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Determination of the carboxylic acids in acidic and basic process samples by capillary zone electrophoresis

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ABSTRACT

Capillary zone electrophoresis (CZE) with indirect UV detection was used in developing a method for the simultaneous determination of inorganic anions, aliphatic and heterocyclic organic acids in various processed samples. The analytes were determined simultaneously in 10 min using an electrolyte containing 20 mM 2,3-pyrazine dicarboxylic acid, 65 mM tricine, 2 mM $BaCl_2$, 0.5 mM cetyltrimethylammonium bromide, and 2 M urea at pH 8.06. Linear plots for the analytes were obtained in the concentration range of 2–150 mg L^{-1}. Relative standard deviations (RSDs) of peak areas during a 3-day analysis period varied from 5.5% for glycolate to 9.5% for oxalate. RSDs of migration times varied between 0.4% and 1.1%. The detection limit (at S/N 3) was 1 mg L^{-1} for all the analytes studied. The proposed method was successfully demonstrated for the determination of carboxylic acids in eight oxygen treated samples of commercial softwood and hardwood kraft lignin and two red wine samples of Pinot Noir grapes. In the kraft lignin samples the concentrations of carboxylic acids correspond to the oxidation time. The acid concentrations of wine varied considerable.

1. Introduction

The analysis of organic acids and inorganic anions is of interest in many fields of industry and research, thus the demand for a simple and reliable method for the fast determination of anions has increased. A number of capillary electrophoretic separation methods for carboxylic acid have been published [1,2]. Generally, the common analytical set-up includes co-electroosmotic separation and indirect UV detection. A cationic surfactant has been added to reverse the electroosmotic flow and an UV absorbing organic aromatic or inorganic chromophore for monitoring non-absorbing analytes by the indirect UV detection mode. Moreover, while the indirect technique is not as sensitive as the direct one, good resolution of the analyte zones in the separation medium and narrow sample zones are needed to detect the low levels of the ions in real samples [3]. Adler et al. compared the chromophoric properties of 2,3-pyrazinedicarboxylic acid (2,3-PyDC) and 2,3-pyridinedicarboxylic acid (2,3-PDC) with the more common probe 2,6-pyridinedicarboxylic acid (2,6-PDC) [4]. The pH was adjusted above 10, where all the analyte are fully ionized. They observed that although the molecular structures were quite similar, the probes were different in respect of sensitivity. The applications of monitoring carboxylic acids by capillary electrophoretic methods cover a wide range of samples, including food and beverage [5–8] and industrial samples [9–13]. Although some CZE electrolytes based on the application of pyromellitic acid [14] or 2,6-pyridinedicarboxylic acid [7] as a chromophore have been commercialized as such or with some modifications [15,16], there is still room for BGE solutions for special purposes. For example, if the sample matrix is very acidic, basic, or highly ionic or the concentrations of the closely migrating analytes varies largely, it may be necessary to develop a new BGE solution to enhance resolution and sensitivity [12,17].

Chromatographic separation techniques offer competitive alternatives for analyzing organic acids of various matrices. Especially liquid chromatographic techniques include various possibilities in acid analyses [6,18–21]. Gas chromatography (GC) coupled with flame ionization (FID) or mass spectrometric (MS) detection offers a second alternative technique for the analysis of organic acids. This technique provides high detection sensitivity. However, most often GC methods require a complicated and time-consuming derivatization step, in which the acids are analyzed typically as trimethyl silyl (TMS) derivatives [22].

Wood contains cellulose, hemicellulose, and lignin. Their mutual portions are different in hardwood and softwood. The percentage portions of cellulotic compounds and lignin in dry wood varied from 52% to 85% and from 21% to 32%, respectively. The objective of the chemical pulping processes is to separate cellulose fibers from each other and to remove lignin from fibers [23,24]. The cooking chemicals react with the lignocellulose causing the lignin

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polymer to break down into smaller fragments [25,26]. At most 92–94% of the fiber lignin is dissolved during the bulk delignification phase and the residual lignin, attached to cellulose fibers, is removed in the following delignification and bleaching stages. The delignification of chemical pulp is often carried out with oxygen in alkaline medium. The oxidation reactions are targeted toward the phenolic structures in lignin. After delignification, the bleaching stages are needed to increase the pulp brightness [25,28].

