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Determination of fumaric and maleic acids by capillary electrophoresis

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The capillary electrophoretic (CE) technique was developed for a rapid determination of fumaric and maleic acids. The optimized separations were carried out in a 20 mmol/l ethylenediamine-sulphate electrolyte (pH 6.5) using a capillary coated with poly(diallyldimethylammonium chloride) and direct UV detection at 214 nm. The calibration curves were linear in the concentration range 0.02–1.00 mmol/l for both anions with the correlation coefficients higher than 0.9996. The detection limits were 0.005 mmol/l for fumarate and 0.006 mmol/l for maleate. The proposed method was applied for a rapid determination of fumaric acid in apple juice and drink powder and of maleic acid in a copper plating electrolyte. The recovery tests established for real samples were within the range 95.0–102%.

Key words: fumaric acid, maleic acid, capillary electrophoresis

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 physical properties that differ due to the *cis* and *trans* configurations about the double bond. For instance, maleic acid a exhibits higher solubility and toxicity, and the melting point of maleic acid (139 °C) is much lower than that of fumaric acid (287 °C) [1].

Maleic acid is an important raw material used in the manufacture of polyester resins, surface coatings, lubricant additives, plasticizers, copolimers and agricultural chemicals [2]. Fumaric acid is a food acidulent used since 1946 because it is non-toxic [3]. It is generally used in beverages and baking powders for which requirements are placed on purity. In addition, fumaric acid is an important indicator of microbial spoilage as well as of the authenticity of juices [4]. The levels of fumaric acid in well-prepared (authentic and not decayed) apple juice 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 [5].

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Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the commonly used techniques for the determination of fumaric and / or maleic acid in a wide variety of samples [6–11]. However, the GC technique needs a derivatization (esterification) step prior to analysis, which is complex and time-consuming [6, 7]. For HPLC methods, additional sample pretreatment (e. g. solid-phase extraction) procedures are often required [8–11].

In the recent two decades, capillary electrophoresis (CE) has been recognized as a powerful separation technique for the analysis of ions because of its good resolution, high speed, simplicity and reduced sample preparation time. Several comprehensive surveys of CE methods developed for the determination of small ionic compounds in a wide variety of matrices have been recently published [12–14]. Usually, the separation of inorganic and small organic anions is carried out in the co-electroosmotic mode in which the anion migration time can be significantly reduced by establishing a co-directional movement of electroosmotic flow (EOF) with the anionic analytes [15]. Most of CE methods are based on adding anionic compounds with UV absorbing groups to the carrier electrolyte, thus making universal indirect UV absorbance detection possible [16–18]. However, separation selectivity and detection sensitivity are the two main problems occurring when indirect UV detection is used. In addition, most separations are carried out in about 10–20 min.

Despite the rather big number of CE methods published in the literature, none suited our objectives of a fast and simple determination of two geometrical isomers of 2-butenedioic acid.

The aim of this work was to develop a rapid and simple CE method for the selective determination of fumaric and maleic acids.

EXPERIMENTAL

Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a UV detector with wavelength filters (200, 214, 230 and 254 nm). A fused silica capillary (Polymicro Technology, Phoenix, AZ, USA) of 75 μm I.D. and 57 cm total length (50 cm to the detector) was used. Samples were introduced in the hydrodynamic mode by overpressure ($3.43 \cdot 10^3$ Pa). System Gold software (Beckman Instruments Inc.) was used for data acquisition. Direct UV detection was employed at 214 nm. All experiments were conducted at 25 °C using a liquid thermostated capillary cartridge.

Fumaric acid, maleic acid and poly(diallyldimethylammonium chloride) (PDDAC, 20 wt. % in water, average molecular weight ~100000–200000) were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used as received. Ethylenediamine (En) was purchased from Merck (Darmstadt, Germany). All other reagents, obtained from Sigma-Aldrich, were of analytical-reagent grade.

All electrolyte and standard solutions were prepared using doubly distilled degassed water. Stock standard solutions (10.0 mmol/l) of the analytes were prepared in water. Working standard solutions were prepared by appropriate dilution with water. Carrier electrolytes were prepared by neutralization of 0.02 mol/l H_2SO_4 solution with En to pH 6.5.

All electrolyte and sample solutions were filtered through a 0.2 μm membrane filter. Each new fused-silica capillary was flushed with 1 mol/l NaOH for 10 min and then with deionized water for 10 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. Finally, the capillary was flushed with the carrier electrolyte (absence of polymer) for 2 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.

