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High-speed separation of carboxylic acids by co-electroosmotic capillary electrophoresis with direct and indirect UV detection

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Abstract

The separation of saturated and unsaturated mono- and dicarboxylic acids which are intermediates and reaction products in the conversion pathway of citric acid and itaconic acid in hot, compressed liquid water and supercritical water is performed with co-electroosmotic high-performance capillary electrophoresis. Both direct as well as indirect photometric detection can be carried out at a wavelength of 185 nm. Co-electroosmotic separation conditions are set up by adding 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (hexadimethrine bromide; HDB; polybrene) as an electroosmotic flow modifier to achieve fast separations in the range of minutes and below. The quantitative results achieved with direct and indirect UV detection are in good comparison with the values of published HPLC data. Using this method, process control is possible by fast component monitoring of the reaction solutions.

Keywords: Detection, electrophoresis; Electroosmotic flow modifiers; Co-electroosmotic capillary electrophoresis; Carboxylic acids; Citric acid; Itaconic acid

1. Introduction

Carboxylic acids have usually been analyzed with chromatographic methods, such as gas–liquid chromatography [1], liquid chromatography using ion-exchange [2–4], ion moderated [5], reversed-phase [6,7] and ion chromatography [8–11], as well as electrokinetic methods like electrophoresis [12–19] and isotachophoresis [20,21]. Generally, organic acids play an important role in biological fluids and are widely used in the nutrition industry. The annual production of citric acid by the fermentation of molasses lies between one- and two hundred thousand metric tons [22], with lesser quantities of itaconic acid produced by similar means. One way to utilize these cheap components is to convert them into high value chemicals (e.g., methacrylic acid). This can be performed in hot, compressed liquid water and supercritical water at high temperatures and pressures [23]. Supercritical and near-critical water at high temperatures have already been investigated as a reaction medium for various conversion reactions: the dehydration of lactic acid to acrylic acid [24], the acid catalyzed reaction of ethanol to ethene [25,26], the conversion of 1- and 2-propanol to propene [27,28], xylose to 2-furaldehyde [29], glucose and fructose to 5-hydroxymethyl-2-furaldehyde [30] and the reaction of dihydroxyacetone and glyceraldehyde to methylglyoxal [31].

In recent years, capillary electrophoresis de-

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veloped into a suitable tool, especially for the analysis of small molecules and ions [32]. In order to obtain high-speed electrophoretic separations of anionic compounds, the electroosmotic flow (EOF) can be reversed with cationic surfactants added to the buffer electrolyte which causes a fast migration as the anionic analytes co-migrate with the EOF. Long-chained alkylammonium salts (cetyltrimethylammounium and tetradecyltrimethylammonium bromide) as well as polycations [1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (HDB)] are suitable for use as osmotic flow modifiers. CE with reversed EOF has already been applied for the separation of proteins [33,34], anions [13,35–43] and phenolic compounds [44–48]. Separations of carbohydrates in acidic media with modified EOF have also been performed using HDB [49] and triethylamine [50].

The goal of the present paper is to analyze the reaction broths of citric acid and itaconic acid in supercritical water by capillary electrophoresis and to develop a method for the fast separation of saturated and unsaturated carboxylic acids. Direct UV detection at 185 nm with a borate–phosphate electrolyte as well as indirect UV detection at 185 nm with phthalate as background electrolyte is performed.

2. Experimental

2.1. Apparatus

The analytical data were obtained using a Quanta 4000 capillary electrophoresis system connected with a system interface module. Data acquisition and processing was carried out with a commercial chromatography software (Maxima 820) on a personal computer. These devices were purchased from Waters Chromatography (Milford, MA, USA). Fused-silica capillaries (Composite Metal Services, Worchester, UK) 32 cm (effective length 24.5 cm)×50 μm I.D. were used. Both direct and indirect UV detection was performed at 185 nm.

2.2. Reagents

All reagents were of analytical grade. Carboxylic acid standard solutions were prepared by dissolving the various compounds (Sigma Chemie, Deisenhofen, Germany; Aldrich-Chemie, Steinheim, Germany; Fluka, Buchs, Switzerland; and Merck, Wien, Austria) in ultrapure water (Barnstead/Thermolyne, Dubuque, IA, USA). 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (polybrene; hexadimethrine bromide; HDB), cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB), cetylpyridinium chloride (CPC), and polyoxyethylene-20-cetyl ether (Brij 58) were obtained from Sigma Chemie.

2.3. Buffers

The buffer solutions for direct UV detection were prepared from sodium tetraborate and phosphoric acid (Merck, Darmstadt, Germany) by dissolving them in ultrapure water with a conductivity of 18.2 MΩ and adjusting the pH with 0.1 *M* NaOH. For indirect UV detection the electrolytes were prepared from solid sodium chromate, sorbic acid and potassium hydrogen phthalate. No adjustment of the pH was necessary to keep pH 3.85 for 5 m*M* potassium hydrogenphthalate.

For direct UV detection the electrolyte mixtures had a concentration of 10 m*M* phosphate, 5 m*M* tetraborate and acidic pH values between 3.7 and 4.1. In all experiments, the HDB concentration was adjusted to 0.001% (w/v).

