly used anionic surfactants in China are linear alkylbenzene sulfonates (LAS) which are composed of 4 linear alkylbenzene sulfonate sodium homologs (carbon chain lengths of 10, 11, 12, and 13, respectively, which are abbreviated as C_{10}–C_{13} in the following), and each homolog has 4 to 6 isomers due to the different attachment positions of benzene sulfonic acid

groups and carbon chains (Ding and Liu 2001). An example of a linear dodeylbenzenesulfonate (C-LAS) isomer is shown in Fig. 1. The decontamination ability of LAS is affected by the hydrophobic chain length.

The LAS without proper treatment will form a foam cover after entering the environmental water, which will consume dissolved oxygen and cause water quality deterioration. When the concentration of LAS is higher than 1 mg/l, it will have a drastic effect on the peroxidase of aquatic organisms and damage the physiologic function of the cells. In severe cases, it can cause organism mass deaths in waters (Ying 2006). The homolog distribution is important for characterizing detergency, biodegradation, and toxicity of LAS mixtures (Heinig et~al. 1999). It is imperative to develop convenient and appropriate analytical techniques for the separation and sensitive detection of these surfactants. The assay methods of LAS in water mainly include methylene blue spectrophotometric method, gas chromatography and mass spectrometry (GC/MS), and high-performance

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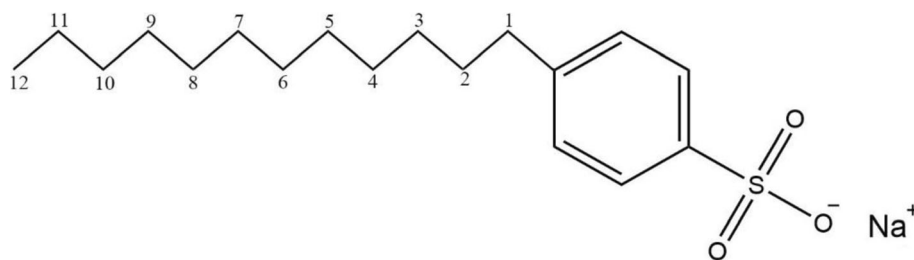Full list of author information is available at the end of the article

Fig. 1 Structure of a C-LAS isomer

liquid chromatography (HPLC). The methylene blue spectrophotometric method is complicated, time-consuming, enormous workload, and involved the high toxicity of organic reagents. HPLC has been extensively employed in the routine detection of LAS contained in different products with good sensitivity and selectivity, while the complicated pretreatment of the sample and high reagent dosage could not be ignored.

Capillary electrophoresis (CE) processes obvious advantage of high resolution, fast, easy operation, and strong anti-interference capability. Importantly, CE significantly reduces the workload of sample collection and transportation in monitoring sites, and the sample needed (<1 mL) is dramatically less than other methods. Though CE and HPLC are both liquid phase separation technologies, they follow different separation mechanisms and can be complemented by each other (Lin 1996). However, few literatures about the analysis of LAS by CE method in environmental monitoring have been reported.

Capillary zone electrophoresis (CZE) has been widely used (Malik et~al. 2016; Sovocool et~al. 2010; Ferey and Delaunay 2015) and reported to be feasible for the determination of LAS (Herrero-Martinez et~al. 2015; Villar et~al. 2009). In many cases, CZE lacks the detection sensitivity due to the limited injection volume of analytes within the column, so large-volume sample stacking (LVSS) and field-amplified sample stacking (FASS) were usually used to effectively increase the detection sensitivity (Ding and Liu 2001; Li and He 2005; Simpson et~al. 2008; Li et~al. 2016). Normal electrostacking usually applies to cations, and cationic surfactant is used to change the electroosmotic flow (EOF) direction when stacking anions (Huang et~al. 2004a; Huang et~al. 2004b). However, it does not work on LAS which easily react with cationic surfactants. A reported CE method for the LAS detection changed the EOF direction by polarity conversion after the sample stacking (Ding and Liu 2001). Since EOF moves in the opposite direction to the anion with faster velocity than the mobility of LAS, only LVSS could be used.

The schematic in Fig. 2 shows the steps of the FASS technique under reversed polarity conditions (outlet positive, therefore reversed direction of EOF). Only requiring the electrokinetic injection of the sample after the introduction of a short plug of a high-resistivity solvent such as water, the anionic analyte could be concentrated as the electrophoretic velocity of the analyte anion is faster in opposite direction than the velocity of EOF because of the higher field strength in the sample solution zone. The principle of this method is based on the fact that ion electrophoretic migration dramatically slows down at the boundary of a low-conductivity solution into a high-conductivity solution (Li 1992). Thereafter, the separation voltage is applied without polarity conversion under low EOF and the electrophoretic migration of the analyte is the opposite migration direction of EOF. In this case, the migration time of the focused anionic analyte zone to the detector becomes longer but gets better stacking and separation. Usually, acidic conditions are employed for this purpose (Simpson et~al. 2008).