Although it is important to study the chemical reactions in various stages of the pulping process, sidestreams of the forest industry can be potential raw material sources for the production of different industrially relevant products. The black liquor obtained from kraft cooking contains a rich mixture of inorganic anions, aliphatic and aromatic carboxylic acids, and phenolic compounds [13,21]. Typically, black liquor solution has been burned to obtain energy and for recycling sulfur anions, which are regenerated through the green liquor phase to white liquor [29]. However, the components of the cooking liquor can be used as a raw material for the production of chemicals [30].

Wine is a widely consumed beverage in the world with thousands of years of tradition.

Determining of authenticity of wine is important in respect to food quality and safety [18,31,32]. Red wine is a rather complex fluid containing for instance water, ethanol, acids, carbohydrates, and phenolic compounds [33]. Among other analyte groups, the total acid content affects the balance of the wine while the levels of an individual acid can impart flavor with direct effect on the taste [6,18,34,35].

The aim of this work was to develop a new background electrolyte (BGE) solution buffered with the zwitterionic tricine for the determination of closely related aliphatic carboxylic acids in strongly alkaline lignin samples. The oxidation of kraft lignin of hardwood and softwood produces a large amount of various carboxylic acids which can be used as raw material for new products. The concentrations of the formed carboxylic acids were analyzed as a function of the oxidation time. This data will be used for a more comprehensive study on the reaction kinetics of lignin oxidation. The applicability of the method was also tested in the determination of aliphatic carboxylic acid compositions in acidic samples. For this application, wine samples made of similar grape but different origins were selected.

2. Experimental

2.1. Materials

Na_2SO_4, $MgCl_2{\cdot}6H_2O$, sodium maleate, and glacial acetic acid were purchased from Sigma (St. Louis, MO, USA). Tricine, fumaric acid, and lactic acid come from BDH (Poole, UK). Glycolic acid, 2,3-pyrazinedicarboxylic acid (2,3-PyDC), succinic acid, and 2-furancarboxylic acid (2-furoic acid) were obtained from Fluka (Buchs, Switzerland). HCl (Titrisol), cetyl trimethylammonium bromide (CTAB), urea, $Na_2HPO_4{\cdot}2H_2O$, citric acid, and $BaCl_2{\cdot}2H_2O$, were purchased from Merck (Darmstadt, Germany). NaOH came from Akzo Nobel (Eka Nobel AB, Bohus, Sweden); formic acid and tartaric acid from Riedel- deHaën (France). Oxalic acid, malonic acid, and malic acid were obtained from Aldrich (Milwaukee, WI, USA).

All chemicals were analytical grade and used as received. Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and capillary electrophoresis method

Uncoated fused silica capillaries of 50 μm I.D. and length 50/60 (effective length/total length) were employed in the experiments. The capillary and samples were thermostatted to +15 and +20 °C, respectively. The samples were injected at a pressure of 0.5 psi (34.5 mbar) and the injection time was set to 10 s. The separation voltage was raised linearly within 1 min from 0 to −15 kV. Before the measurements, the new capillaries (from Teknolab Trollåsen, Norway) were conditioned by rinsing sequentially with 0.1 M sodium hydroxide, 0.1 M HCl and ultra pure water, each solution for 20 min and then with electrolyte solution for 5 min. Between analyses, the capillaries were rinsed with 0.1 M NaOH solution for 2 min and the electrolyte solution for 5 min, both using the pressure of 20 psi (1380 mbar).

Separations of the standards and samples were performed with a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a photodiode array (PDA) UV/vis detector. Monitoring of carboxylic acids was carried out using the indirect UV detection mode at wavelength 281 nm with a bandwidth of 10 nm.