2.4. Procedure

Prior to analysis the fused-silica capillary was purged for 5 min with an electrolyte consisting of the same composition as the running electrolyte but without electroosmotic flow modifier (purging electrolyte I). The capillary was then rinsed with the running electrolyte (purging electrolyte II). Between the runs a purging sequence consisting of 30 s with electrolyte I followed by 60 s with electrolyte II was performed. To further shorten the purging time high voltage (20–30 kV) was applied during purging. The pre-conditioning of the capillary and the purging sequence between two runs were essential to obtain reproducible results.

Sample injection was carried out either electromigratively for qualitative analysis or hydro-

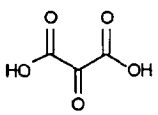
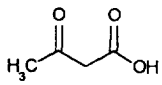
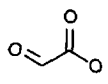
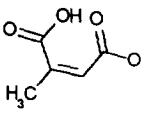
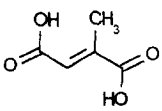
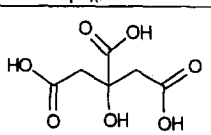
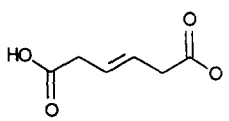
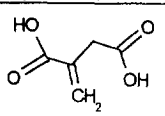
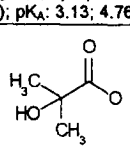
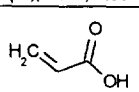
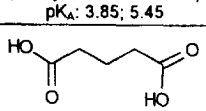
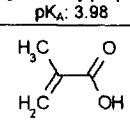
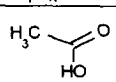
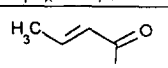
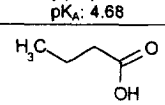
statically for quantitative purposes by raising the sample vial to an elevation of 10 cm for 10 s. The samples were prepared simply by diluting the reaction broths with water.

3. Results and discussion

The supercritical water reaction broths of citric and itaconic acid contain both saturated and unsaturated aliphatic acids. Due to the low UV absorptivities of saturated compounds indirect detection methods are usually employed for the analysis of organic acids [13,14,41,42]. However, at wavelengths below 200 nm most of the investigated acids can be also detected by direct UV detection.

Some of the investigated acids differ only slightly in their structure, e.g., citraconic and mesaconic acid are the *cis* and *trans* isomers, respectively, of 2-methyl-2-butenedioic acid. In Table 1 the structures, Chemical Abstract names and the pK_a values of the investigated acids are specified [51–54].

Table 1
Structures, chemical abstract names and pK_a values of the investigated carboxylic acids [51–54]

 **1. mesoxalic acid** (oxopropanedioic acid) pK_A: 1.82; 3.52	 **2. pyruvic acid** (2-oxopropanoic acid) pK_A: 2.49	 **3. glyoxylic acid** (oxacetic acid) pK_A: 3.34
 **4. citraconic acid** (*cis*-2-methyl-2-butenedioic acid) pK_A: 2.95; 5.98	 **5. mesaconic acid** (*trans*-2-methyl-2-butenedioic acid) pK_A: 3.09; 4.75	 **6. citric acid** (2-hydroxy-1,2,3-propanetricarboxylic acid); pK_A: 3.13; 4.76; 6.40
 **7. glutaconic acid** (3-hexendioic acid) pK_A: 3.77; 5.08	 **8. itaconic acid** (methylenesuccinic acid) pK_A: 3.85; 5.45	 **9. 2-hydroxyisobutyric acid** (2-hydroxy-2-methylpropanoic acid) pK_A: 3.98
 **10. acrylic acid** (2-propenoic acid) pK_A: 4.25	 **11. glutaric acid** (pentanedioic acid) pK_A: 4.34; 5.41	 **12. methacrylic acid** (2-methylpropenoic acid) pK_A: 4.68
 **13. acetic acid** pK_A: 4.75	 **14. crotonic acid** (*trans*-2-butenoic acid) pK_A: 4.69	 **15. butyric acid** pK_A: 4.81

3.1. Direct UV detection at 185 nm

The composition of the electrolyte for direct UV detection was chosen in order to obtain sufficient resolution between critical peak pairs to enable quantification as well as short separation times. This is important to ensure accurate reaction control in industrial applications and short sample turnover times. On the one hand, variations of the separation temperature did not exhibit a significant influence on the electrophoretic mobilities of the acids, although the migration times of the components were shifting with changing temperature. On the other hand, an increase of the separation efficiencies expressed in heights of theoretical plates (HETP) was observed with increasing temperature which is partly due to lower buffer viscosities and shorter migration times (data not shown). Resolution between some peak pairs was slightly decreasing with increasing temperature because of the reduced migration time and an overall improvement of the separation compared to room temperature could be observed at 32.5°C.

The optimum pH value was found to be between pH 3.7 and pH 4.1 with an electrolyte consisting of a mixture of tetraborate and phosphate. For preliminary experiments borate was chosen rather for historical reasons than for practical purposes. However, although no ionic borate species are expected at these acidic pH values, slight variations in the resolution of some peak pairs could be observed on the variation of the borate concentration between 2.5 and 10 m*M* which justifies its use. A phosphate concentration of 10 m*M* was found to be most suitable in terms of fast migration time and high resolution.