In this study, we focused on the separation and detection of four LAS isomers by reversed migration capillary electrophoresis (RMCE) with the FASS technique under reversed polarity conditions. The technique is quite simple and stable without polarity conversion, as well as more anionic analytes injected electrokinetically. A separate buffering system with acidic pH (lower than 4) was examined to ensure that the analytes could be successfully stacked at the column. The buffering system and separation conditions were systematically optimized. RMCE has also been employed for the analysis of practical domestic sewage under the optimal conditions, and the analytical results were evaluated by the reported HPLC.

Experimental
Chemicals and reagents
The Sinopharm Chemical Reagent Co., Ltd. provided main chemicals and reagents, just as analytical reagent linear alkylbenzene sulfonates, sodium dihydrogen phosphate, sodium hydroxide, and acetonitrile. Guarantee reagent phosphoric acid and hydrochloric acid were also provided by the Sinopharm Group. HPLC grade methanol was purchased from Burdick & Jackson (Morris
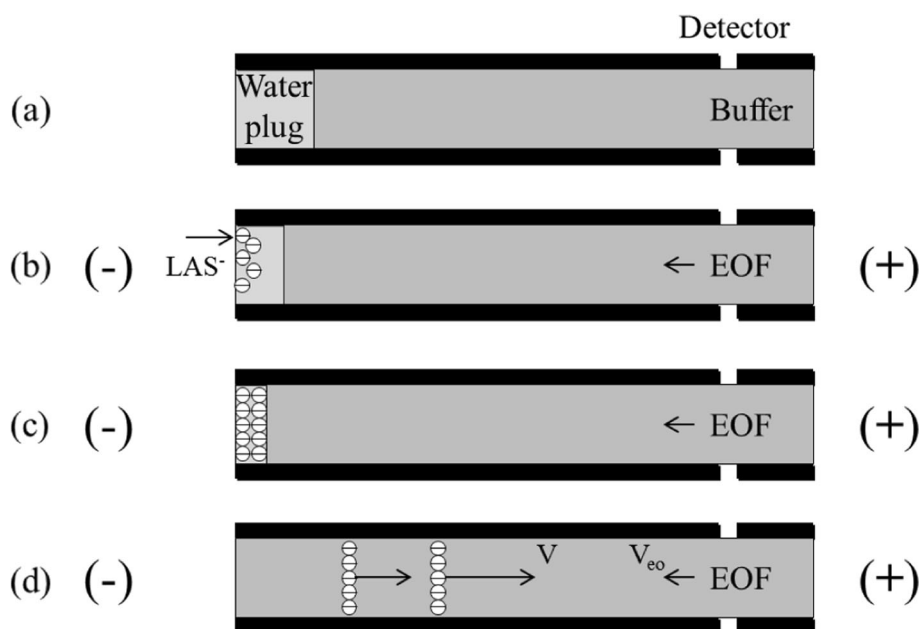
Fig. 2 The schematic of RMCE with FASS under reversed polarity conditions. **a** A small water plug was injected, **b** electrokinetic injection of a sample under reversed polarity with low voltage, **c** anionic analytes were focused on passing through the concentration boundary, and **d** the separation voltage was still applied with reversed polarity for the analytes' separation and detection

County, American). Stock standard solution (200 mg/l) of LAS mixture was obtained by dissolving LAS mixture with ultrapure water. Working and standard solutions were prepared by diluting the stock standard solution with ultrapure water and methanol solution to appropriate concentrations. The C_{10}–C_{13} distribution of LAS homologs given by the manufacturer are 21%, 31%, 29%, and 19%, respectively. Phosphate background electrolyte (BGE) with specific pH (between 2 and 4) was prepared with different proportions of sodium dihydrogen phosphate (0.1 M NaH_2PO_4) and phosphoric acid (0.1 M H_3PO_4) in ultrapure water. Then, a certain volume fraction of acetonitrile (ACN) was added to BGE. The domestic sewage was sampled for method validation. To prevent capillary blockage, all solutions and samples were filtered through a 0.45-μm membrane filter prior to use.

Equipment
Separations were performed on a P/ACE MDQ system (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV-Vis detector. Untreated fused silica capillaries of 60-cm in length (50 cm to the detection window) and 50 μm I.D. were used. The UV detector was operated at 200 nm. All electrophoresis runs at a temperature of 25°C and a humidity below 56%.

Electrophoresis and stacking procedures
The newly installed capillary should be conditioned with methanol for 30 min, followed by 30 min with 1 M HCl,

30 min with 1/3 M H_3PO_4, and 30 min with separation buffer. It was rinsed with ultrapure water for 10 min between each conditioning step. Between runs, the capillary was rinsed with 1/3 M H_3PO_4, ultrapure water, and electrolyte solution for 3 min at 20 p.s.i.

Procedures for FASS have been described elsewhere (Li 1992) and were used here with minor modifications. Briefly, the capillary was filled with BGE for 2 min at 20 p.s.i, and then, a small water plug was hydrodynamically injected into the capillary over an injection time of 5 s at 5 p.s.i. After that, an electrokinetic injection was carried out by applying a reversed polarity at 10 kV for 30 s to introduce and stack the samples. When the sample injection procedure finished, the inlet of the capillary was inserted into BGE till the separation finished. The detection steps were done automatically and controlled by Beckman P/ACE MDQ 32 Karat software.

