



Microemulsion Electrokinetic Chromatographic Analysis of Potassium Sorbate and Saccharin Sodium Using Trimethylchlorosilane Double Coating

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A novel microemulsion electrokinetic chromatography with photo diode array detection method was developed, using trimethylchlorosilane double coating for the analysis of potassium sorbate and saccharin sodium. The influences of wavelength, buffer pH, buffer concentration and applied voltage on the separation were studied. A running buffer of 30 mmol/L sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$) + 10 % microemulsion, pH 9.2 and voltage 12 kV were applied after a series of optimization. The coating on the capillary surface helped to speed up the separation. Potassium sorbate and saccharin sodium were separated within 2.6 min. Under the optimized experimental conditions, real samples were determined with good results.

Keywords: MEEKC, Photo diode array detection, Trimethylchlorosilane, Double coating, Potassium sorbate, Saccharin sodium.

INTRODUCTION

Artificial food additives were added to food for making our vision and taste buds happy. In daily life, a person had to take dozens of food additives including caramel, sodium glutamate, thickeners, sodium benzoate, potassium sorbate, sodium saccharin, *etc.* Recent years some illegal food additive processing events were revealed. In other words, they had been prohibitive to the consumers¹. Our body would suffer from different degrees of damage when they were over-used. Therefore, the establishment of simultaneous separation and determination methods for analysis of all kinds of food additives was essential. Chromatography technology was modern separation techniques widely used in functional foods and additives industry, including ion chromatography², high performance liquid chromatography (HPLC)³ and electrokinetic chromatography⁴ which belongs to capillary electrophoresis (CE)⁵. With some efficient separation modes, capillary electrophoresis can meet the complex food matrix analysis requirements⁶. Because capillary electrophoresis methods had good compatibility with sample preparation techniques, capillary electrophoresis analysis in the application of food safety was increasingly used.

Microemulsion electrokinetic chromatography (MEEKC) is a new capillary electrophoresis technology, which was developed on the basis of the micellar electrokinetic chromatography (MEKC). The oil in microemulsion acts as a separation media. Analytes separate according to the differences of distribution coefficient between the solute in the microemulsion

droplets and water phase⁷. Microemulsion electrokinetic chromatography can separate water-soluble, fat-soluble, charged or uncharged substances at the same time⁸.

This paper used a simple trimethylchlorosilane double-coating method⁹ for the analysis of potassium sorbate and saccharin sodium, making the separation time short and greatly improving the efficiency of separation. In mild conditions, the coating on the capillary surface can be generated or removed at any time, so it is very convenient.

EXPERIMENTAL

Experiments were performed on a P/ACE™ MDQ CE system from Beckman Coulter (Fullerton, CA, USA) equipped with a photo diode array (PDA) detector using a 32 Karat software (Version 8.0 Beckman). Applied detection wavelength was 230 nm.

Sodium tetraborate (CP, $\text{Na}_4\text{B}_2\text{O}_7$) was the product of Kaifeng No. 4 Chemical Plant (Henan, China). Sodium dodecyl sulfate (CP, SDS) and trimethylchlorosilane (CP, TMCS) were purchased from Zhanyun Chemical Co., Ltd. (Shanghai, China). Saccharin sodium (AR) was produced by Guangfu Fine Chemical Institute (Tianjin, China). Potassium sorbate (AR) was provided by Fu Chen Chemical Reagent Factory (Tianjin, China). All other reagents were of analytical reagent grade. Drink sample Orange Guolishuang was purchased from a local supermarket.

The separation was carried out on a 31.2 cm (21 cm to the detector) × 75 μm id fused-silica capillary from Ruifeng

Chromatographic Device Co., Ltd (Yongnian, Hebei, China). The capillary was pretreated before its first use by flushing with 0.6 M HCl, 0.5 M NaOH and distilled water for 15 min, respectively. Then washed twice with acetonitrile with each wash for 5 min and coating solution (acetonitrile:trimethylchlorosilane = 9:1) was used for 5 min and running buffer rinsed for 5 min, respectively. Between two runs, a rinse-coating cycle, HCl, NaOH and distilled water for 0.5 min respectively, acetonitrile for 0.5 min, acetonitrile again for 0.5 min, coating solution and the running buffer for 1 min, respectively, was used.

The microemulsion was prepared by mixing 4 components with the ratio of $\omega(\text{heptane}):\omega(\text{SDS}):\omega(n\text{-butyl alcohol}):\omega(\text{distilled water}) = 3.24:13.24:26.44:57.08$. 100 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$ was prepared in distilled water by dissolving accurate amount of corresponding solute.

A certain amount of potassium sorbate or sodium saccharin, dissolving in distilled water and transferred to a volumetric flask without loss, the standard solutions were then stored for use.

After being diluted and filtrated, real samples of orange guolishuang were prepared using ultrasonication for 5 min.

RESULTS AND DISCUSSION

Photo diode array detection can detect a wide wavelength range, which was its great advantage. In this experiment, 227, 230 and 234 nm were used for the measurement at the same time. According to the absorption of potassium sorbate and sodium saccharin and some other factors, the final selection was 230 nm for the determination of potassium sorbate and sodium saccharin.

