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Determination of fumaric and maleic acids with stacking analytes by transient moving chemical reaction boundary method in capillary electrophoresis

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ABSTRACT

The paper presents an on-line transient moving chemical reaction boundary (MCRB) method for simply but efficiently stacking analytes in capillary electrophoresis (CE). The CE technique was developed for a rapid determination of fumaric and maleic acid. Based on the theory of MCRB, Effects of several important factors such as the pH and concentration of running buffer and the conditions of stacking analytes were investigated to acquire the optimum conditions. The optimized separations were carried out in a 20 mmol/L sulphate neutralized with ethylenediamine to pH 6.0 electrolytes using a capillary coated with poly (diallyldimethylammonium chloride) and direct UV detection at 214 nm. The optimized preconcentrations were carried out in 50 mmol/L borax (pH 9.0). The calibration curves were linear in the concentration range of 1.0×10^{-7}–1.0×10^{-4} mol/L and 5.0×10^{-7}–1.0×10^{-4} mol/L for fumaric and maleic acid with correlation coefficients higher than 0.9991. The detection limits were 5.34×10^{-8} mol/L for fumaric acid and 1.92×10^{-7} mol/L for maleic acid. This method was applied for determination of fumaric acid in apple juice and of fumaric and maleic acid in DL-malic, the recovery tests established for real samples were within the range 95–105%. This work provided a valid and simple approach to detect fumaric and maleic acid.

1. Introduction

Fumaric (*trans*-2-butenedioic) and maleic (*cis*-2-butenedioic) acids are two geometrical isomers of the composition $C_4H_4O_4$. Both acids have different physical properties due to the *cis* and *trans* configurations about the double bond [1]. For example, maleic acid exhibits higher solubility and toxicity, the melting point of maleic acid (139 °C) is much lower than that of fumaric acid (287 °C).

Maleic acid is an important industrial raw material used in the manufacture of polyester resins, surface coatings, lubricant additives, plasticizers, copolymers and agricultural chemicals, for instance, used as an raw material for the production of glyoxylic acid by ozonolysis [2].

Fumaric acid is found in fumitory, bolete mushrooms, lichen and iceland moss, on the other hand, different with the toxicity of maleic acid, it has been used as a food acidulent since 1946 because it is non-toxic. It is generally used in beverages and baking powders for which requirements are place on purity. It is also usually used as a substitute for tartaric acid, occasionally in place of citric acid, at a rate of 1.36 g of citric acid to every 0.91 g of fumaric acid to add sourness, similar to the way malic acid is used [3]. Fumarate is an intermediate in the citric acid cycle used by cells to produce energy in the form of adenosine triphosphate from food. In addition, fumaric acid is an important indicator of microbial spoilage as well as of the authenticity of juice. The levels of fumaric acid in well-prepared (authentic and not decayed) apple juices usually do not exceed 3 mg/L. A higher content of fumaric acid in apple juices indicates their microbial spoilage or the processing of decayed fruits. Another source of fumaric acid in juice can be addition of synthetic malic acid which contains fumaric acid as a contaminant. It is, therefore, necessary to develop simple, economical, and efficient methods for qualitative and quantitative analysis of fumaric and maleic acid in real samples [4].

Chromatographic methods such as high-performance liquid chromatography (HPLC), ion chromatography (IC), gas chromatography (GC), and ion-exclusion chromatography (IEC) have been used for the determination of fumaric and/or maleic acid in a wide variety of samples. In which, HPLC and IC are the most popular methods, since GC analysis requires additional derivatization procedures to enhance sample volatility, which is complex and time-consuming. But for HPLC methods, additional sample pretreatment procedures are often required [5].