2.3. Samples

Commercial softwood and hardwood lignin originated from kraft black liquor and were donated by MeadWestvaco (MWV, Corp., Appomattox, USA). These kraft lignins were dissolved in 0.1 M sodium hydroxide solution in concentration of 7.5 g L^{-1}. After dissolution samples were oxidized at a temperature of 90 °C. The initial oxygen pressure was set to 5 bars and the total reaction time was 240 min. Liquid samples were taken from a closed vessel through a sampling valve as a function of reaction time and the carboxylic acid quality and concentration were determined carefully with CE to reveal the degradation reaction kinetics. Prior to CE analyses, lignin samples were diluted to 1:10 (v/v) with ultra pure water.

Two red wine samples made of Pinot Noir grape from different continents and countries were purchased from a local liquor store. Wines were Pinot Noir Reserva del Fin del Mundo (Bodega del Fin del Mundo, Argentina, Patagonia 2007) and Pinot Noir du Valais (Cave St Pierre AC Valais, Switzerland 2007). Prior to analyses the wines were diluted 1:20 (v/v) with ultra pure water.

For quantitation, standards and samples were analyzed in triplicate. For method validation, four consecutive injections of standards were performed.

2.4. Standard solutions for CZE analyses

For method development and quantification, stock solutions of 10,000 mg L^{-1} in ultra pure water were prepared for each analyte. The working standard solutions with a concentration range of 1–150 mg L^{-1} were made by appropriate dilutions of the stock solutions with ultra pure water. The stock solutions were stored in a refrigerator (+4 °C), except fumarate, which was prepared in 40% methanol and stored in room temperature. The limit of detection (LOD, S/N of 3) was determined with standards in water, while the limit of (LOQ S/N of 10) was determined in matrix, for all the analytes.

2.5. Preparation of the electrolyte solution for CZE analyses

An optimized electrolyte solution consisted of 20 mM 2,3-PyDC, 65 mM tricine, 2 mM $BaCl_2$, 0.5 mM CTAB, and 2 M urea. Stock solutions of 100 mM of 2,3-PDC, tricine, CTAB, and barium chloride were prepared, and a suitable volume of each component was added to the solution, while the appropriate amount of urea was weighed for each portion of the electrolyte. The pH value of the electrolyte was adjusted to 8.08 with 0.1 M NaOH. The pH measurements were carried out using a Denver model 20 pH meter with combination electrode (Denver Instrument Company, CO, USA). The

electrode was calibrated with pH 4.00 (±0.01), 7.00 (±0.01), and 10.00 (±0.01) commercial buffers (Reagecon, Shannon, Ireland and Reagena, Toivala, Finland).

3. Results

3.1. Optimization of the BGE solution used in the analysis of carboxylic acids

In this study, 2,3-PyDC was chosen for monitoring the aliphatic organic acids by an indirect UV detection. The effective mobility of chromophore was similar to the organic aliphatic acids under analysis conditions. In addition, a high sensitivity and a stable baseline with low noise were achieved. The pH and additives and some physical parameters were modified to reach optimum resolution with fast analysis time.

The preliminary tests showed that the best suited pH value of the electrolyte solution is between 8.0 and 8.5. This pH allows the use of organic buffers such as TRIS (Tris(hydroxymethyl)aminomethane), TAPS (N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid), and tricine (N-[tris(hydroxymethyl)methyl]glycine), since their useful working ranges are at pH scale 7–9, 7.7–9.1, and 7.4–8.8, respectively [36]. The buffering capacity of an electrolyte limits the possible variations in migration of ions caused by changes in pH and thereby aids to obtaining results with better reproducibility [37]. A zwitterionic tricine was chosen from these three organic buffers, since it gave the most repeatable consecutive runs. The concentration was optimized to 65 mM and the pH was adjusted to 8.06 with sodium hydroxide. At lower pH, maleate and succinate migrate together and phosphate disturbs the separation of acetate. However, at a pH above 8.5, the migration of lactate slows down and it merges with a negative system peak which migrates after all anionic analytes. The optimized separation of two inorganic anions and twelve aliphatic and one heterocyclic carboxylic acid under optimized electrophoretic conditions is presented in Fig. 1. Closer examination of the electropherogram indicates that the mobility of the probe is close to that of tartrate and phosphate. This can be observed in peak profiles: analytes having a higher mobility than phosphate are slightly fronting while the analytes migrating after acetate have tailing peak profile [38].