Samples of apple juice and drink powder were obtained from a local market.

RESULTS AND DISCUSSION

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 [19]. In addition, buffering of the electrolyte is essential for reproducible and rugged separations [20]. 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 nature and pH were optimized.

To examine the influence of different electrolyte co-ions on the separation efficiency, chloride, sulphate and phosphate anions were compared. All experiments were performed in an electrolyte containing 20 mmol/l of appropriate acid (HCl, H_2SO_4 or H_3PO_4) neutralized with En to pH 7.0. This weak base exhibits buffering properties in the pH range 6–8, is transparent in the UV range and well suitable for the preparation of CE electrolytes [21]. Table 1 compares the theoretical plate numbers obtained for each analyte in the electrolytes studied. As can be observed, slightly higher efficiencies for fumarate and maleate peaks using sulphate co-ion were obtained. Based on these results, ethylenediamine sulphate was chosen as a carrier electrolyte.

Table 1. **Effect of electrolyte co-ion on the peak efficiency of fumarate and maleate anions (n = 3). Electrolyte, 20 mmol/l of appropriate acid neutralized with En (pH 7.0)**

Electrolyte co-ion	Number of theoretical plates	
	Fumarate	Maleate
Chloride	21500	19600
Sulphate	24400	22700
Phosphate	10250	13300

In CE, ionic analytes are separated both by their charge and size. For a weakly acidic analyte, its mobility is pH-dependent because the dissociation of the analyte is controlled by electrolyte pH. Because the pK_as values of fumaric (pK_{a1} = 3.03 and pK_{a2} = 4.44) and maleic (pK_{a1} = 1.83 and pK_{a2} = 6.07) acids differ significantly, their separation can be further improved by varying the electrolyte pH. Its effect on the separation is summarized in Fig. 1 in which the electrophoretic mobilities of both analytes are plotted against the pH. As expected, the increased ionization of acids at pH values near and above their pK_a increased their net charges and, consequently, effective mobilities. At pH ≥ 8, both acids were totally ionized and therefore comigrated. An acceptable resolution was observed in the pH range 4–7. Taking the buffering capacity into account, pH 6.5 was considered to be the best value for the carrier electrolyte.

Preliminary separations were performed under counter-electroosmotic conditions without reversal of the electroosmotic flow (EOF). To achieve a fast separation, the EOF should move in the same direction as anionic analytes (co-EOF mode). The EOF reversal requires a chemical modification of the capillary surface which can be achieved by adsorption of the cationic modifier onto the activated silica surface. Such kind of surface modification is called dynamic coating. Traditional EOF-modifiers such as tetradecyltrimethylammonium bromide or cetyltrimethylammonium hydroxide must be present in the electrolyte at relatively high concentrations to maintain an equilibrium between the free modifier cations and those adsorbed at the wall [15]. Whitesides and coworkers [22] have demonstrated the use of cationic polymers as semi-permanent coatings in which the separation takes place in an electrolyte containing no polymer additive. The cationic polymer adsorbs to the capillary surface so that the excess polymer can be flushed from the capillary before separation. Poly(diallyldimethylammonium chloride) (PDDAC) was tested to determine their effectiveness in providing a stable reversed EOF for the separation of fumarate and maleate anions. This cationic polymer had been previously employed for EOF reversal in CE separation of inorganic ions [23–26].