The recent advances of capillary electrophoresis (CE) provide a more rapid, economic, simple, and highly efficient separation

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method that can solve the matrix interference problem as experienced in IC and HPLC separation [6,7]. Having the advantages of high efficiency, reduced sample preparation time, high resolving power as well as low mass detection limit, CE is becoming a powerful analytical tool for isomerism separations [8,9]. There are many kinds of detectors such as fluorescence spectroscopy, mass spectrometry, and ultraviolet-visible (UV) detector used for isomerism detection in CE. Among them, UV detector is the most commonly used one, because it is applicable for a wide variety of analytes and cheaper than other detectors. However, the technique often suffers from poor sensitivity due to its short optical path length and small injection volume. Similar to other techniques, the relatively poor sensitivity is one of the most serious problems that CE has to solve [10–12].

On-line sample preconcentration is considered to be the most convenient way for improving detection sensitivity, because it can be easily accomplished by carefully controlling the operation conditions on a commercially available CE instrument equipped with a UV detector [10,13]. So far, many on-line sample preconcentration approaches have been developed and used [14,15]. Stacking analyte is one of the most widely used methods [16–18]. A variety of stacking techniques have been used as an effective strategy for improving detection sensitivity in CE [19–22].

On-line stacking, videlicet, preconcentration, of analytes has become a simple, convenient, and economical but very powerful tool used to greatly improve the detection sensitivity of CE. In 1988–1992, Boček et al. [23] developed the preconcentration of transient isotachophoresis (tITP) in CE and Jandik and Jones [24] achieved over 100 fold sensitivity increase by tITP. Almost at the same time, Chien and Burgi [25,26] created the field amplification sample injection (FASI) and real concentration analytes up to 1000 fold in CE. In 1998, Quirino and Terabe successfully enhanced the sensitivity of micellar electrokinetic chromatography over 5000 fold [27]. From 1998 to 2002, Quirino et al. [28,29] and Palmer et al. [30] pictured a novel sweeping procedure for the stacking of neutral and charged analytes by the interaction between micellar molecular and analytes. During the period 2000–2003, Britz-McKibbin et al. invented the pH junction stacking for the preconcentration of analytes in a sample matrix [31]. In 1996–2003, Lunte's group advanced the pH mediated stacking method for analyses of drugs in a biological sample matrix [32].

In 2002, Cao et al. developed the stacking procedure of moving chemical reaction boundary (MCRB) for the enhancement of separation efficiency of CE and realized higher than 200 fold improvement of detection sensitivity of analyte in the sample matrix with high salt [21,33–35]. The pioneer idea of MCRB, termed "precipitate reactive front", was evolved by Deman and Rigole, and the valuable concept of "stationary neutralization reactive boundary" was advanced for electrically controlled electrofocusing in CE by Pospichal et al. From the works above, the theory of MCRB has been developed by Cao et al. [36,37], and opened new horizons for the investigation electrophoresis. MCRB is a new and useful boundary system, and the theory of MCRB for a strong electrolytic system has been proved by some experiments quantitatively [38,39].

However, the relative studies on stacking of geometrical isomers by the MCRB method have not been carried out up to now. In addition, there are still, to authors' knowledge, no investigations of quantitative method design on conditions of sample stacking and separation of two geometrical isomers. Therefore, based on above factors, the main purpose in this paper is that applying the theory of MCRB to develop a rapid, simple and sensitive method for the selective determination of fumaric and maleic acid by CE. The advantages of CE and MCRB were united to improve the detection sensitivity. This method possesses obvious benefit such as lower detection limit and good resolution.

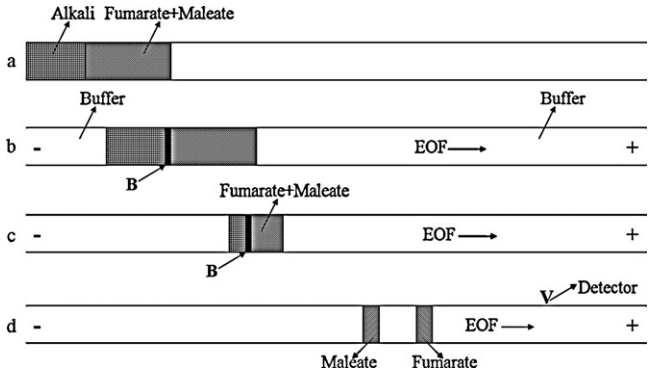

Fig. 1. General procedure of MCRB for stacking analytes. (a) Sample and alkali were injected. (b) Displacing the sample with the cathodic vial holding pH 6.0 20 mmol/L ethylenediamine sulphate buffers, and the power supply was turned on; a transient MCRB indicated by the "B" was created. (c) Applying electric field for the MCRB stacking of analytes. (d) CZE separation of fumarate and maleate.