Three negative peaks can be seen in the electropherogram, one before the signal of sulphate, the second before acetate and the third after lactate. The sources of the peaks are chloride in the barium salt, absorbed carbonate, and tricine. Tricine produces a negative system peak since it acts as a co-ion of 2,3-pyrazine dicarboxylic acid. The addition of co-ion should be avoided as is stated by Doble and Haddad [38]. It will hinder the analysis of very low mobility analyte anions and secondly, analyte ions can displace the co-ions rather than the probe, which may lead to reduced detection signals. However, this non-recommendable choice to prepare BGE solution worked excellently for our purposes.

The bivalent metal cation can form weak complexes with dicarboxylic acids. Alkaline earth metal cations such as Ca^{2+}, Mg^{2+}, or Ba^{2+} can be employed in complexation reactions to enhance separation selectivity between closely related dicarboxylic acids [39,40]. In this study, barium cation was used for the complexation. Barium chloride was added to the electrolyte solution at the concentration of 2 mM. It has a positive effect especially on to the resolution between acetate and glycolate, which are unresolved from each other without Ba^{2+}. Moreover, the addition of a bivalent metal cation to the electrolyte solution improves the selectivity and resolution between dicarboxylic acids with a four carbon backbone, such as fumaric, maleic, succinic, malic, and tartaric acids. Fumaric acid is a *trans*-isomer and maleic acid a *cis*-isomer of butenedioic acid [41]. Another finding was that Ba^{2+} interacts with carbonate. Therefore, it is supposed that under the experimental conditions barium forms chelates with carbonate. $BaCO_3$ has low water solubility [42], and carbonate concentrations below 150 mg L^{-1} are not detectable (unpublished data). In addition, the low solubility of $BaCO_3$ may produce precipitations on to capillary surface and thereby affect to the repeatability of analysis. Therefore, to improve the capillary performance, rinsing step with a 0.1 M sodium hydroxide was added in analysis method. It is a notable finding that also the chromophore 2,3-pyrazinedicarboxylic acid forms complexes with bivalent cations [43]. This ability has a positive effect when organic acids are to be determined, since BGE masks the metal impurities which otherwise may repel on complex formation with the analytes [44].

Traditionally, urea has been used as a denaturant in the field of proteomics [45]. In the applications of capillary gel electrophore-