To investigate the influence of certain types of EOF modifiers, various surfactants, such as HDB, CTAB and TTAB were used. With CTAB as EOF modifier, higher electrophoretic mobilities of all the acids compared to HDB and TTAB were obtained (Fig. 1) and, at the same time, the separation window was significantly reduced. With TTAB a better resolution was achieved than with CTAB, however, important compounds could not be resolved (e.g., citraconic and mesaconic acid). In addition, the use of electrolyte additives (e.g., organic solvents) which is necessary for specific purposes is limited with EOF modifiers of the alkylammonium type like CTAB or TTAB [47,48]. This is due to the fact that the positive coating is dependent on the formation of dimeric alkylammonium hemimicelles by hydrophobic interaction of the alkyl chains and the physicochemical properties of the wall coating. The addition of organic solvents affects these equilibria which itself impairs the EOF. Other EOF modifiers, such as polyoxy-20-cetyl ether (Brij 58) and cetylpyridinium chloride (CPC) do not show advantages over HDB, CTAB or TTAB. Possible improvements are made by a dramatic reduction of sensitivity in the case of CPC and long migration times in the case of Brij 58. HDB renders the best overall results in terms of separation speed and resolution of relevant acids which cannot be resolved with CTAB or TTAB as EOF modifier.

Cationic EOF modifiers also have the capability to dynamically coat fused-silica in acidic media down to pH 3. This is remarkable as below pH 4 the degree of dissociation of the silanol groups in fused-silica is considerably lower than in alkaline media. In acidic electrolytes the attachment of the EOF modifier molecules onto the silica surface does not only occur by pure electrostatic interaction of the silanolate anions and the positively charge head groups of the surfactants. In addition, interactions also take place by Van der Waals forces and hydrophobic interactions of the methylene groups as well, resulting in a positively charged surface coating. This is also partly supported by the observation (data not shown) that the anodic EOF obtained with HDB increases with lower pH values over the range from pH 13 to pH 3. A general explanation for this behavior is the higher ionic strength of alkaline electrolytes which reduces the EOF. In addition, for dynamically coated capillary surfaces, the competing interaction of sodium and surfactant cations for the negatively charged sites on the silica surface is another presumable reason for this behavior. Due to the fact that in alkaline media the concentration of sodium ions exceeds the concentration of EOF modifier cations by a factor of 10 to more than 1000 (depending on the pH value, the type of EOF modifier and the electrolyte composition) a higher adsorption rate of sodium onto the surface occurs [55]. With decreasing sodium ion concentrations at lower pH values the EOF modifier becomes the predominant cationic species for wall interactions,

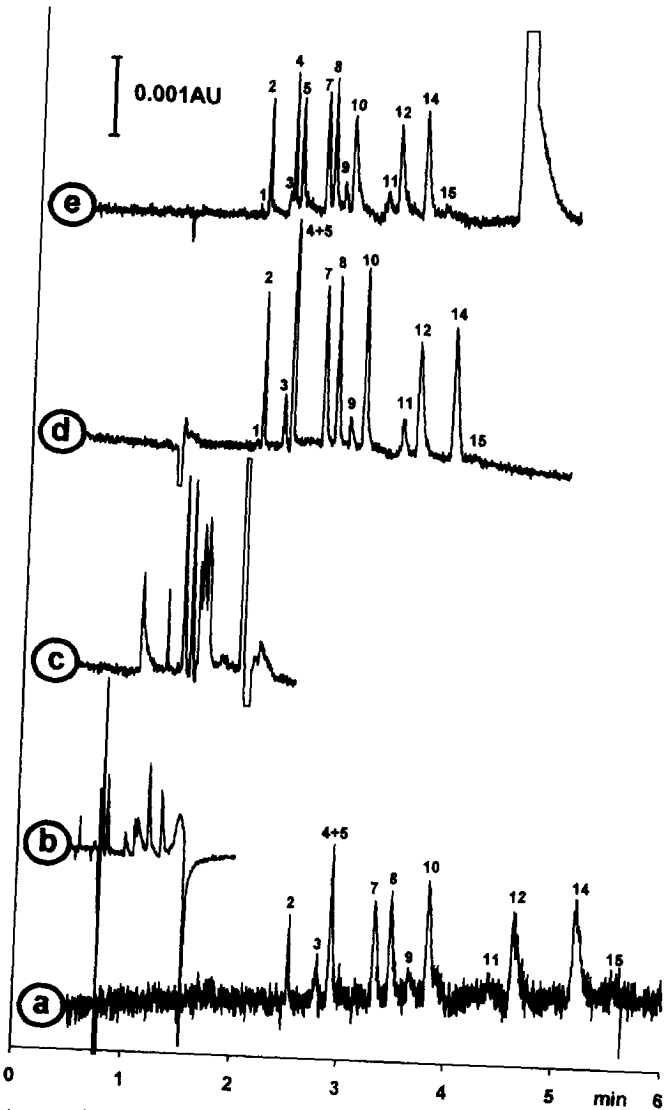


Fig. 1. Influence of various electroosmotic flow modifiers on the migration behavior of carboxylic acids. (a) Polyoxy-20-cetyl ether (Brij 58); (b) cetylpyridinium chloride (CPC); (c) CTAB; (d) TTAB; (e) HDB. Separation conditions: fused-silica capillary 32 cm (effective length 24.5 cm)×50 μm I.D.; U=−10 kV, I=5.5–8.5 μA; direct UV detection at 185 nm; electromigrative injection −2 kV for 2 s.

which partly explains the higher EOF velocity at lower pH values.

Working with acidic electrolyte systems is thus advantageous by two means: (a) a fast EOF towards the anode is established and (b) carboxylic acids can be separated as their respective anions at pH values in the range of the pK_a values. As a consequence, a fast co-electroosmotic separation with high resolution is possible.