2. Materials and methods

2.1. Apparatus

The CE experiments were performed on a CL1020 HPCE system from Beijing Cailu instrumental (Beijing, China) equipped with a HW-2000 chromatography data acquisition unit (Qianpu Software, Shanghai, China) and a UV detector (190–700 nm). A fused silica capillary (Yongnian Optical Fiber Factory, Hebei, China) of 50 μm I.D. and 62 cm total length (50 cm to the detector) was used. Direct UV detection was employed at 214 nm. All experiments were performed at ambient temperature.

2.2. Reagents and solutions

Maleic acid, fumaric acid, borax, sodium formate, sodium acetate and ammonia were obtained from Beijing Chemical Plant, sodium hydroxide and sodium oxalate were obtained from Beijing Xingguang Chemical Reagent factory. Poly (diallyldimethylammonium chloride) (PDDAC, 20 wt.% in water, average molecular weight ~ 100,000–200,000) was purchased from Sigma–Aldrich. Ethylenediamine was purchased from Sinopharm Chemical Reagent Co. Ltd. All reagents were of analytical grade or better.

All electrolyte and standard solutions were prepared using triple distilled water. Carrier electrolytes were prepared by neutralization of 20 mmol/L H_2SO_4 solution with ethylenediamine to pH 6.0. All electrolytes and sample solutions were filtered though a 0.45 μm membranes filter before CE analysis.

Sample of DL-malic acid (C.P) was obtained from Beijing Chemical Plant; apple juice was purchased from a local market.

2.3. Electrophoresis procedure

Each new fused-silica capillary was conditioned with methanol for 20 min, 1.0 mol/L HCl for 20 min, 1.0 mol/L NaOH for 20 min, and then 0.1 mol/L NaOH for 20 min. After preconditioning, the capillary was coated with a polymer by flushing the capillary with a 0.1% (W/V) PDDAC solution in water for 5 min, thus, when the voltage is applied, the EOF direction is reversed (i.e. towards the anode). Finally, the capillary was flushed with the carrier electrolyte for 3 min; between all electrophoretic separations the capillary was rinsed with polymer solution for 1 min, followed by flushing with the carrier electrolyte for 1 min.

The running buffer was 20 mmol/L H_2SO_4 solution neutralized with ethylenediamine to pH 6.0. In the CZE experiment, sample was injected by electrokinetic injection at −16 kV and the injection was 20 s. In the stacking–separation section, sample and alkali were injected successively by electrokinetic injection at −16 kV. The injection times were 20 and 10 s, respectively. Analysis was performed at a potential of −16 kV and ambient temperature.

2.4. General stacking procedure of MCRB

Fig. 1 gives the general evolution of on-line preconcentration of anionic analytes by transient MCRB. First, the sample and alkali are successively introduced to the capillary by electrokinetic injection (see Fig. 1a). Then, the sample cell was displaced with the cathodic vial holding the running buffer. After this step, the power supply was turned on, and a transient MCRB indicated by the "B" was created between the sample which was dissolved in the pH 6.0 acidic running buffer (holding H^+) and the pH 9.0 weak alkali (holding OH^-) in the capillary (see Fig. 1b). After the reaction between the H^+ and OH^-, the original alkali in the matrix plug in the capillary was gradually neutralized by the acidic running buffer and sample. The transient MCRB was design to move toward the anode, and the most important was that the movement of transient MCRB is slower than that of sample; only the condition the MCRB can well stack the sample as a sharp zone. So the boundary could effectively stack the analytes (maleic and fumaric acid) moving toward the anode all together as shown in Fig. 1c. After the end of transient MCRB, the stacked zones of maleic acid and fumaric acid electrically migrated as a manner of capillary zone electrophoresis (CZE), further were separated in accordance with their mobilities, and passed through the detector (see Fig. 1d).

