

A Convenient Trimethylchlorosilane Rinse-Coating Procedure for Rapid Microemulsion Capillary Electrokinetic Chromatography with Photo Diode Array Detection of Melamine and Sodium Benzoate

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A convenient trimethylchlorosilane rinse-coating procedure was studied for microemulsion capillary electrokinetic chromatography with photo diode array detection for the first time and a new method based the coating was developed for rapid simultaneous determination of melamine and sodium benzoate. In the tested ranges, good linear relationships between peak heights and concentrations were observed (correlation coefficients for melamine and sodium benzoate were 0.9995 and 0.9959, respectively) and the relative standard deviations ($n = 5$) of migration time were 0.68 % for melamine and 0.71 % for sodium benzoate, respectively. The detection limits for melamine and sodium benzoate were 0.06 and 0.02 mg/L, respectively. All peaks appeared less than 2 min under optimized conditions.

Key Words: Trimethylchlorosilane, Double coating, Rinse-coating procedure, Microemulsion capillary electrokinetic chromatography, PDA, Melamine, Sodium benzoate.

INTRODUCTION

High attention has been paid to illegal food addition of chemicals such as melamine^{1,2} and excessive sodium benzoate³ after SANLU baby-formula scandal. Microemulsion capillary electrokinetic chromatography (MEEKC) is a high performance capillary electrophoresis (HPCE) mode using microemulsion as buffer, where the analytes separate according to the difference of partition coefficients between the oil and water phase. Microemulsion capillary electrokinetic chromatography has been applied by researcher more than a decade for its wide application, high separate efficiency, stability and reproducibility⁴. But to our best of knowledge, there has no analysis methods (including no MEEKC method) reported for simultaneous determination of melamine and sodium benzoate.

Covalently bonded/cross-linked polymer is one of the most ordinarily used wall coating material for modifying the charge on the capillary surface⁵. But it does have some disadvantages such as high price, complicated coating procedure and long coating time. So it is not easy for general researchers to use. Furthermore, it may be not suitable for UV detection, especially for photo diode array (PDA) detection using continuous UV scanning because of its coating frangibility and its irreproducibility under UV photo. Trimethylchlorosilane (TMCS) with simple molecular structure is a commonly used protective reagent for hydroxyl groups in organic synthesis⁶.

Under a mild condition, trimethylchlorosilane reacts with hydroxyl to form trimethylsilyl protective group. The trimethylsilyl group can be removed under another mild condition as necessary. It was just based on this principle that we tried a new double coating (DC) derived by trimethylchlorosilane. This coating can be regenerated on line with a simple rinse-coating procedure and suitable for microemulsion capillary electrokinetic chromatography analysis of melamine and sodium benzoate with photo diode array detection.

EXPERIMENTAL

Experiments were performed on a P/ACETM MDQ capillary electrophoresis system from Beckman Coulter (Fullerton, CA, USA) equipped with a photo diode array detector using a 32 Karat software (Version 8.0 Beckman). Applied detection wavelength was 214 nm.

Chemicals are of analytical-reagent grade (AR) or chemical-pure grade (CP), including melamine (AR, Tianjin Basf Chemical Co. Ltd., Tianjin, China), sodium benzoate (CP, Nanxin Reagent Factory, Yixing, China), sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$, CP, Kaifeng No.4 Chemical Plant, Kaifeng, China), Sodium dodecyl sulfate (SDS, CP, Zhanyun Chemical Co. Ltd., Shanghai, China) and trimethylchlorosilane (CP, Zhanyun Chemical Co. Ltd., Shanghai, China). All other chemicals were also made in China and of analytical reagent grade. Distilled water was used throughout. Trimethylchlorosilane is diluted

4.5 times with acetonitrile to form trimethylchlorosilane solution for future use.

The stock microemulsion was prepared by mixing heptane (3.24 %, w/w), sodium dodecyl sulfate (13.24 %, w/w), butanol (26.44 %, w/w) and distilled water (57.08 %, w/w). The run buffers were prepared by mixing the stock microemulsion and 50 mM $\text{Na}_2\text{B}_4\text{O}_7$ solution. The stock 1 mg/mL melamine solution is prepared in 100 mmol/L acetic acid. The stock 2 mg/mL sodium benzoate solution is prepared in distilled water.

Lab water waste was collected in a polypropylene plastic tube. After being shaken forcibly, blended and 20 min of 4000 rpm centrifuged and filtered through a 0.45 μm membrane filter, supernatant fluid was selected for MEEKC analysis.