Fig. 2 shows an optimized separation of a standard mixture containing 15 carboxylic acids. Note that citric acid and acetic acid are not detected at these particular separation conditions, as they do not exhibit a sufficiently high UV absorptivity. Monocarboxylic acids generally migrate according to their pK_a values, as do the dicarboxylic acids with respect to the first dissociation step. However, no relation of migration order and first pK_a is found

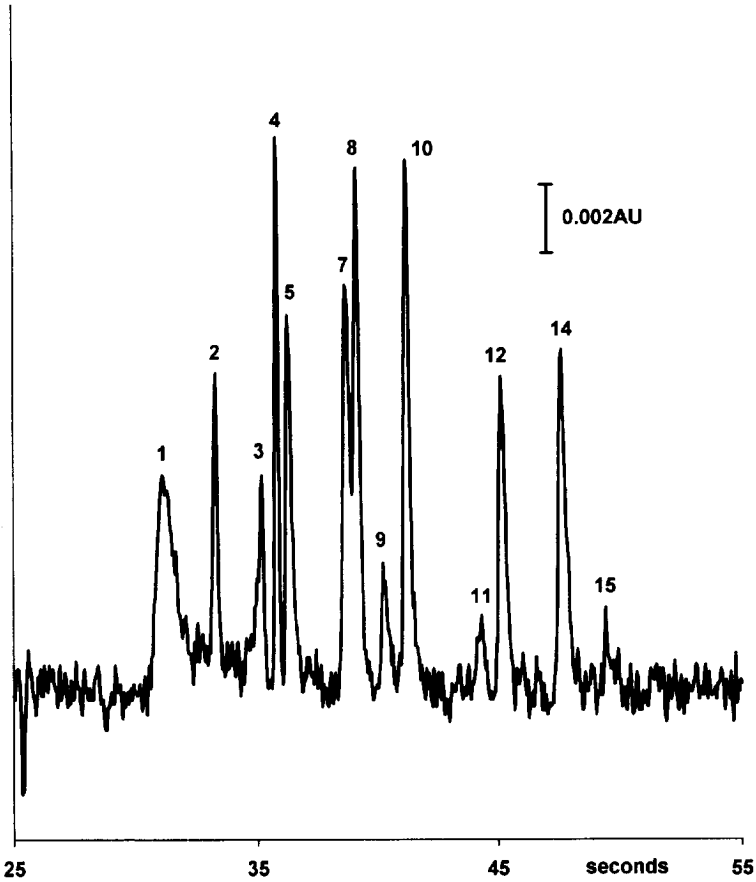


Fig. 2. Optimized separation of a standard mixture of 15 carboxylic acids with direct UV detection. Separation conditions: fused-silica capillary 32 cm (effective length 24.5 cm)×50 μm I.D.; U=−27 kV, I=20 μA; direct UV detection at 185 nm; electromigrative injection −2 kV for 2 s; buffer: 0.001% (w/v) polybrene; pH 3.9; 10 m*M* phosphate; 5 m*M* tetraborate; peak assignment as in Table 1.

between mono- and dicarboxylic acids. In the case of the 2-methyl-2-butenedioic acid isomers, steric distinctions cause decisive differences in the second dissociation step of citraconic (*cis*-isomer) and mesaconic acid (*trans*-isomer) of more than one pH unit.

Despite short separation times required for standard mixtures, very high separation efficiencies of the single zones are obtained. Expressed in heights equivalent to theoretical plates, values of plate heights between 3 and below 1 μm were obtained. This is partly due to the fact that separation efficiencies can be increased, when the apparent mobilities of the analytes are high [32,56], which is achieved when the inherent electrophoretic analyte mobility and the electroosmotic mobility vectors have the same direction and thus add up to a high apparent mobility.

One important question which had to be answered in this work was whether some of the acids were present in trace levels or not. This qualitative information was necessary to propose mechanisms of the reaction chemistry of citric and itaconic acid in hot, compressed liquid water and supercritical water. The reaction solutions were first injected electromigratively to preconcentrate compounds present even at very low concentrations by electrostacking. As a consequence, unsaturated acids can be detected down to 25 ppb, saturated acids exhibit detection limits in the range of 100 ppb.

However, for quantitative analysis, the hydrostatic injection mode was chosen because electromigrative

Table 2
Concentration of reaction products of citric acid and itaconic acid in supercritical water. Comparison of HPLC (values in mol% from Ref. [23]) with CE results (values in absolute mol% = 100×mol of product/mol of reactant)