3. Results and discussion

3.1. Optimum conditions of separation

In order to obtain well-shaped and symmetrical peaks, the mobility of the electrolyte anion should match the mobility of the analytes as closely as possible. In addition, buffering of the electrolyte is essential for reproducible and rugged separations. This factor should be especially important in the CE analysis of weak acid anions such as fumarate and maleate. In order to obtain a high efficiency and pH stability with a short analysis time, the electrolyte type, pH and concentration were optimized.

3.1.1. Influence of different electrolyte on the separation efficiency

For the purpose of examining the influence of different electrolyte on the separation efficiency, chloride, sulphate and phosphate anions were compared. All the experiments were performed in an electrolyte containing 20 mmol/L of appropriate acid (HCl, H_2SO_4 and H_3PO_4) neutralized with ethylenediamine to pH 6.0. Fig. 2 compares the three different electrolytes, as can be observed, slightly higher efficiencies for fumarate and maleate peaks using sulphate were obtained. Based on these results, ethylenediamine sulphate was chosen as a carrier electrolyte.

3.1.2. Effect of pH and concentration of electrolyte

The acidity and concentration of the running buffer play an important role in CE for the effects on zeta potential, the electroosmotic flow (EOF), as well as the overall charge of all the analytes, which affect the migration time and the separation of the analytes. Therefore, it is important to study their influences on CE in order to obtain optimum separations. The effect of the running buffer pH on the peak height of the investigated analytes is shown in Fig. 3. The running buffer is 20 mmol/L H_2SO_4 solution neutralized with ethylenediamine at seven different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0). As shown in Fig. 3, the peak height of both analytes is poor at pH 2.0. When the running buffer pH increases, the peak height of analytes is improved. It is also found that the peak height is low and the peak shape became poor at pH value above 6.0. According to the results, pH 6.0 was considered to be the best value for the carrier electrolyte.

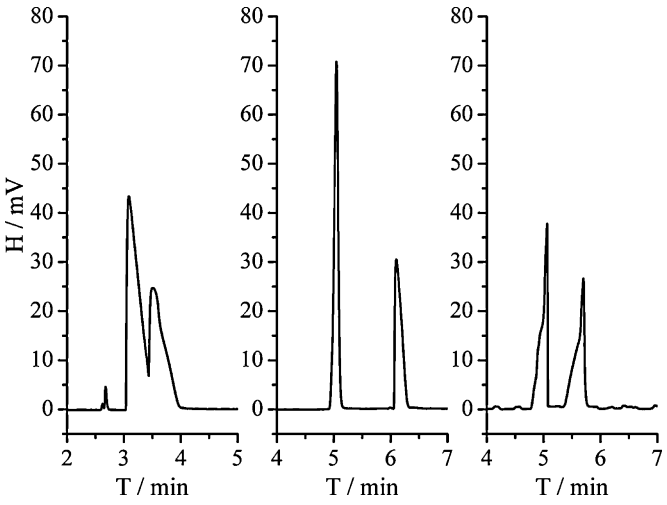

Fig. 2. The comparisons of different electrolytes in the separation efficiency on CE. Concentration of mixture sample, 0.02 mmol/L. Working voltage, −16 kV. Electrokinetic injection time, 20 s (−16 kV). Running buffer, 20 mmol/L HCl (a), H_2SO_4 (b), H_3PO_4 (c) neutralized with ethylenediamine to pH 6.0.

As the buffer concentration influences the viscosity coefficient of the solution, the diffusion coefficient of analytes and the zeta potential of the inner surface of capillary tube as well, it affect not only the resolution and migration time of the analytes, but also the peak height. The migration time and the resolution increase with increasing buffer concentration. However, if buffer concentration is too high, it will also cause a negative effect on the detection limits because the peak heights of both analytes decrease and the effect of Joule heat becomes more pronounced. The effect of buffer concentration is shown in Fig. 4, on the basis of the results, 20 mmol/L ethylenediamine sulphate buffers with pH 6.0 was chosen as the optimized running buffer in considering the peak height, resolution and analytical time and buffer capacity.