Microemulsion [10 % (v/v) was set in the buffer solution] plus 10 mmol/L, 20 mmol/L, 30 mmol/L, 40 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$ were tested respectively while buffer pH was 8.9 and applied voltage was 12 kV. With the increasing of borax concentration, the current was increased, leading to increasing baseline noise. If the concentration of borax was too low, the current would be too small and the migration time would be too long. After comparing of peak height, column efficiency and separation time, 30 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$ was selected. Under this condition, the peak shape and the separation resolution of potassium sorbate and sodium saccharin were satisfactory and the column efficiency was high.

Buffer pH had a great impact on the separation of analytes, so it was important to control it strictly. The pH was tested at the value of 8.3, 8.6, 8.9, 9.2 and 9.5. At the same electrophoresis conditions, the results showed that the lower pH, the smaller electroosmotic flow and the longer the migration time. And the greater pH, the greater the Joule heat generated, causing air bubbles that could easily interrupt in the capillary experiments. At buffer pH 8.9, or pH 9.2, potassium sorbate and saccharin sodium can be separated well. But at pH 9.2,

the separation was better to some extent and the separation efficiency was higher, so pH 9.2 was selected.

When the voltage value was changed, the current value was changed correspondingly. The greater the voltage was, the shorter the separation time was. But the voltage could not be too large. Otherwise, it would produce large amounts of Joule heat in the electrophoresis process, leading to current leakage and the baseline noise also increased. In this experiment, the voltage was tested at 8, 12, 16 and 20 kV, respectively. It was found that the lower the voltage, the longer migration time, but it would cause band broadening and worsened the separation of the analytes. Taking account of all these factors, 12 kV was selected as the applied voltage.

For evaluation of the quantitative applicability of the method, five standard solutions of potassium sorbate in the range of 300.0-1.0 mg/L and saccharin sodium in the range of 500.0-2.5 mg/L were analyzed under optimized separation conditions. 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 potassium sorbate and saccharin sodium at the top concentrations of the linear ranges. The relative standard deviations (RSD, $n = 5$) of the migration time and peak height for potassium sorbate and saccharin sodium 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 potassium sorbate and saccharin sodium in the orange guolishuang. The typical electropherograms for the standard and the real samples are illustrated in Figs. 1 and 2. The average determination recoveries are 101.6 % for potassium sorbate and 110.3 % for saccharin sodium, respectively.

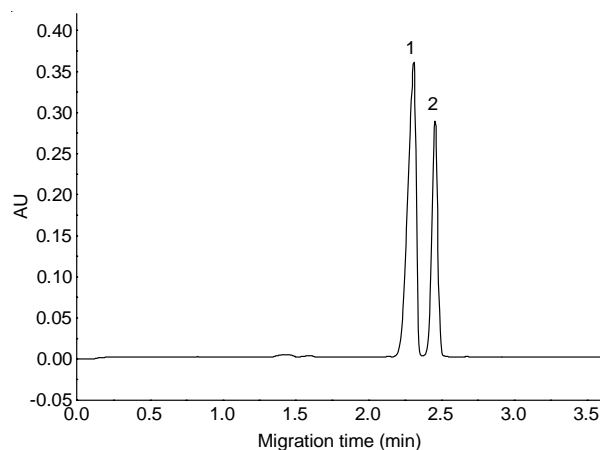


Fig. 1. Electropherogram for standard of the analytes; Peak identification: (1) potassium sorbate; (2) sodium saccharin. Capillary electrophoresis conditions: 30 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$, 10 % microemulsion (v/v), running buffer pH 9.2, separation voltage 12 kV

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

Analyte	Regression equation*	Correlation coefficient	RSD (%)		Detection limit (mg/L)
			Time	Height	
Potassium sorbate	$Y = 0.9084X + 0.009980$	0.9975	0.28	0.99	0.044
Saccharin sodium	$Y = 0.5897X + 0.007170$	0.9972	0.83	1.04	0.063

*Y was peak height (Au); X was analyte concentration (mg/L)

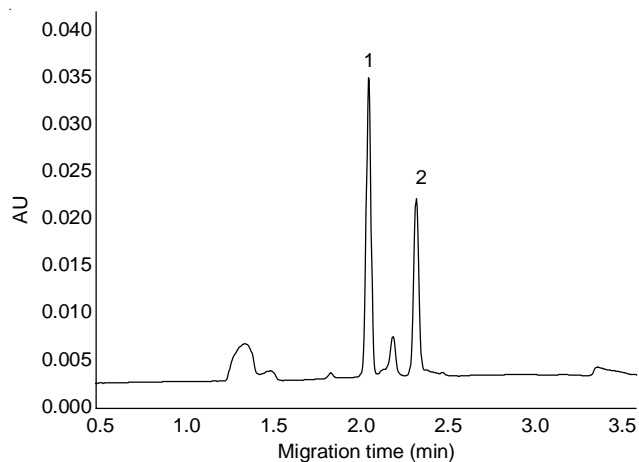


Fig. 2. Electropherogram for orange guolishuang; Peak identification: (1) potassium sorbate; (2) sodium saccharin. Capillary electrophoresis conditions: 30 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$, 10 % microemulsion (v/v), running buffer pH 9.2, separation voltage 12 kV

Conclusion

A novel microemulsion electrokinetic chromatography with photo diode array detection and trimethylchlorosilane double coating method was developed for the fast separation and determination of potassium sorbate and saccharin sodium with good selectivity, reproducibility and sensitivity.

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