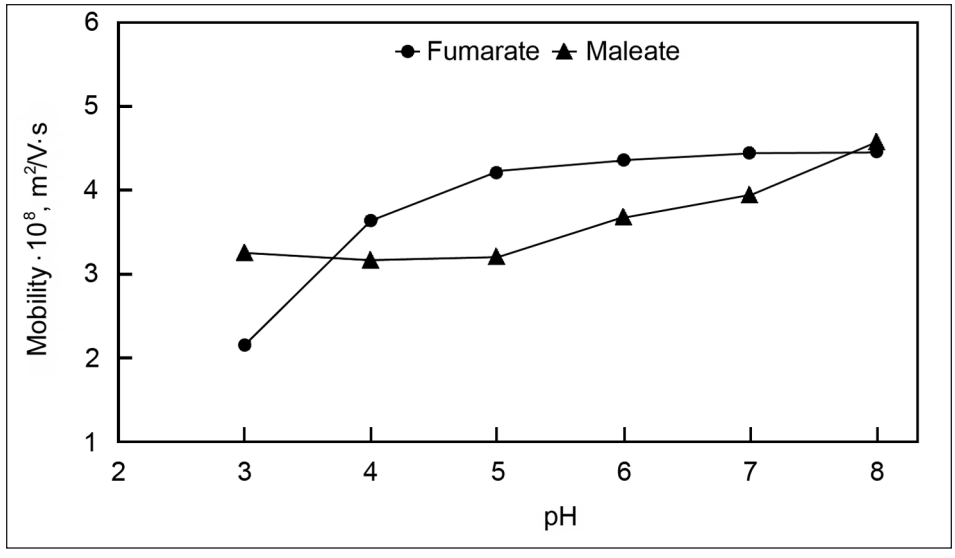

Fig. 1. Effect of electrolyte pH on the effective mobility of the analytes. Electrolyte, 20 mmol/l of H_2SO_4 neutralized with En to desired pH. Voltage, 25 kV. Direct UV detection at 214 nm

The capillary coating protocol used in this work involves flushing the capillary with 0.1% (w/v) PDDAC aqueous solution for 5 min, followed by flushing the capillary with a carrier electrolyte containing no polymer for 2 min. In addition, for the best performance, the capillary was additionally rinsed with polymer solution between runs for 1 min. Using this coating procedure, the cationic polymer yielded a very stable and fast anodic EOF. Under co-EOF conditions, the separation time was reduced by a factor of 2.5, while the resolution remained quite satisfactory. The electropherogram obtained under optimum conditions for a standard solution is shown in Fig. 2. As can be seen, both anions are well resolved in less than 4 min. Owing to the adequate choice of the electrolyte system, the peaks are relatively symmetrical, with the asymmetry factors close to 1 for both compounds.

Once the optimized conditions were selected, the method was validated with respect to the following parameters: stability of the solutions, linearity, method limit of quantification, precision and accuracy.

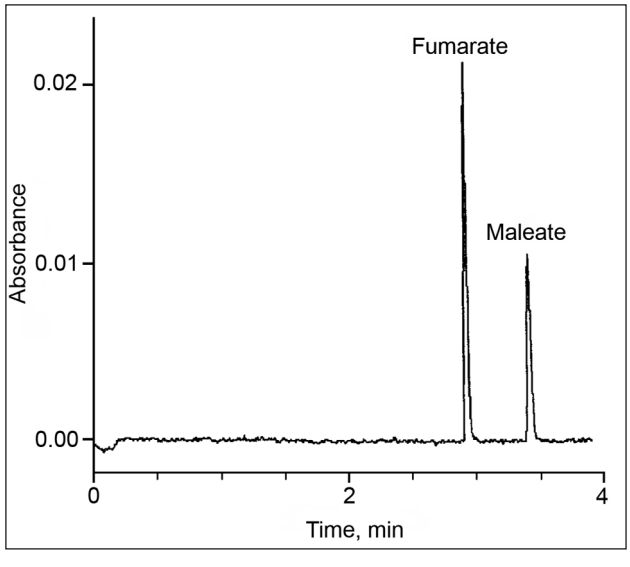

Fig. 2. Electropherogram of a standard anion solution using capillary preconditioning with 0.1% (w/v) PDDAC solution followed by carrier electrolyte flushing. Electrolyte pH 6.5. Other conditions as in Fig. 1

Although the stability test is often considered as part of the ruggedness of the procedure, it should be carried out at the beginning of the procedure validation because it conditions the validity of the data of the other tests. The response factors of standard solutions were found to be unchanged for at least to one month. Less than a 4.5% concentration difference was found between a freshly prepared solution and solutions kept for one month. The solutions can therefore be used within this period without the results being affected.

The linearity of the method was tested by preparing a calibration curve for each analyte with six points (Fig. 3). The test concentration ranged from 0.02 to 1 mmol/l, and each concentration level was injected (15 s) three times. The assay showed linearity with a relative standard deviation (RSD) ≤3.6% for the relative responses (peak area divided by concentration) obtained in the test concentration range, and the correlation coefficients >0.9996 were found for both analytes. The intercepts were not significantly different from zero. In addition, at the lower limit of the analytical range, defined as the method limit of quantification, the analytical performances were satisfactory. Consequently, for the first concentration (0.02 mmol/l) of the analytical range, the signal-to-noise ratio was measured and the RSD of the peak area was calculated with five replicates. The signal-to-noise ratios were 16 and 12, and the repeatability RSD was 6.4% and 7.8% for fumarate and maleate, respectively. Thus, for both compounds the signal-to-noise ratio was higher than 10 and the RSD less than 10%. The detection limits (three times the baseline noise) were 0.005 and 0.006 mmol/l for fumarate and maleate, respectively. These data support the suitability of the proposed method for its application to real samples.