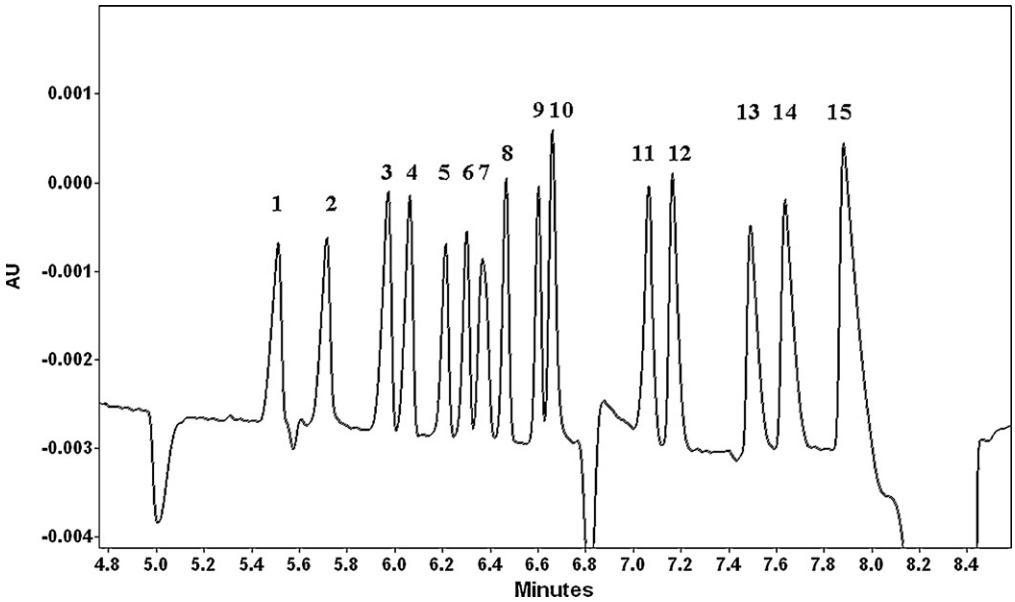

Fig. 1. Electropherogram of standard mixture of anionic analytes, 50 mg L^{-1} each. Peak numbering: 1, SO_4^{2-}; 2, oxalate; 3, formate; 4, malonate; 5, fumarate; 6, maleate; 7, succinate; 8, malate; 9, tartrate; 10, HPO_4^{2-}; 11, acetate; 12, glycolate; 13, citrate; 14, lactate, and 15, 2-furoate. Separation conditions: 250 V/cm; detection, 281 nm indirect mode; injection pressure, 0.5 psi for 10 s; capillary, 50/60 cm (L_{det}/L_{tot}), i.d. 50 μm; separation temperature 15 °C.

sis and capillary isoelectric focusing, urea is included in relatively high concentrations, up to 8 M, in the sample and/or run buffers to reduce conformer formation and prevent aggregation [45]. In our optimized BGE for carboxylic acids, 2 M urea was added to the electrolyte to increase the viscosity of the solution and even further improve the resolution between maleate, succinate, and malate. Polyethylene glycol, PEG 8000 at the concentrations of 0.01–0.1% (v/v), was also tested for enhancing the resolution between the comigrating acids, but a desirable effect was not obtained. During the analyses, traces of urea tend to crystallize on the vial caps, but this was mainly avoided by dipping the capillary ends in water immediately after the BGE rinse and by diminishing the running electrolyte volumes from 1.5 to 1.3 mL.

The detection wavelength for indirect UV detection was set to 281 nm, where the molar absorptivity of 2,3-PyDC was the highest. The developed electrolyte solution works well also for the monitoring of 2-furancarboxylic acid (2-furoic acid). This heterocyclic carboxylic acid is an oxidation product of dehydration of pentose sugars [11]. Typically, it has been analyzed with CE with some other aromatic carboxylic acids using direct UV detection [46]. The optimum wavelength for detecting 2-furancarboxylic acid with direct UV is 252 nm when the current BGE solution was employed. However, its signal is almost equal in sensitivity to the indirect UV detection. This finding allows us to use the developed BGE solution also for the analysis of 2-furoic acid with aliphatic acids.

Instrumental parameters were optimized in lesser extent than chemical parameters. However, the separation temperature and capillary length were tuned to appropriate values in order to maintain a fast separation and a high resolution. The effect of the separation temperature was studied in the range of 15–20 °C. The best suited separation temperature was 15 °C, where the quartet of fumarate, maleate, succinate, and malate were separated from each other. At the higher temperature the total migration time was slightly faster than at 15 °C, but the resolution of closely migrating analytes, i.e. between formate and malonate and also between tartrate and phosphate was poorer. During the method optimization, the total capillary length varied from 60 to 80 cm. The comparison of shorter and longer capillary revealed, that lengthening of the capillary does not improve remarkably the selectivity or resolution; it only unnecessarily slowed total analysis time. Thus, the optimized physical parameters in regards of fast separation and an appropriate resolution include the separation temperature of 15 °C and the total capillary length of 60 cm.