High-performance liquid chromatography analysis
The procedure used for HPLC analysis was carried out similar to Cao et~al. (2011). Detection was performed on a Waters Alliance e2695 high-performance liquid chromatograph system connected to Waters 2475 UV-Vis detector operating at 224 nm. A Waters Xbridge C_{18} column (25×0.46 cm I.D., 0.5 μm packing) with a guard column was used at 30°C with an eluent flow-rate of 1 ml/min and an injection volume of 20 μl. The mobile-phase solvents were methanol (eluent A)-water containing 0.5 mM NaAc (eluent B) (volume ratio=77:23), and

the flow rate was 0.5 ml min^{-1}. For quantitation, LAS mixtures (AR) as standard solutions were prepared by dissolving the mixtures (AR) in ultrapure water to construct an external calibration curve covering the range 0.5–5.0 mg/l.

Results and discussion

Optimization of separation conditions

BGE concentration and pH

BGE pH is an important factor in CE separation because it affects both the charge of the analyte and the strength of EOF (Ding and Liu 2001). The curve in Fig. 3 shows the relation between EOF and pH, indicating that EOF increases with pH in the range of 4 to 8, and almost keeps unchanged after pH 8.

To decrease EOF, only lower pH of less than 4 could be considered. The separation results of four LAS homologs at different BGE pH were displayed in Fig. 4, and it showed obvious changes in migration time and peak area. As shown in Fig. 5, the peak area decreased and the migration time increased as the BGE pH increased from 2.4 to 3.6. A BGE pH of 2.4 was the optimal result.

BGE concentration is another important effect on the stacking and separation of LAS homologs. Figure 6 displays the electropherograms of LAS mixtures measured in the phosphate BGE with different concentrations. A low concentration did not work for the separation, and the optimal separation effect was observed in 20 mM phosphate BGE, while the worse resolution and high background were observed when the phosphate concentration further increased to 30 mM.

Acetonitrile content

As can be seen from Section 3.1.1, the lower the pH of BGE is, the larger the peak area is, but the resolution is relatively poor. It is reported that organic modifiers to the BGE in CE can affect both the EOF and electrophoretic mobility (Heinig and Vogt 1998). By several organic reagents compared in this study, it was found that an appropriate amount of acetonitrile significantly improved the peak resolution and had little effect on the peak area. Figure 7 shows the effect of different contents of ACN in BGE. It is shown in Fig. 8 that the migration time decreased and better peak separation could be obtained as ACN content increased. The addition of ACN could decrease the absorption of analytes on the capillary wall to make a better resolution. The decrease in reversed EOF is mainly due to an increase in viscosity (Li 1992), which makes analytes move more quickly to the detection window. Nevertheless, the buffer system of BGE became unstable and the stacking effect reduced when ACN content was greater than 50%. An ACN content of 50% was the optimal result.

Injection time of water plug

A short water plug injected before sample injection improved sample enrichment obviously and ensured good reproducibility of the detection results (Liu and Wang 2011). Figure 9 shows the separation effects of different injection times of water plugs. As shown in Fig. 10, a better peak resolution and a greater peak area were obtained when the water plug injection
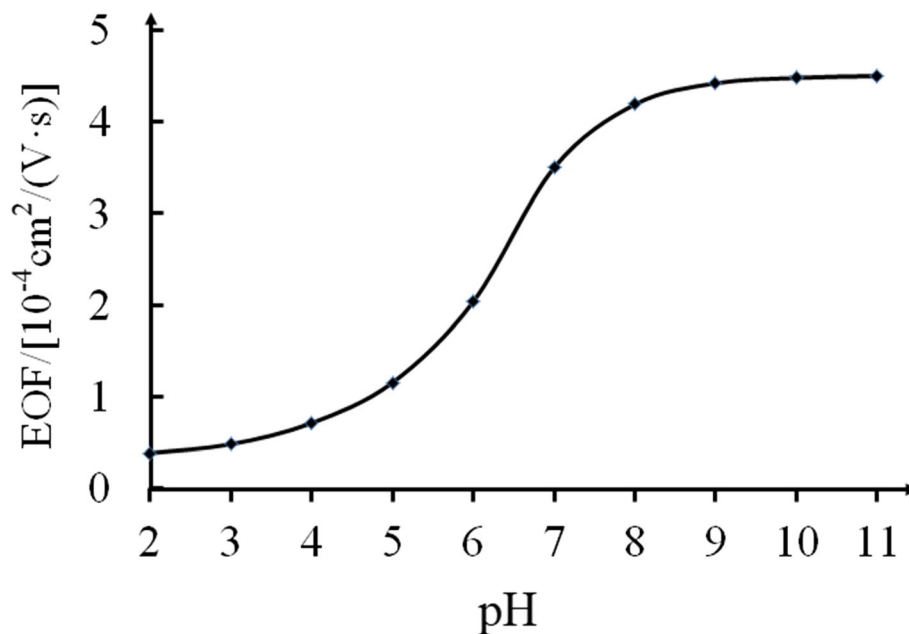