The accuracy of the method was determined by measuring the repeatability and intermediate accuracy (between-day accuracy). To determine the repeatability of the method, six replicate injections of two standards at three concentration levels (0.05, 0.20 and 0.50 mmol/l) were carried out. The intermediate precision was evaluated over 3 days by performing six successive injections daily. A relatively low dispersion was observed

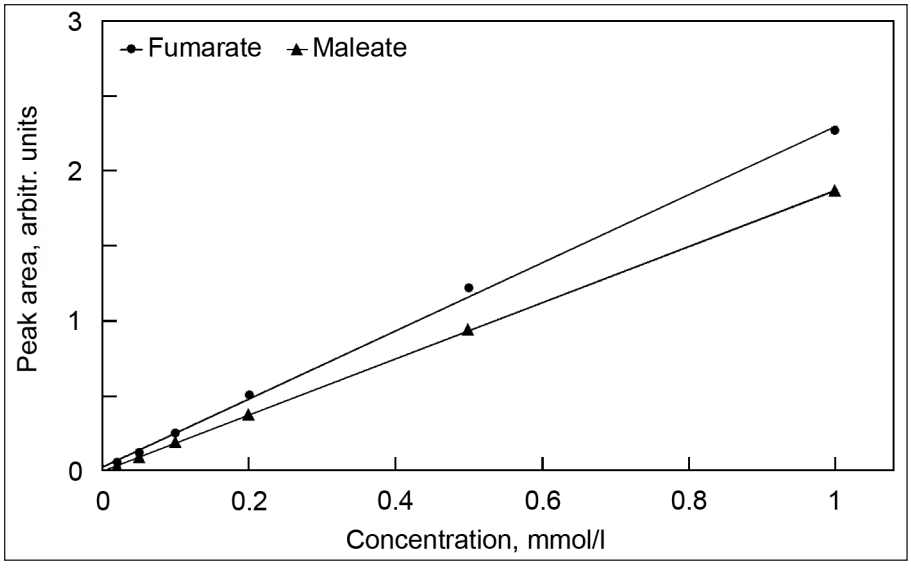

Fig. 3. Calibration curves obtained for fumarate and maleate anions

for migration times because both the repeatability and the intermediate accuracy levels were less than 1.2%. For the peak areas, repeatability was in the range 1.8–4.6%, whereas the intermediate accuracy ranged from 3.5 to 5.3%, depending on the concentrations.

To evaluate the suitability of the proposed CE system for real samples, it was applied to determine the content of fumaric acid in apple juice and drink powder samples and of maleic acid in the copper plating electrolyte. Apple juice was analysed undiluted, whereas a 1 : 50 dilution of the plating electrolyte sample with water was necessary. Drink powder samples were prepared in water according to the procedure depicted on the label. The potential of the proposed system for the determination of fumarate and maleate in real samples is demonstrated in Figs. 4 and 5. To evaluate the accuracy of the method, a recovery study was carried out with four samples, and the results are summarized in Table 2. One can see that the actual concentrations were generally in good agreement with the added concentrations, the recoveries being between 95% and 102%. 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.

The analysis does not require any preliminary treatment of the samples, except dilution. In comparison with the GC and