To demonstrate the applicability of the developed analysis method for the determination of selected anionic compounds, two red wine samples of various origins and two series of lignin processing samples were chosen.

3.2. Validation of the method

Linearity, repeatabilities, and detection limits were determined for each analyte. The calibration was made for 13 carboxylic acids and two inorganic anions. The percentage relative standard deviations for migration time and peak area were calculated for each analyte from the absolute values of intra- and interday runs (4 days, four repetitions/concentration level). The average of RSD% was calculated using the results of all calibration levels. The results are compiled in Table 1. All the calibrations were linear over the range announced, which can be seen from the coefficients of the regression lines. The concentration varied between analytes ranging from 2 to 75 mg L^{-1} of malonate and succinate to 2–150 mg L^{-1} of formate, acetate, and glycolate. In the studied concentration scales of analytes, the linearity of the calibration lines were good while the correlation coefficients ranged from 0.99 to 0.9994. The results show excellent reproducibility of the absolute migration times, and acceptable reproducibility of the absolute peak areas for all the analytes. The relative standard deviations (RSDs) of intraday results of peak areas varied from 4.3% for glycolic acid to 8.1% for lactic acid while the same values of interday results settled down to 5.3% for malonate and 8.8% for oxalate. The limits of detection (LOD) and quantitation (LOQ) were 1 and 2 mg L^{-1}, respectively, for all the analytes when injection of 0.5 psi for 10 min was employed. These concentrations are equivalent with approximately 60 and 150 fmol of injected amount per acid. The alkali rinse before analysis has a considerable effect on the reproducibility of absolute migration times: the RSD% varied from 0.2% to 1.1%. Without rinsing, the RSD values varied from 1.8 for oxalate to 5.6% for lactate indicating that the migration of last eluting analytes becomes slower due the adsorbed residues.

Addition of the sodium hydroxide rinsing step with stabilizes the migration time variation. This indicates that some compounds, analytes, or complexation products tend to attach to the capillary wall and are effectively removed by using a harsh rinsing step.

Table 1
Repeatability of the CZE analytical procedure: regression equations of standard mixtures, coefficient, and the average relative standard deviations (RSD%) of migration time and peak area for intra- and interday results.

Analytes in migration order	Peak area[a] (regression)			Average RSD% of standards, intraday (n = 4/concentration level)		Average RSD% of standards, interday (n = 4/concentration level, 3 days)	
	Equation ($y = ax + b$)	Coefficient (r^2)	Range (mg L^{-1})	Migration time	Area	Migration time	Area
SO_4^{2-}	$y = 118.12x - 94.299$	0.9983	2–100	1.0	6.6	1.2	7.8
Oxalate	$y = 117.41x - 83.865$	0.9986	2–100	0.2	6.0	0.6	8.8
Formate	$y = 159.16x - 72.137$	0.9994	2–150	0.2	5.2	0.6	5.7
Malonate	$y = 133.19x - 111.37$	0.9967	2–75	0.2	4.8	0.6	5.3
Fumarate	$y = 120.35x - 148.74$	0.9986	2–100	0.2	5.4	0.6	6.9
Maleate	$y = 93.841x - 92.285$	0.9988	2–100	0.2	6.0	0.6	6.2
Succinate	$y = 131.71x - 71.576$	0.9963	2–75	0.3	6.8	0.6	6.7
Malate	$y = 119.04x - 107.69$	0.9978	2–100	0.2	4.9	0.6	5.5
Tartrate	$y = 111.09x - 127.65$	0.9985	2–100	0.3	4.4	0.6	6.3
HPO_4^{2-}	$y = 126.26x + 52.746$	0.995	2–100	0.3	5.3	0.7	7.0
Acetate	$y = 171.6x - 287.45$	0.9953	2–150	0.3	6.4	0.6	6.2
Glycolate	$y = 192.36x - 503.56$	0.995	2–150	0.3	4.3	0.7	5.3
Citrate	$y = 148.71x - 203.3$	0.9962	2–100	0.3	4.8	0.7	7.9
Lactate	$y = 219.77x - 49.306$	0.9982	2–100	0.4	8.1	0.8	8.1
2-Furoate	$y = 454.29x - 32.011$	0.99	2–100	0.4	5.8	0.5	5.6

[a] Eight concentration levels of standards, four injections/concentration level.