The separation was carried out on a 31.2 cm (21 cm to the detector) \times 75 μm id fused-silica capillary from Beckman Coulter (Fullerton, CA, USA). The capillary was treated prior to its first use by flushing with 1.0 M HCl for 20 min, 0.5 M NaOH for 20 min, distilled water for 10 min, acetonitrile for 10 min and trimethylchlorosilane acetonitrile solution for 10 min. Between MEEKC (no DC) runs, a rinse-cycle, 0.5 M NaOH for 0.5 min, distilled water for 0.5 min and run buffer for 1 min was used. In MEEKC-DC separation, a coating procedure was described subsequently. After optimized, the run buffer included 30 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$ and 18 % (v/v) stock microemulsion. The pH of run buffer was 9.3. The applied voltage was 14 kV. The capillary was maintained at 25 $^\circ\text{C}$. Sample was injected by applying a pressure of 0.5 psi (1 psi = 6894.76 Pa) for 5 s.

New capillaries were treated prior to their first coating by flushing with 1.0 M HCl for 20 min, 0.5 M NaOH for 20 min, distilled water for 15 min, acetonitrile for 5 min, TMCS solution for 10 min, respectively. At the beginning of each day, the capillary was rinsed with 0.5 M NaOH for 2 min, distilled water for 2 min, acetonitrile for 3 min, trimethylchlorosilane solution for 3 min, respectively. At the end of each day, the capillary was rinsed with 0.5 M NaOH for 1 min, distilled water for 2 min, acetonitrile for 2 min, respectively. Capillaries were stored dry with the ends kept in acetonitrile. Between two runs, a rinse-coating-cycle, 0.5 M NaOH for 0.5 min, distilled water for 0.5 min, acetonitrile for 0.5 min, another acetonitrile for 0.5 min, TMCS solution for 1 min and run buffer for 1 min was used after optimized.

RESULTS AND DISCUSSION

Effect of trimethylchlorosilane rinse-coating procedure was studied. In ordinary MEEKC (no DC) separation [Fig. 1(A)], good separation resulted in long analysis time. When the trimethylchlorosilane rinse-coating procedure was used, the separation time was shortened [Fig. 1(B)]. Furthermore, because trimethylsilyl is more hydrophobic than silanol, the adsorption of sodium dodecyl sulfate to the surface increased and this produced another dynamic coating and resulted in an increase of zeta potential and then the increasing of electroosmotic flow (EOF). The addition of the total combinative DC procedure resulted in no change of migration order. To obtain reproducible separation, microemulsion concentration and sodium dodecyl sulfate coating time in the rinse-coating cycle was investigated.

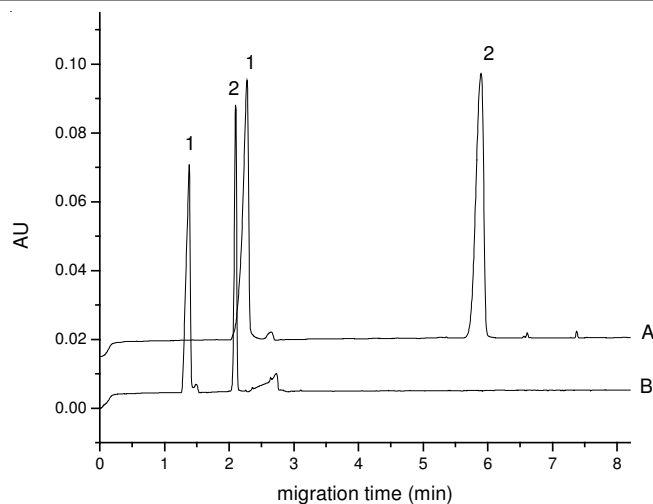


Fig. 1. Electropherogram of (A) ordinary MEEKC (no DC); and (B) MEEKC (using DC). Peak identification: 1, melamine 0.330 mg/mL; 2, sodium benzoate 0.130 mg/mL. HPCE conditions: 30 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$, 12 % microemulsion (v/v), separation voltage 12 kV, length of capillary 31.2 cm (21 cm to the detector) \times 75 μm i.d., sample injected at 0.5 psi for 5 s

The effect of the microemulsion concentration (expressed as volume percentage of the stock microemulsion added to the run buffer) was investigated in the range of 9-21 % while borax buffer solution was maintained 30 mmol/L. It was observed that the percentage was crucial for improving separation. When the ratio was less than or equal to 15 %, melamine peak was interfered by followed impurity peak. When the ratio was 21 %, there was big current led to large Joule heat and then low column efficiency. The final choice of microemulsion percentage was 18 %.

The effect of applied voltage on the migration time of the analytes in this MEEKC method was also investigated in the range 10-16 kV. The electropherograms showed that, with the increase in applied voltage, the migration time became shorter. However, the resolution between the peak of melamine and its followed impurity peak reduced. In this paper, 14 kV was applied for the final separation of the two analytes.