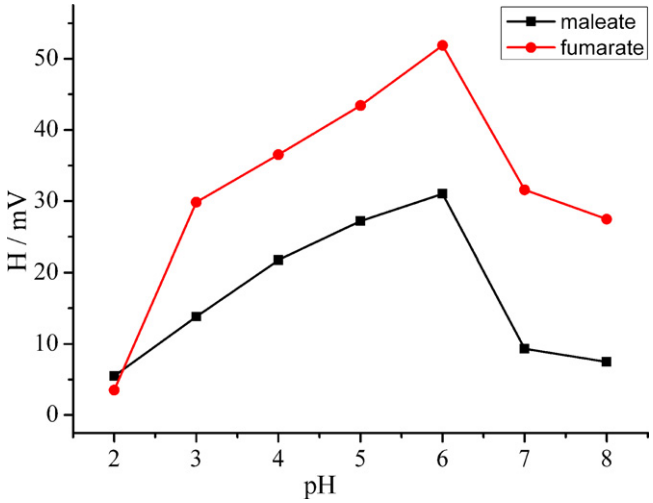

Fig. 3. Effect of buffer pH on separation of analytes. Running buffer, 20 mmol/L H_2SO_4 neutralized with ethylenediamine to desired pH.

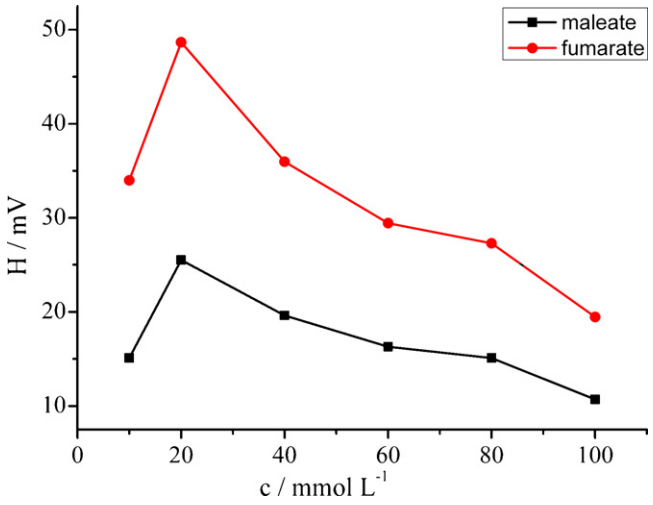

Fig. 4. Effect of buffer concentration on separation of analytes. Running buffer, appropriate concentration of H_2SO_4 neutralized with ethylenediamine to pH 6.0.

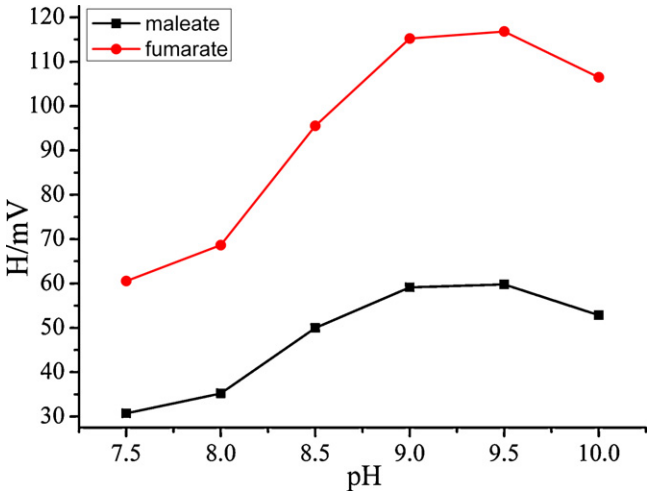

Fig. 6. Influence of borax pH on stacking result in MCRB. Concentration of borax, 75 mmol/L, modulated with H_2SO_4 or NaOH to desired pH.