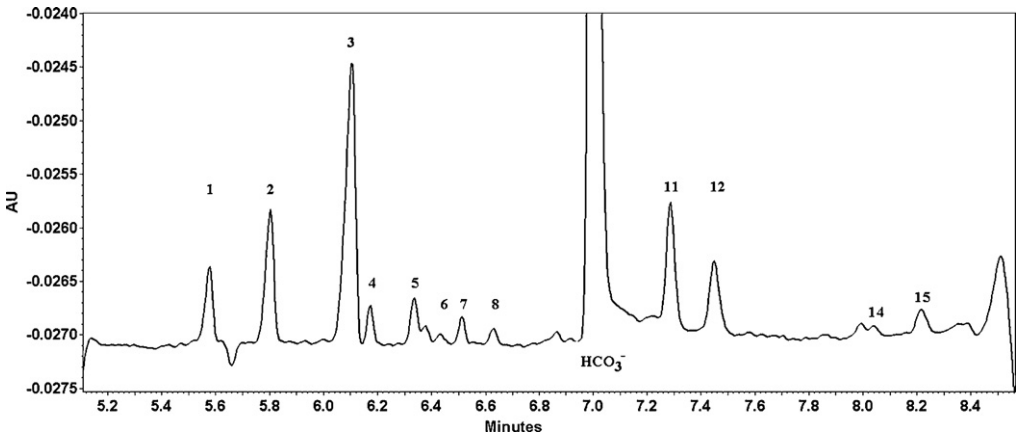

Fig. 2. Lignin degradation sample. The electropherogram is a typical example of lignin samples, only concentrations varied between the samples. Peak identification as in Fig. 1. The bicarbonate mainly originates from the alkaline treatment.

3.3. Analysis of real samples

3.3.1. Quantitative results of lignin samples

Softwood (SW) and hardwood (HW) kraft lignin samples were oxidized using a temperature of 90 °C and at 5 bar initial oxygen pressure. The carboxylic acid content of lignin degradation samples was measured at four points during the total 4 h reaction time. Fig. 2 shows a typical electropherogram of lignin samples. The detected carboxylic acids are both volatile and nonvolatile in nature. The previous group includes formic and acetic acids while the latter one contains dicarboxylic acids and hydroxy acids. Signals of oxalate, formate, acetate, and glycolate together with bicarbonate dominate the profile of the electropherogram. The pH of the oxidized samples is above 9. In those solutions carbon dioxide is solubilized to bicarbonate or carbonate. In Section 3.1 it was noted that carbonate concentrations below 150 mg L^{-1} are not detectable due to their complex formation with barium cation. The concentration of HCO_3^- is well above this limit; therefore its signal is visible. The main source of carbonate is sodium hydroxide which absorbs carbon dioxide. Malonate, fumarate, succinate, malate, and lactate can be found in smaller quantities. 2-Furancarboxylic acid has also been quantified in trace amounts, more in softwood samples than in hardwood samples. The amounts of 2-furoic acid found in the samples are at lower concentration levels than found in bleached effluents [27]. Some unidentified signals, next to fumarate and lactate, can be seen in the electropherogram. The results from the calculated concentrations of identified anions are compiled in Table 2. The development of concentrations of four major acids in respect to reaction time is presented in Fig. 3. Generally, the amount of individual acid increased during the first 3 h of reaction time and the evolution decreased during the last hour of oxygen treatment. These results lead to the assumption that under the presented oxidizing conditions the degradation of lignin will stabilize in 180 min, and only minor changes occur after this.