Fig. 3 Relation curve between EOF and pH in capillary untreated fused-silica capillaries (Perrett 2002)
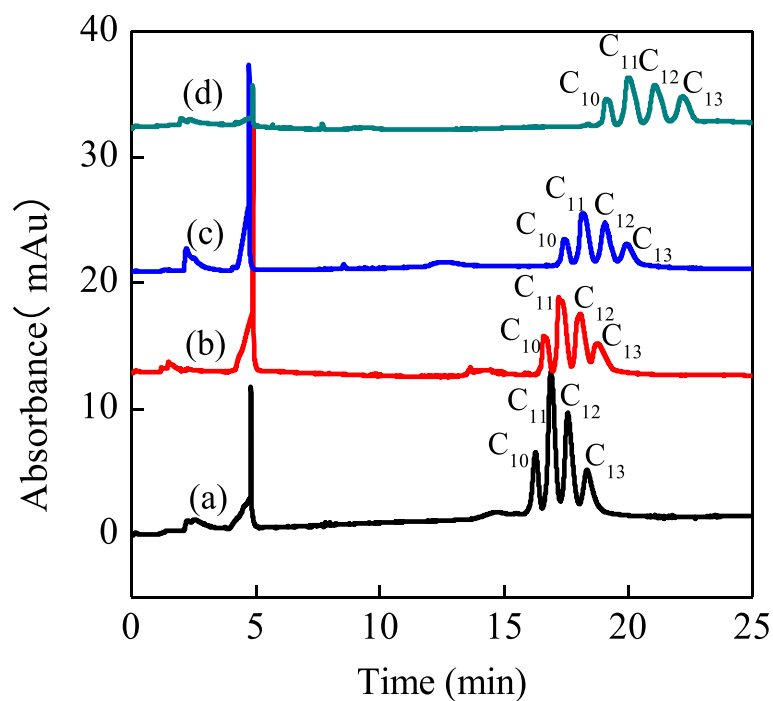
Fig. 4 The electropherograms of LAS in 20 mM phosphate BGE with different pH **a** 2.4, **b** 2.8, **c** 3.2, and **d** 3.6. LAS (1ppm) in 50% methanol aqueous solution; BGE: 20mM phosphate-30% acetonitrile; voltage: 20kV; temperature: 25℃; detection wavelength: 200nm

time was 10 s, which broaden the effective distance of the separation zone. Because too long, the water plug would share much voltage gradient in the stacking stage, which led to a reduction of sample injection amount, and a water plug injection time of 10 s was chosen.

Different organic solvents and content in the sample

Adding organic solvent to the sample can prevent analytes from being absorbed on the capillary wall and improve detection efficiency. We studied the effect of methanol, ethanol, and acetonitrile under the optimal conditions obtained above. Methanol was
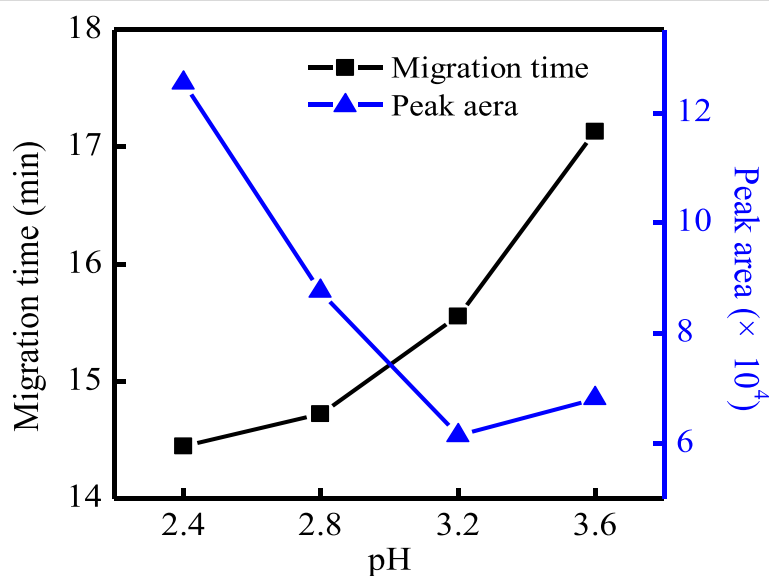
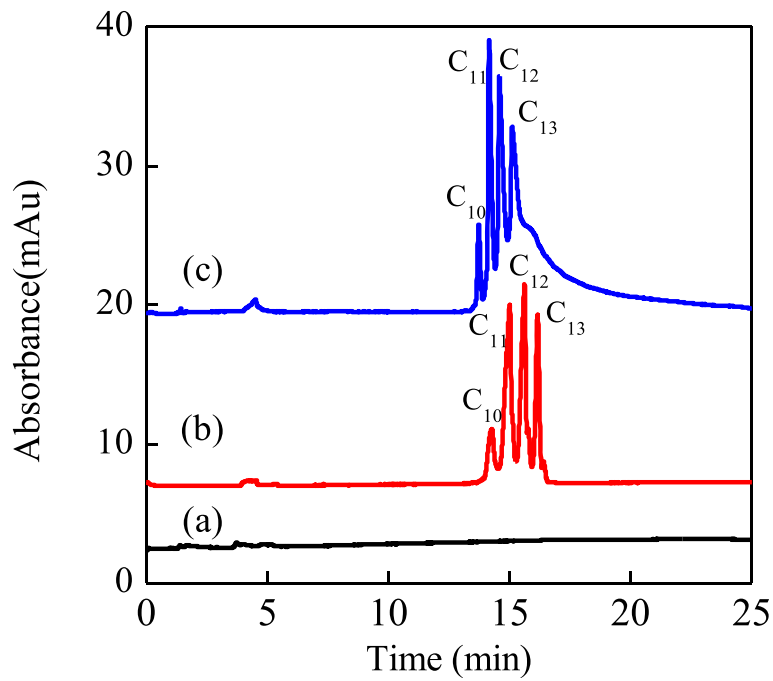**Fig. 5** Changes in migration time and peak area with pH of BGE