3.2. Optimized conditions of preconcentration

Once the best conditions of separation were selected, the following steps were optimizing preconcentration conditions. Condition such as alkaline type, alkaline concentration and pH, sample injection time, and alkali were studied to improve the stacking efficiency and achieve good separation.

3.2.1. Comparisons of different alkalis on stacking result in the MCRB system

Different alkalis probably have different preconcentration effects on the analytes because of their different pK_a and buffering capacity. Seven alkalis (sodium hydroxide, ammonia, borax, sodium formate, sodium acetate, disodium hydrogen phosphate and sodium oxalate) at the same concentration and pH were compared (see Fig. 5), with the main consideration being analyte peak height. The experimental results showed that borax resulted in the highest preconcentration factor and sodium hydroxide the lowest. Borax was therefore chosen before optimization of the other conditions.

According to the theory of MCRB described mainly by Cao's group [36,37], it is important to choose a proper velocity of MCRB. If the boundary velocity is much faster than the sample velocity, the MCRB cannot stack the sample plug, the stacking of sample plug is poor; if the velocity of MCRB is slightly less than that of sample, an excellent stacking must be achieved. The previous experimental results showed that borax resulted in the highest preconcentration factor and sodium hydroxide the lowest. The reason was that, firstly, a weak alkali is of much superiority to a strong alkali in the stacking procedure, which has been proved by Cao et al., that was why the sodium hydroxide had the lowest preconcentration factor; secondly, the borax has high molecular weight which would induce slow migrate velocity; thus, borax can stack sample more excellent than other alkali.

3.2.2. Influence of pH and concentration in MCRB system

In the MCRB method, the boundary acts a solid barrage blocking migration of the analytes and causing their concentration by an acid–base reaction. It is, therefore, certain the concentration and alkalinity of the alkali affect stacking. To study the effect of pH, six alkaline barrages were investigated, at pH ranging from 7.5 to 10 at the same concentration. The results revealed that at the range from 7.5 to 10, the best pH value of borax was 9.0. Above or below this value the peak height was lower (see Fig. 6). With pH 9.0 as the MCRB the effect of concentration on stacking was also evaluated.

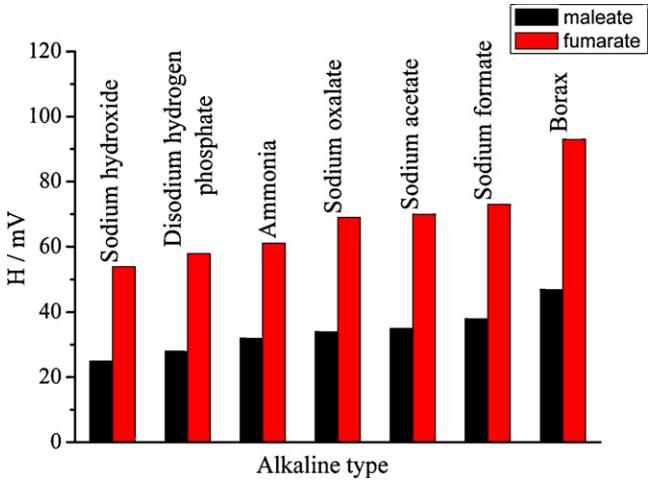

Fig. 5. The comparisons of seven alkalis on stacking result in the MCRB system. Concentration of alkali, 75 mmol/L, modulated with H_2SO_4 or NaOH to pH 8.5.