Peak	Compound	LOD	0.5 *M* itaconic acid 10 m*M* NaOH 58 s at 370°C (D3)				0.5 *M* itaconic acid 10 m*M* NaOH 71 s at 350°C (A4)				0.5 *M* citric acid no catalyst 41 s at 320°C (N16)				0.5 *M* citric acid 10 m*M* NaOH 43 s at 280°C (N10)			
			CE			HPLC	CE			HPLC	CE			HPLC	CE			HPLC
			time	area	conc	conc	time	area	conc	conc	time	area	conc	conc	time	area	conc	conc
Direct detection																		
2	Pyruvic acid	5.7	–	–	–	0	66.2	108	0.9±0.2	1	67.9	339	2.4±0.6	2	67.2	309	2.2±0.5	2
4	Citraconic acid	3.1	74.2	566	1.5±0.2	1	73.8	3022	7.3±0.8	7	75.9	8054	19.2±0.8	19	75.4	9557	22.7±1.1	23
5	Mesaconic acid	2.0	78.3	683	1.1±0.2	1	75.7	4689	7.3±0.8	3	77.4	11 048	17.1±0.8	14	77.7	3234	5.0±0.3	4
8	Itaconic acid	1.6	81.5	1136	1.5±0.2	1	79.0	6338	8.1±0.9	7	81.4	21 032	26.4±1.1	26	79.6	35 968	45.1±2.3	46
9	Hydroxybutyric acid	9.6	83.1	519	4.8±0.1	5	84.0	557	5.2±1.8	4	–	–	–	0	–	–	–	0
12	Methacrylic acid	1.2	89.8	32 051	45.6±2.1	44	88.4	21 932	31.2±4.4	34	104.6	6017	8.6±0.4	7	107.8	676	1.0±0.2	1
14	Crotonic acid	1.2	111.6	800	1.6±0.2	2	112.4	328	0.7±0.2	1	–	–	–	0	–	–	–	0
Indirect detection																		
2	Pyruvic acid	1.1	–	–	–	0	37.1	61	1.4±0.2	1	36.9	116	2.3±0.3	2	37.3	260	3.2±0.2	2
4	Citraconic acid	0.8	40.3	281	1.6±0.2	1	40.9	1689	8.4±0.6	7	40.8	4211	20.6±0.9	19	41.4	5178	25.3±1.2	23
5	Mesaconic acid	0.7	41.0	221	1.3±0.2	1	41.5	1526	6.3±0.4	3	41.5	4045	16.0±0.7	14	41.9	1055	4.5±0.3	4
6	Citric acid	1.6	42.3	141	1.0±0.2	1	42.7	1046	6.5±0.5	7	–	–	–	0	42.8	2577	15.8±0.9	14
8	Itaconic acid	1.0	44.8	237	1.8±0.2	1	44.8	1323	7.8±0.7	7	44.2	4385	24.6±1.0	26	44.5	7870	43.8±2.2	46
9	Hydroxybutyric acid	0.5	45.9	1154	4.5±0.2	5	46.4	1033	4.1±0.4	4	–	–	–	0	–	–	–	0
12	Methacrylic acid	0.5	49.5	10 044	44.0±0.9	44	50.3	7397	32.6±2.2	34	52.6	1841	8.5±0.2	7	54.7	298	1.9±0.2	1
13	Acetic acid	0.3	54.7	785	2.7±0.1	3	56.0	835	2.9±0.2	3	55.3	1194	4.0±0.3	4	56.9	923	3.2±0.5	3
14	Crotonic acid	0.6	55.2	454	2.3±0.1	2	56.8	56	0.3±0.1	1	–	–	–	0	–	–	–	0

The peak areas (μV s) and migration times (s) are the mean values of six injections of the diluted reaction broths. Confidence intervals were calculated at a confidence level of 95%. Limits of detection (LOD) are stated in mg/l at a *S/N* ratio of 3 and were determined by hydrostatically injecting the respective standard solutions for 10 s.

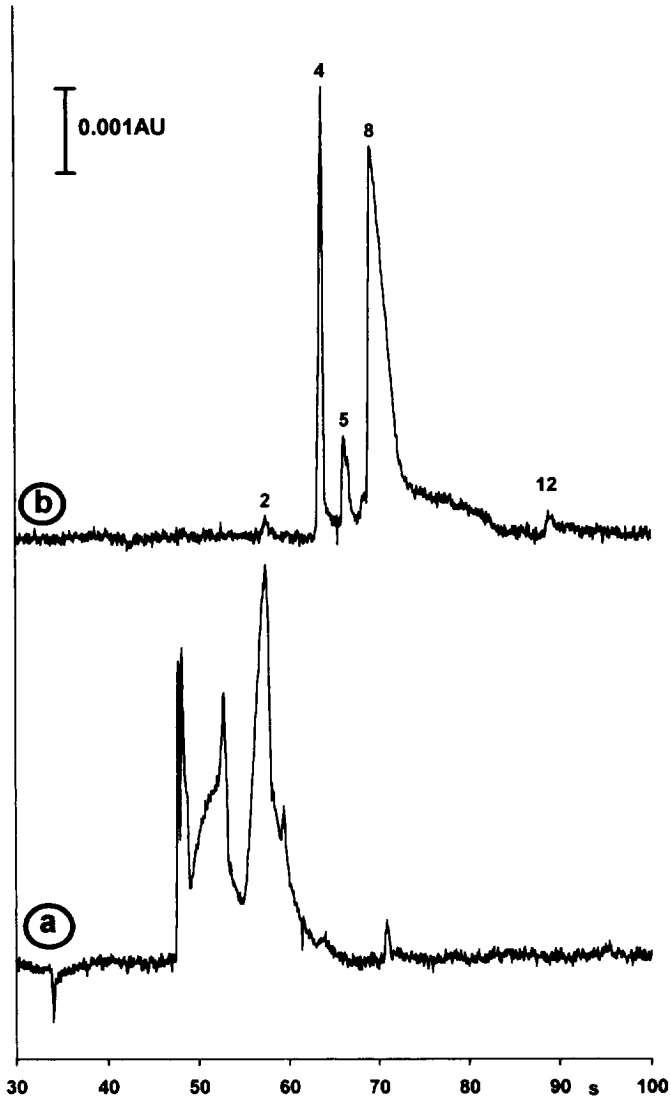


Fig. 3. Influence of methanol on the separation of a citric acid reaction solution (N10). (a) Without ethanol; (b) 30% ethanol. Separation conditions: fused-silica capillary 32 cm (effective length 24.5 cm)×50 μm I.D.; U=−30 kV, I=20 and 12 μA for (a) and (b), respectively; direct UV detection at 185 nm; electromigrative injection −5 kV for 2 s; buffer: 0.001% (w/v) polybrene; pH 3.9; 10 m*M* phosphate; 5 m*M* tetraborate; peak assignment as in Table 1.