Fig. 6 The electropherograms of LAS in buffer with different phosphate concentrations **a** 10mM, **b** 20mM, and **c** 30mM. LAS (1ppm) in 50% methanol aqueous solution; BGE: phosphate-30% acetonitrile (pH 2.4); for other conditions, see Fig. 4
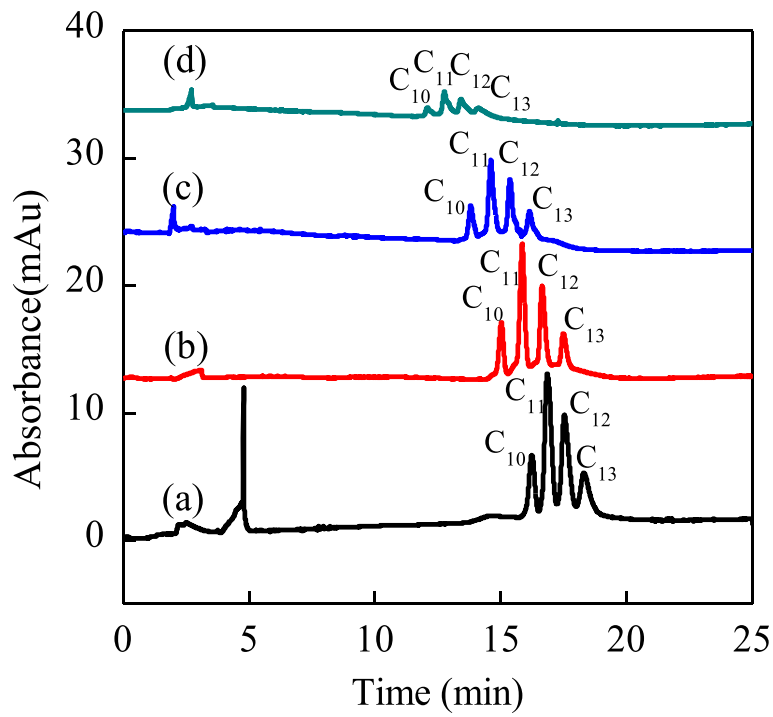

Fig. 7 The electropherograms of LAS mixtures in BGE containing different ACN contents **a** 30%, **b** 50%, **c** 70%, and **d** 100%. LAS (1ppm) in 50% methanol aqueous solution; BGE: 20mM phosphate (pH 2.4); for other conditions, see Fig. 4
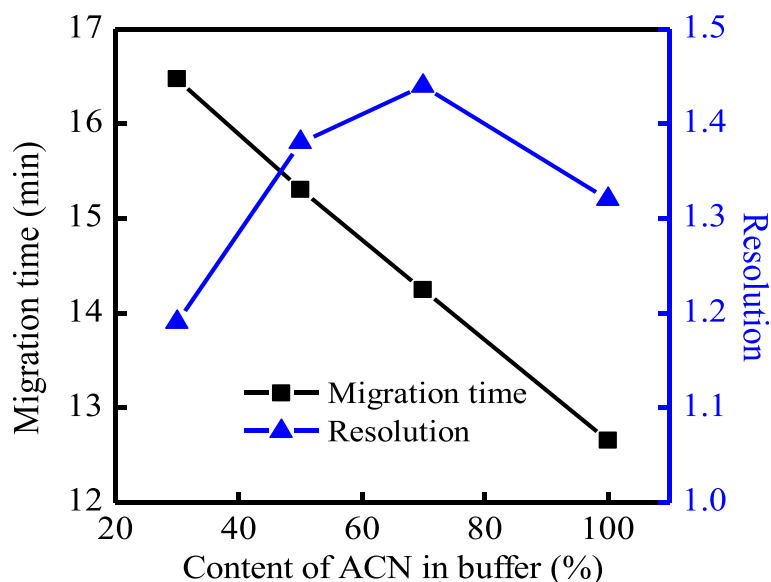
Fig. 8 Changes in migration time and resolution with different content of ACN in BGE