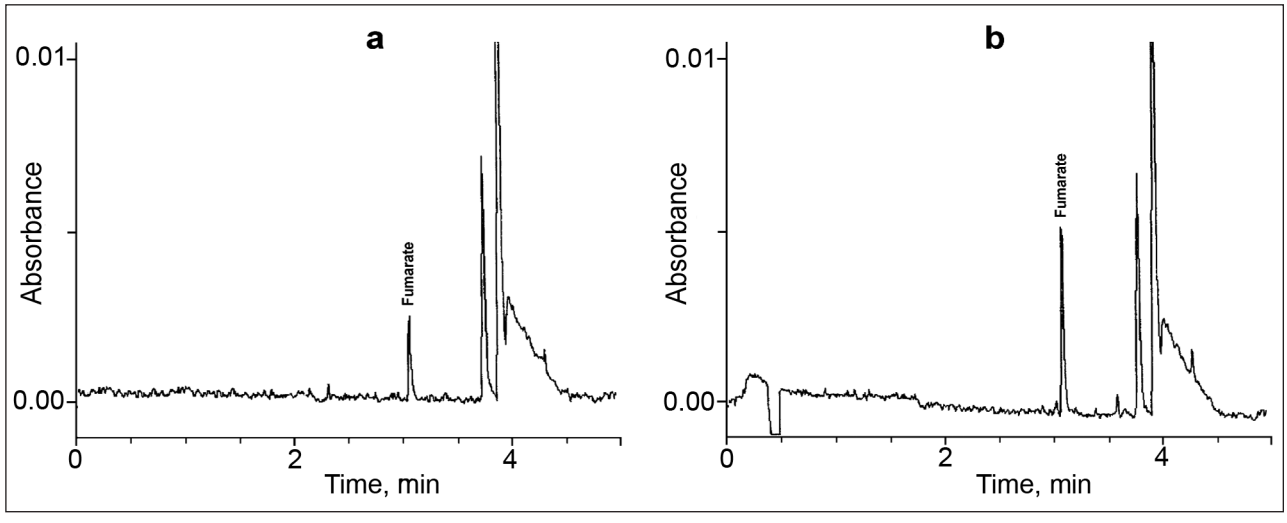

Fig. 4. Electropherograms of (*a*) apple juice sample and (*b*) apple juice sample spiked with 0.02 mmol/l fumarate standard

Table 2. **Results of the determination of fumarate and maleate in real samples (n = 3)**

Sample	Analyte	Found	Added	Found total	Recovery, %
Apple juice	Fumarate	23.5 μmol/l	20.0 μmol/l	43.9 μmol/l	102.0
Drink powder "Bolero"	Fumarate	2.19 mg/g	2.00 mg/g	4.12 mg/g	96.5
Fresh copper plating electrolyte	Maleate	38.2 mmol/l	10.0 mmol/l	47.7 mmol/l	95.0
Used copper plating electrolyte	Maleate	36.8 mmol/l	10.0 mmol/l	46.6 mmol/l	98.1

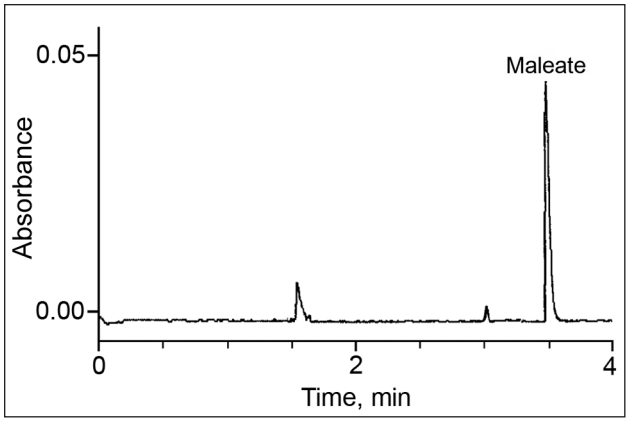

Fig. 5. Electropherogram of copper plating electrolyte sample diluted 1 : 50

HPLC techniques, the CE method is characterized by a minimal set-up time, a lower sample matrix dependence, reduced costs and reagent consumption and gives better separation efficiencies in a shorter time of analysis.

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FUMARO IR MALEINO RŪGŠČIŲ NUSTATYMAS KAPILIARINĖS ELEKTROFOREZĖS METODU

S a n t r a u k a

Optimizuotas kapiliarinės elektroforezės metodas fumaro ir maleino rūgštims nustatyti. Atskyrimas atliekamas poli(dialildimetilamonio chloridu) padengtame kapiliare 20 mmol/l etilendiamino sulfato elektrolite (pH 6,5) detektuojant (214 nm). Abiems anijonams kalibracinės kreivės yra tiesinės koncentracijų intervale 0,02–1,00 mmol/l, koreliacijos koeficientai ≥0,9996, aptikimo ribos – 0,005 mmol/l fumaratui ir 0,006 mmol/l maleatui. Metodas pritaikytas fumaro rūgščiai nustatyti obuolių sultyse ir gėrimo milteliuose bei maleino rūgščiai nustatyti variavimo elektrolite. Analičių išgavos iš realių mėginių siekia 95,0–102%.