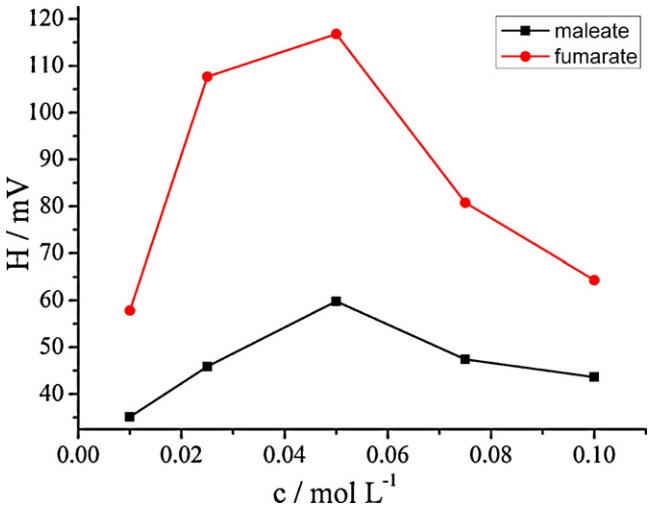

Fig. 7. Influence of borax concentration on stacking result. Running buffer, 20 mmol/L H_2SO_4 neutralized with ethylenediamine to pH 6.0. Borax, pH 9.0.

Table 1
Results of the analytes in real samples and recovery ($n = 3$)[a].

Sample	Found (μmol L^{-1})[b]		Added (μmol L^{-1})		Found total (μmol L^{-1})		Recovery (%)		RSD (%)	
	Maleate	Fumarate	Maleate	Fumarate	Maleate	Fumarate	Maleate	Fumarate	Maleate	fumarate
DL-malic acid	1.78	3.14	3.0	3.0	4.86	6.05	102.7	97.0	3.4	1.8
Apple juice	–[b]	2.28	2.0	2.0	1.96	4.19	98.0	95.5	2.7	1.5

[a] The experiment was carried out under optimum conditions.
[b] "–" means "not found".

The study revealed that when the concentration of borax was below 50 mmol/L the peak height of fumaric and maleic acid increased linearly with increasing concentration of borax; when the concentration was greater than 50 mmol/L, the increase was much lower (see Fig. 7). On the basis of stacking efficiency, 50 mmol/L borax and pH 9.0 were used in the experiment.

The velocity of MCRB holds a key importance to the design of MCRB stacking of sample and should be properly chosen. The pH value and concentration of the weak alkali should be balanced between the boundary velocity and the constituent velocity of hydroxyl ion. Different pH values, different concentration of borax solution led to different velocities of MCRB. From Fig. 6 we can see that the stacking result improved rapidly when the pH value of borax solution improving from 7.5 to 9.0, and then reach a plateau. At the same time, because the concentration of borax solution is directly affected the pH value, in Fig. 7, it is revealed that the stacking result improved when concentration of borax solution increased from 0.01 to 0.05 mol/L and then decreased. The reason was that the pH value and concentration of borax solution affects the boundary velocity.

3.3. Effect of separation voltage and injection time

The separation voltage affects the electric field strength, which in turn affects the EOF and the migration velocity of charged particles, which determine the migration time of the analytes. Moreover, higher separation voltage may result in higher Joule heating. The effect of separation voltage on the migration time of the analytes is that, increasing the voltage gives shorter migration times but also increases the background noise, resulting in a higher detection limit. Although the resolution of analytes can be improved to some extent, too low a separation voltage will increase the analytical time considerably, which in turn causes severe peak broadening. Based on experiments, −16 kV was chosen as the optimum voltage to accomplish a good compromise.

The effect of injection time on separation was investigated by different sampling time (5, 10, 20, 30, 40 s at a voltage of −16 kV). The injection time determining the amount of sampling affects peak shape. It was found that the peak height increases with increasing sampling time, and it was also found that the peak width increases with increasing time. When the injection time is more than 20 s, the peak shape levels off and peak broadening becomes more severe. 20 s (−16 kV) was, therefore, selected as the optimum injection time.

According to the previous experiment, the optimized conditions can be extracted. The optimized separations were carried out in a 20 mmol/L sulphate neutralized with ethylenediamine to pH 6.0 electrolytes. The optimum preconcentration was carried out in 50 mmol/L borax (pH 9.0). Sample and alkali were injected successively by electrokinetic injection at −16 kV and the injection times were 20 and 10 s, respectively.