proved to be the best one. Then, the separation effects of sample solutions with different volume fractions of methanol were studied. Results in Fig. 11 showed that the peak area of LAS increased quickly while the resolution reduced as the content of methanol in the sample increased. The reason was

that the absorption of analytes decreased. However, when the content of methanol exceeded 50%, the system became unstable and the resolution decreased to less than 1.1 with a peak area of 1.75×10^5. The optimal result of methanol content in the sample solution was 50%.
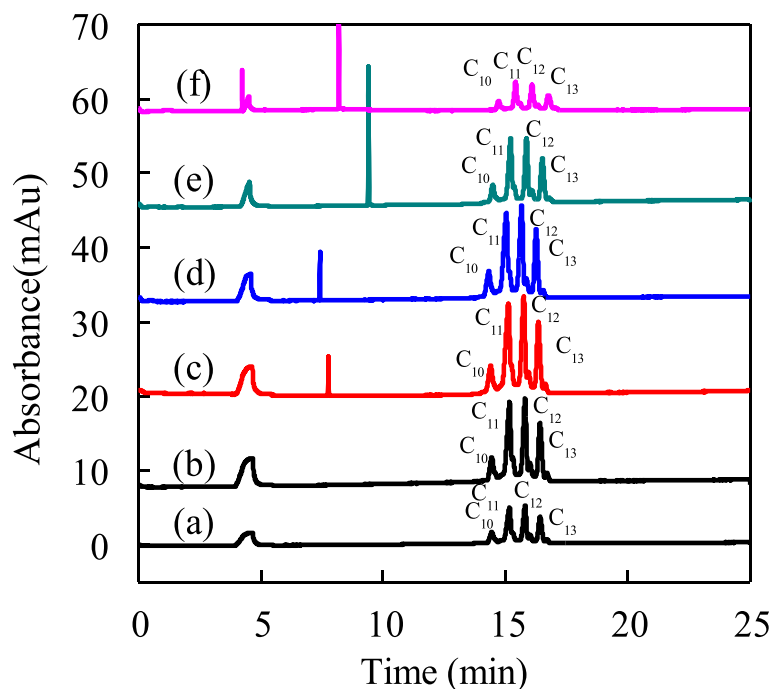

Fig. 9 Comparison of the electropherograms of LAS mixtures by injection time of water plug **a** 0s, **b** 5s, **c** 10s, **d** 20s, **e** 40s, and **f** 80s. LAS (1ppm) in 50% methanol aqueous solution; BGE: 20mM phosphate (pH 2.4)-50% acetonitrile; for other conditions, see Fig. 4
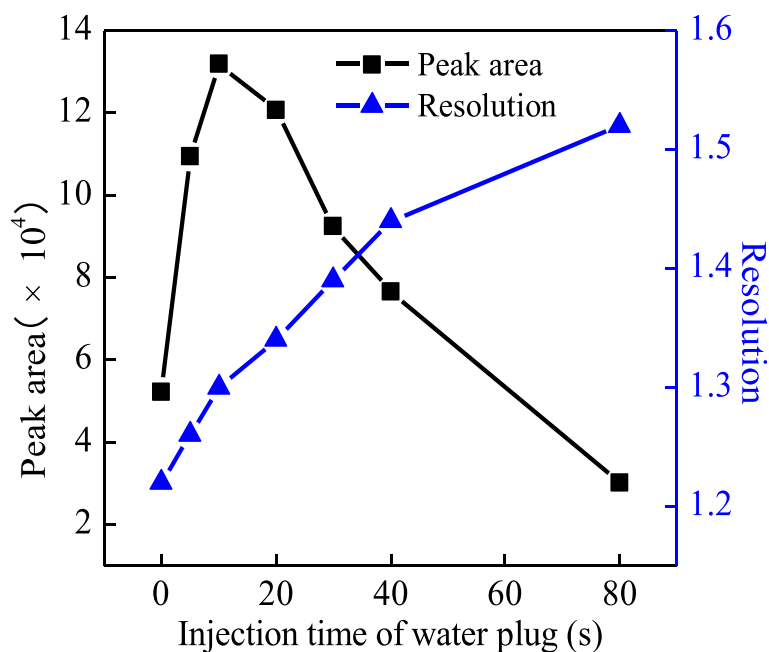
Fig. 10 Changes in peak area and resolution with a different injection time of water plug

Separation voltage

Separation voltage had a significant influence on migration time and peak resolution. To get a better peak shape and shorter migration time, separation voltage was varied in the range of 15–25 kV. As shown in Fig. 12, peak area decreased as the applied voltage increased, which might be caused by the fact that larger voltage strengthened the EOF which led to a reduction of sample migrated to detection window. Electrophoretic velocity increased with the increase of separation voltage, which led to a shorter migration time but worse peak resolution. Therefore, the separation voltage of 15 kV was chosen under a comprehensive consideration.