injection did not render reliable calibration data for this purpose. This is due, on one hand, to different conductivities of the sample solutions and, on the other hand, to a saturation of the amount of ion sample constituents driven into the capillary with increasing injection time caused by an increased conductivity of the sample zone. The reproducibility of the hydrostatic injection was determined in terms of confidence intervals at a confidence level of 95%. Limits of detection for hydrostatic injection were considerably higher than for electromigrative injection (Table 2).

For some purposes organic solvents are useful electrolyte additives in order to increase separation efficiencies [46–48] or to improve the resolution of various peak zones with samples containing high

amounts of matrix components [41], e.g., in reaction broths. For this particular purpose it was necessary to modify the composition of the buffer when working with the reaction broths. When using standard solutions, the addition of organic solvents (methanol, ethanol) to the running electrolyte did not markedly improve the separation. However, the reaction solutions contained analytes with considerably different concentration ratios, which caused broad and overlapping peak zones in addition to other sample matrix influences (Fig. 3).

The presence of 30% ethanol in the electrolyte caused an improved resolution of the components in the reaction solution compared to the pure aqueous buffer system. In addition, undesired matrix effects which caused a reduction of the EOF and fluctuations in migration times could be avoided. This behavior is partly due to the fact that matrix components exhibit less tendency to interact with the capillary wall modifier in the presence of organic solvents as the solvation properties are different than with pure aqueous electrolytes [46,47]. In addition, a manual purging protocol was applied which required shorter purging sequences. This procedure is described in more detail at the end of Section 3.2.

Fig. 4 depicts the overlay of electropherograms obtained from a standard mixture (a) and reaction solutions of 0.5 *M* citric acid (b) and 0.5 *M* itaconic

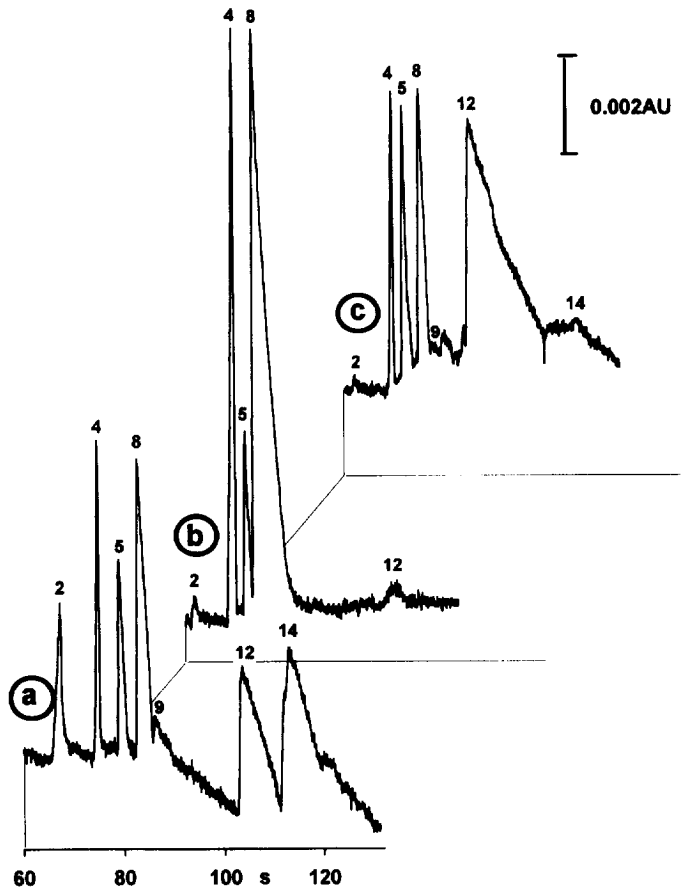


Fig. 4. Overlay of capillary electropherograms of a standard mixture and two reaction solutions with 30% ethanol and direct UV detection. (a) Standard mixture; (b) 0.5 *M* citric acid, sample N10; (c) 0.5 *M* itaconic acid, sample A4. For reaction conditions see Table 2. Separation conditions: fused-silica capillary 32 cm (effective length 24.5 cm)×50 μm I.D.; direct UV detection at 185 nm; U=−30 kV, I=12 μA; hydrostatic injection at 10 cm for 25 s; buffer: 0.001% (w/v) polybrene; pH 3.9; 10 m*M* phosphate; 5 m*M* tetraborate; 30% ethanol; peak assignment as in Table 1.

acid (c) with an electrolyte containing 30% ethanol. The shift of the methacrylic acid peak towards faster migration times in the reaction broth of itaconic acid (c) is due to the considerable high concentration of this compound.