3.4. Reproducibility, linearity and detection limits

A standard mixture solution of 1.0×10^{-5} mol/L for both analytes was analyzed for six times to determine the reproducibility of peak height and migration time for both analytes under the optimum conditions in this experiment. The relative standard deviations (RSD) of peak height and migration time are 1.6% and 0.7% for fumaric acid, 2.1% and 0.9% for maleic acid.

The correlation between the peak height (H, mV) and concentration of analytes (c, mmol/L) was investigated. A series of the standard mixture solutions of fumaric and maleic acid with a concentration range of 1.0×10^{-8}–1.0×10^{-3} mol/L were tested to determine the linearity for both analytes in this method. The peak height was linear to concentration of analytes in the range of 1.0×10^{-7}–1.0×10^{-4} mol/L and 5.0×10^{-7}–1.0×10^{-4} mol/L for fumaric and maleic acid, respectively. The linear regression equation was H (mV) = 0.79546 + 2146.44553c (mmol L^{-1}) ($r^2 = 0.9991$) for maleic acid and H (mV) = 0.37408 + 5915.90469c (mmol L^{-1}) ($r^2 = 0.9995$) for fumaric acid. The calculated detection limit ($S/N = 3$) were 5.34×10^{-8} and 1.92×10^{-7} mol/L for fumaric and maleic acid. The results of regression analysis on calibration curves are presented in Fig. 8.

3.5. Sample analysis and recovery

Under optimum conditions, the determination of fumaric and maleic acid in real samples was carried out according to the procedures described earlier. It was applied to determine the content of fumaric and maleic acid in DL-malic acid and of fumaric acid in apple juice. Apple juice was diluted 1:10 by electrolytes. To evaluate the accuracy of the method, a recovery study was carried out with two samples, and the results are summarized in Table 1. We found that the actual concentrations were generally in good agreement with the added concentrations, the recoveries being between 95% and 105%. These results show that the interferences by the other matrix components are not significant and the CE conditions are suitable for obtaining an adequate accuracy of the method.

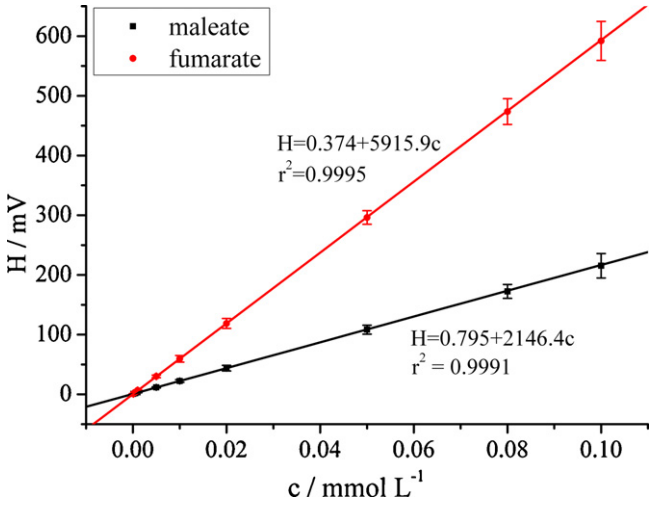

Fig. 8. Calibration curves obtained for fumarate and maleate anions. The calculated detection limit ($S/N = 3$) were 5.34×10^{-8} and 1.92×10^{-7} mol/L for fumaric and maleic acid.

4. Conclusions

From the above results and discussion, a rapid and sensitive CE method for determining fumaric and maleic acid with normal UV detection was established by coupling with MCRB procedure. The linearity, accuracy and precision of the method in analysis of apple juice and malic acid were readily validated. Compared with the normal CZE, the MCRB-based stacking can generally improve the detection sensitivity of 80–100-fold. It is clear that the MCRB is a powerful tool for stacking of analytes. This technique could be useful to broaden the application of CE in the trace analysis of pharmaceutical, environmental, and biological samples.

Conflict of interest

The authors have declared no conflict of interest.

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