In conclusion, the optimal separation condition of LAS homologs was 20 mM phosphate buffer with 50% acetonitrile at pH 2.4 and a separation voltage of 15 kV.
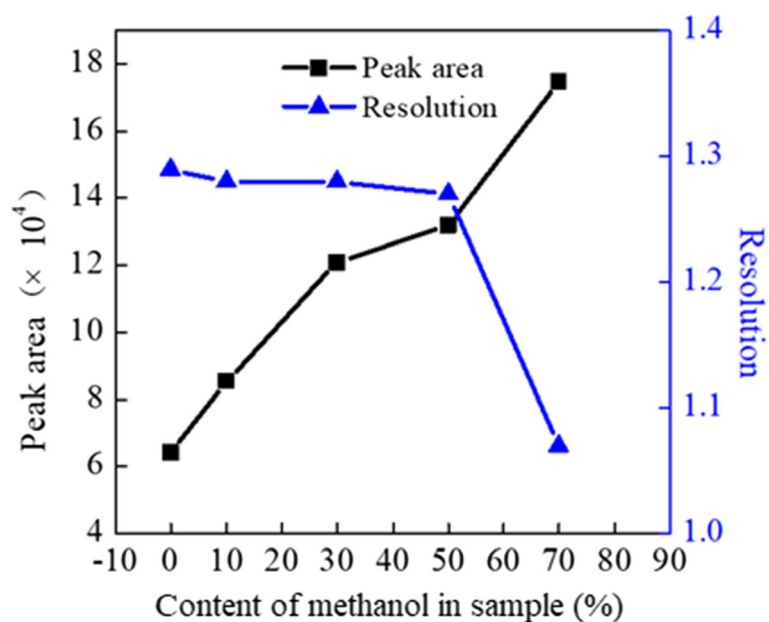

Fig. 11 Changes in peak area and resolution with different content of methanol in sample
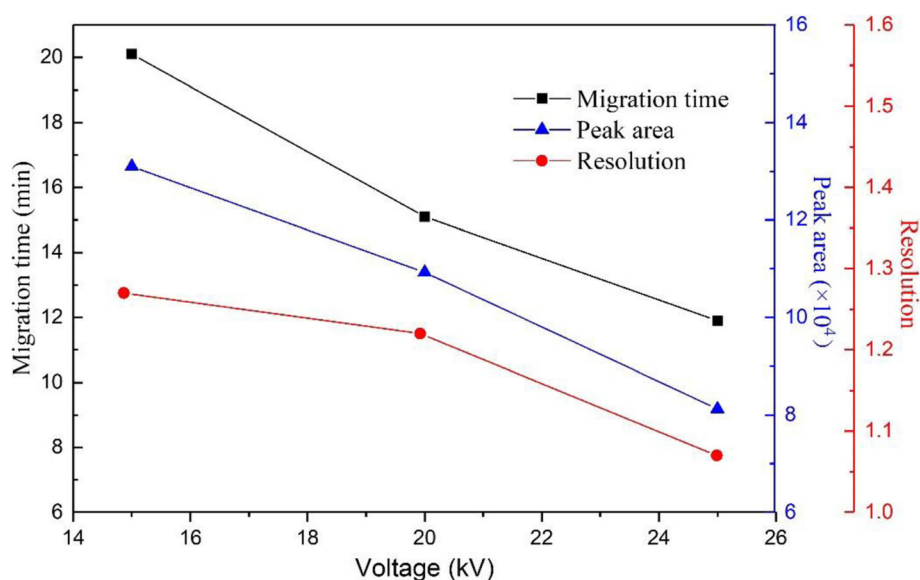
Fig. 12 The variation of peak area, resolution, and migration time with separation voltage

The most effective stacking condition was to solve the samples in solutions with 50% methanol and hydrodynamically inject a water plug into the capillary before sample stacking for 10 s at 0.5 p.s.i. Samples should be electrokinetically injected into the capillary injection over 30 s at a reversed voltage of 10 kV.

Evaluation of the method

A validation process was carried out to confirm the suitability of the method for the intended use. The linearity with standard solution mixtures, precision (in terms of relative standard deviations (RSD), determined by analyzing a LAS sample with a concentration of 1 mg/l for six times), and limit of detection (LOD, S/N=3) under the optimal conditions described above were investigated (see Table 1).

A series of standard solutions of different concentrations containing four LAS homologs were separated and detected under the optimal conditions. The external standard method was used for the quantification of different components by peak area. The linear dynamic

Table 1 Linear dynamic ranges, regression coefficients (r^2), LOD, and RSD of the method

LAS	Linear dynamic ranges (mg/l)	Regression coefficient (r^2)	LOD (mg/l)	RSD (%)	
				Peak area	Migration time
C_{10}	0.1–2.0	0.990	0.004	2.07	0.67
C_{11}	0.1–3.0	0.996	0.002	4.40	0.69
C_{12}	0.1–3.0	0.999	0.002	2.68	0.72
C_{13}	0.1–2.0	0.995	0.001	4.28	0.75
C_{10-13}	0.1–10	0.997	0.002	2.25	/

ranges are shown in Table 1, and the regression coefficients were between 0.999 and 0.990. The LODs of LAS homologs reach 0.001–0.004 mg/l. The linear calibration range of the total LAS mixture could reach 0.1–10 mg/l with the LOD of 0.002 mg/l. The variation range of peak area was 2.25–4.40%, and it was 0.67–0.75% for migration time. Over 100-fold enrichment was achieved with the comparison of CZE.