Trimethylchlorosilane reacts with hydroxyl of capillary inner wall to generate the first coated layer and then sodium dodecyl sulfate adsorbs hydrophobically trimethylsilyl to form the second layer coating. The coating time of second layer coating (sodium dodecyl sulfate coating time, namely the rinse duration of separation buffer) was optimized. Duration of 0.5, 1.0, 2 and 4 min was selected to investigate. It was found that electropherogram baseline was instable when 0.5 min, probably because the coating was not enough, making the second coating layer unstable. The electropherogram has high repeatability when 1, 2 or 4 min. In order to save the total analysis time, 1 min was selected. Finally, a rinse-coating cycle, 0.5 mol/L NaOH for 0.5 min, distilled water for 0.5 min, acetonitrile for 0.5 min, another acetonitrile for 0.5 min, trimethylchlorosilane solution for 1 min and run buffer for 1 min was used between two runs.

For evaluation of the quantitative applicability of the method, five standard solutions of melamine in the range of 330.0-10.0 mg/L and sodium benzoate in the range of 130.0-4.0 mg/L were analyzed under optimized separation conditions.

TABLE-1
REGRESSION DATA AND DETECT LIMITS OF THE ANALYTES (n = 5)

Analyte	Regression equation*	Correlation coefficient	RSD (%)		Detection limit (mg/L)
			Time	Height	
Melamine	$Y = 0.178x + 0.01065$	0.9995	0.68	1.10	0.06
Sodium benzoate	$Y = 0.552x + 0.0097$	0.9959	0.71	0.63	0.02

*Y was peak height (Au); x was analyte concentration (mg/mL).

The linearity between the peak height and the concentration was investigated and the linear regression equations are illustrated in Table-1.

The reproducibility of the method was studied by measuring the migration times and peak heights of melamine and sodium benzoate at the top concentrations in the linear ranges. The relative standard deviations (RSD, n = 5) of the migration time and peak height for melamine and sodium benzoate and the detection limits (based on signal-to-noise ratio $S/N = 3$) were also shown in Table-1.

The method was applied to the analysis of melamine and sodium benzoate in the lab water waste. The typical electropherograms for the standard and the water waste are illustrated in Fig. 2. The average determination recoveries are 100.3 % for melamine and 103.9 % for sodium benzoate, respectively.

Conclusion

The DC derived by trimethylchlorosilane can increase EOF like other organosilicon reagents^{6,7} and shorten analyte migration time and then renovate MEEKC methods for the determination of melamine and sodium benzoate.

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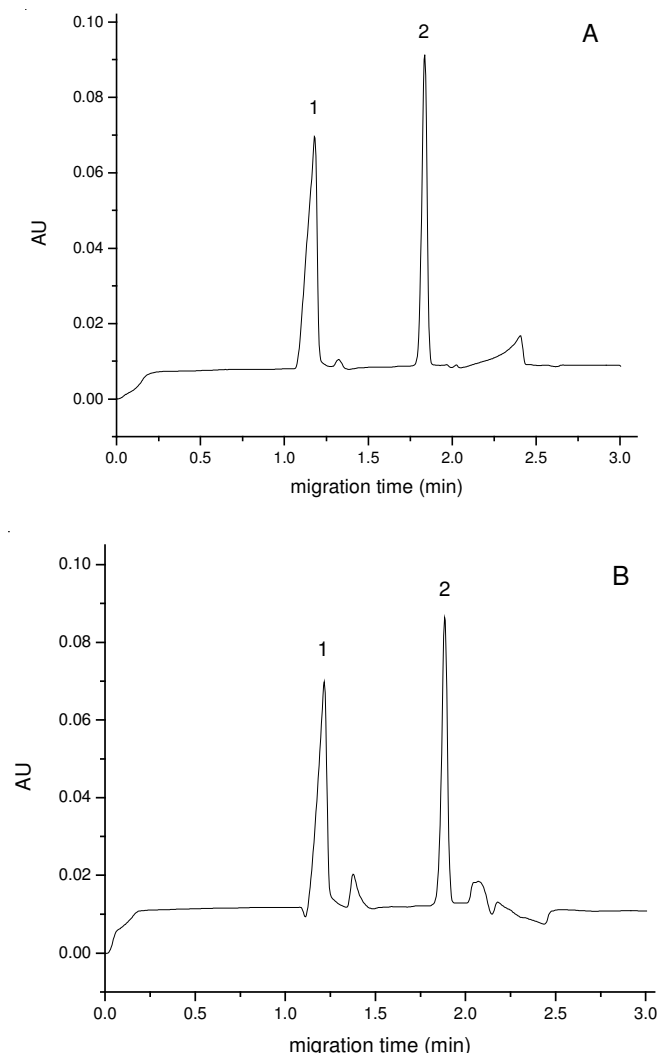


Fig. 2. Electropherogram of (A) standard of melamine and sodium benzoate; (B) lab water waste. Peak identification: 1, melamine 0.330 mg/mL; 2, sodium benzoate 0.130 mg/mL. HPCE conditions: 18 % microemulsion (v/v), separation voltage 14 kV; for other conditions see Fig. 1