The uncatalyzed reaction of citric acid causes the formation of itaconic acid along with citraconic and mesaconic acid (see Table 2). When the reaction is carried out in the presence of 10 m*M* sodium hydroxide a larger amount of itaconic acid is formed. The concentration of the respective reaction products depends both on reaction temperature and reaction time as well as on the presence of specific catalysts. A higher temperature favors the formation of more methacrylic acid, whereas lower temperatures shift the equilibrium towards the formation of citraconic and mesaconic acid.

3.2. Indirect UV detection at 185 nm

In this particular investigation the method with direct UV detection was bearing some disadvantages because of the poor detection limits of saturated acids. To determine whether and to which extent reaction products of citric and itaconic acid occur, a modification of the method was made. In a first attempt, chromate was used as a background electrolyte as it proved to be quite useful for the separation of inorganic anions [13,40,41]. However, indirect UV detection cannot be satisfactorily per-

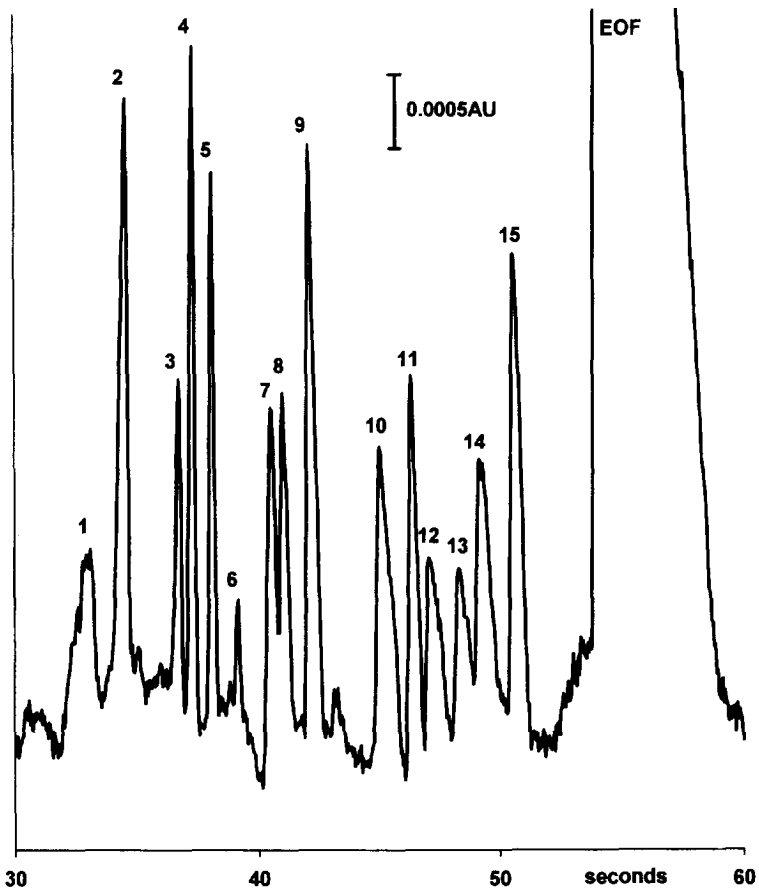


Fig. 5. Optimized separation of a standard mixture of 15 carboxylic acids with indirect UV detection. Separation conditions: fused-silica capillary 32 cm (effective length 24.5 cm)×50 μm I.D.; U=−30 kV, I=13.5 μA; indirect UV detection at 185 nm; electromigrative injection −2 kV for 2 s; buffer: 0.001% (w/v) polybrene; pH 3.85; 5 m*M* phthalate; peak assignment as in Table 1.

formed for this purpose with chromate as background electrolyte due to the acidic conditions required for the separation. At these pH conditions chromate is partly present as dichromate and causes the EOF modifier HDB to precipitate. Furthermore, dichromate may show disadvantageous redox behavior with sensitive analytes. Although sensitivity is sufficient, a complete separation of the investigated compounds was not possible with chromate as background electrolyte at neutral or alkaline conditions (pH 7 and above). A separation of the mono- and dicarboxylic acids can be performed but the selectivity of the system is too poor to completely resolve the single mono- and di-acids.

As a consequence, additional background electrolytes had to be investigated. Sorbate, which is known to work reasonably well for the separation of underivatized carbohydrates at alkaline conditions [57], proved to be too insoluble especially at acidic pH values. Furthermore, the molar absorptivity was too low to obtain acceptable detection limits. For some of the components, negative peaks appeared in the electropherogram which means that some of the analytes exhibit a larger UV absorptivity at this particular wavelength. Thus further investigation was not performed with the sorbic acid system.

Various other background electrolytes are described in the literature for indirect photometric detection of anions and carboxylic acids [58–60]. In this investigation, phthalate was used as a background electrolyte. This compound is described as exhibiting good properties for the separation of organic acids in the presence of alkyltrimethylammonium salts [14,61]. However, these methods work at neutral and alkaline pH values which proved to be unsuitable for this investigation. Fig. 5 shows an optimized separation of a standard mixture of carboxylic acids under acidic conditions with phthalate