Applications

A sample from domestic sewage was analyzed by this RMCE method, and a HPLC method established by Cao et~al. (2011) was also applied to determine the total LAS. The wastewater was filtered through a 0.45-μm membrane filter prior to be analyzed. The results obtained are in good agreement. Table 2 compares the quantitative results obtained from the two methods. The electropherogram of RMCE and chromatogram of HPLC for the sample were shown in Fig. 13. The C_{10}-LAS and solvent peak were not separated by the HPLC method and were not shown in the chromatogram. The response of every component in LAS mixtures detected by RMCE was much better than HPLC.

Table 2 Quantification of LAS in sewage by RMCE and HPLC

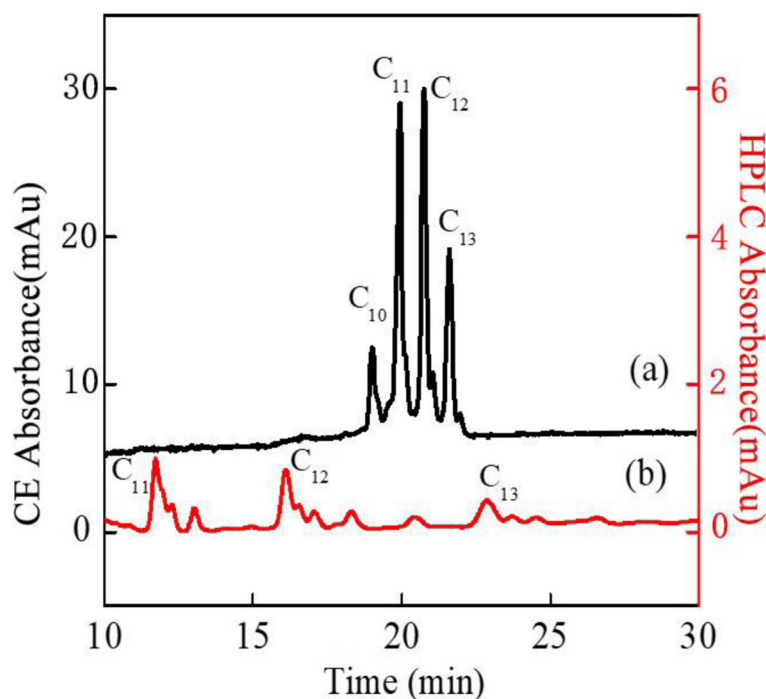| Sample	Results of RMCE (mg/l)					Results of HPLC (mg/l)				
	C10	C11	C12	C13	Total	C10	C11	C12	C13	Total
1	0.41	0.61	0.57	0.37	1.97	0	0.69	0.66	0.45	1.80
2	0.43	0.63	0.60	0.38	2.04	0	0.71	0.67	0.47	1.85
3	0.41	0.65	0.60	0.40	2.07	0	0.71	0.69	0.48	1.88
RSD (%)	2.77	3.17	2.94	3.98	2.53		1.64	2.27	3.27	2.19

Fig. 13 Electropherogram (**a**) and HPLC chromatogram (**b**) of the LAS in sewage

Conclusion

In this work, we developed a novel quick analytical method of LAS in waters. BGE component, injection time of water plug, and separation voltage are considered and have been optimized for the separation of LAS homologs. The sensitivity of the RMCE method with the FASS technique and reversed separation voltage has been significantly improved. This new method leads to a better peak shape, a higher resolution, and a good sensitivity compared with the HPLC method reported in the literature by UV detector, which linear calibration is in the range from 0.1 to 3.0 mg/l with the LOD of 0.001–0.004 mg/l for the LAS homologs. It is worth emphasizing that the RMCE method just needs less solvent and almost no sample pretreatment, which demonstrates obvious advantages and a promising application to meet requirements for trace pollutant monitoring.

The great potential of the CE method encourages us to develop the sample pretreatment or in-column preconcentration technology for further improvement of its analytical sensitivity, which will greatly promote the wide application of the method in water environment monitoring.

Abbreviations
RMCE: Reversed migration capillary electrophoresis; LAS: Linear alkylbenzene sulfonates; RSD: Relative standard deviation; HPLC: High-performance liquid chromatography; GC/MS: Gas chromatography and mass spectrometry; CE: Capillary electrophoresis; CZE: Capillary zone electrophoresis; LVSS: Large-volume sample stacking; FASS: Field-amplified sample stacking; EOF: Electroosmotic flow; BGE: Background electrolyte; ACN: Acetonitrile; LOD: Limit of detection; UV: Ultraviolet

Authors' contributions
All authors contributed to the study conception and design. The idea and experiment scheme of this study were proposed by Zhuo Huang. Material preparation, data collection, and analysis were performed by Weike Wang and Linxian Xie. The first draft of the manuscript was written by Weike Wang and revised by Zhuo Huang. Li Lin gave some experiment advice. All authors commented on previous versions of the manuscript. The authors read and approved the final manuscript.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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