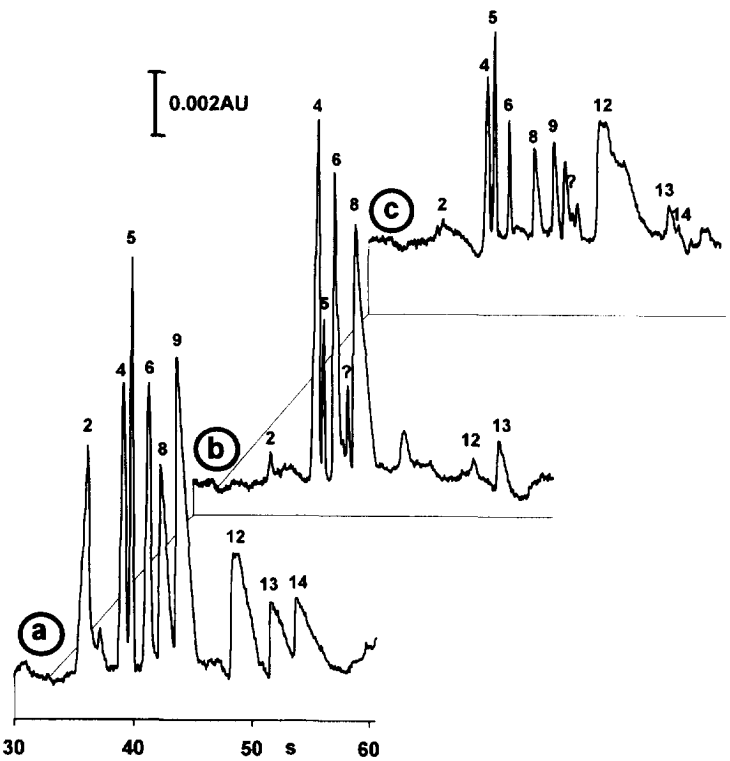


Fig. 6. Overlay of capillary electropherograms of a standard mixture and two reaction solutions with indirect UV detection. (a) Standard mixture; (b) 0.5 *M* citric acid, sample N10; (c) 0.5 *M* itaconic acid, sample A4. For details see Table 2. Separation conditions: fused-silica capillary 32 cm (effective length 24.5 cm)×50 μm I.D.; indirect UV detection at 185 nm; U=−30 kV, I=13 μA; hydrostatic injection at 10 cm for 25 s; buffer: 0.001% (w/v) polybrene; pH 3.85; 5 m*M* phthalate; peak assignment as in Table 1.

as background electrolyte for indirect detection. One significant advantage of phthalate over other background electrolytes is caused by its electrophoretic mobility which approximately matches the mobilities of the investigated compounds. This can be partly attributed to the smaller ionic equivalent conductivity of phthalate compared to phosphate at this particular pH value, which results in better peak symmetries for some components. This is particularly crucial for the compounds with smaller electrophoretic mobilities (e.g., itaconic and methacrylic acid) which are present in high concentrations in the real reaction samples.

As a consequence, it was not necessary to add any organic solvents to the buffer in order to obtain a satisfactory separation of the real reaction samples compared to the direct detection electrolyte system with borate and phosphate (Fig. 6) and faster separation times are possible.

Standard mixtures with the phthalate system and electromigrative injection exhibit detection limits of approximately 25 ppb for both unsaturated and saturated compounds. Calibration using hydrostatic injection renders detection limits in the range of 0.1–1 ppm (Table 2).

To reduce the turnover time, manual purging between runs was performed and, at the same time, high voltage was applied. As a result, matrix components which otherwise cause poor reproducibilities of subsequent runs could be more effectively removed from the capillary surface than by conventional purging without voltage. Also, the overall purging time could thus be reduced to approximately 30–60 s. Thus, turnover times can be significantly shortened to 2–3 min for the analysis of carboxylic acids in real reaction mixtures than by purging the system without high voltage. This enables a more effective process control by rapid monitoring the sample composition which cannot be easily accomplished by traditional methods. However, it has to be emphasized that currently only few commercial instruments supply the option to routinely purge the capillary between runs and, at the same time, apply high voltage.

One should note that in the course of this investigation some additional components were detected in the reaction solutions using indirect UV detection which were not yet identified. The identification of these compounds is planned to be carried out with CE coupled to a tandem mass spectrometer.

3.3. Quantitative analysis

A quantitative evaluation of the electropherograms essentially confirmed the published results obtained with HPLC data [23]. All components which have been detected by HPLC were also found in the CE method. A comparison of the concentrations obtained with CE employing both direct and indirect UV detection and the published HPLC data is displayed in Table 2. Especially in the range of low concentrations the compounds which were determined with HPLC could also be identified with the CE methods. Furthermore, some compounds, though unidentified at this time, can be detected with CE using indirect UV detection. The deviations between the direct and the indirect detection especially for components which are present at considerably low concentrations are due to their small peak areas (e.g., crotonic acid). On the contrary, by choosing a smaller dilution ratio and working with higher component concentrations the chance of overlapping peak zones occurs which further reduces the accuracy. However, for the first step in mechanistic studies it is more desirable to obtain as much information as possible about the qualitative composition of the samples to evaluate or omit specific models. With standard HPLC methods this is often a wearisome task as analysis times may be quite long whereas for this particular purpose CE offers specific advantages.

4. Conclusion

It is demonstrated in this investigation that with capillary electrophoresis a fast separation of saturated and unsaturated carboxylic acids which are present in the reaction solutions of citric and itaconic acid in supercritical water is possible. Conditions for the complete separation of the constituents in both standard mixtures and reaction solutions with considerable concentration differences of the single components could be established. It is thus possible to rapidly determine carboxylic acids in a fraction of the time required for HPLC (2–3 min with CE and high-voltage purging compared to 35 min with

HPLC using ion-exchange columns) which suggests employing CE for process control purposes.

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