In the case of lignocellulose sample, it is assumed that the low molecular acids such as acetic acid and glycolic are degradation compounds of carbohydrates and only minority of acids come from the disruption of lignin [25,27]. However, the origin of the acidic products is difficult to interpret and the present experimental set-up enables unambiguous differentiation of the lignin derived products.

3.3.2. Quantitative results of wine samples

Two wine samples from different origins were used as the model samples of acidic matrix. The common denominator for these samples is Pinot noir grape and production year. Aliphatic carboxylic acids were analyzed from these samples after 1:20 (v/v) dilution with Milli-Q water. Fig. 4 shows a typical electropherogram obtained from the wine samples. Peak identification was performed by standard addition and by comparing the migration times with those obtained with a standard solution.

The major acids found in samples were succinic, tartaric, acetic, and lactic acids. Malic and citric acids have been found in minor quantities. Two inorganic anions, namely sulphate and phosphate, were found. Similar acid contents have been presented by Fung and Lau [5] and Tanyanyiwa et al. [47]. The compiled results of wine samples are presented in Table 3.

As can be seen, there were noticeable variations in acid concentrations between the wines. This may be an indication of the differences in fermentation conditions. More citric, malic, succinic, and tartaric acids were found in the Patagonian product, while the amount of lactic acid was larger in the Swiss wine. Both wines contained sulphate and phosphate.

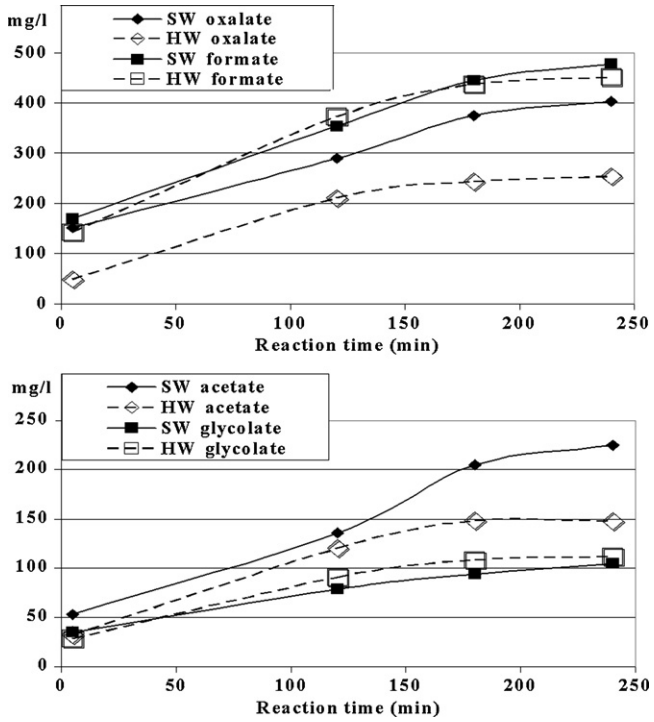

Fig. 3. The change of concentrations of four major anions in softwood and hardwood samples in relation to reaction time.

Table 2
Quantitative results of lignin samples after different processing times at oxidation conditions of 5 bar and 90 °C.

Sample	Time (min)	Anion (mg L^{-1})										
		SO_4^{2-}	Oxalate	Formiate	Malonatec	Fumarate	Succinate	Malate	Acetate	Glycolate	Lactate	2-Furoate
Softwood												
SW 5	5	77	49	143	<LOQ	ND	<LOQ	ND	32.4	28.2	<LOQ	ND
SW 120	120	98	211	372	29.9	49.6	26.7	15.8	120	90.5	ND	21.2
SW 180	180	99	243	436	36.6	69.2	29.7	15.9	145	108	ND	<LOQ
SW 240	240	104	254	451	37.5	70.9	27.9	15.7	148	112	<LOD	<LOQ
Hardwood												
HW 5	5	129	152	170	34.1	22.1	ND	ND	53.0	35.0	ND	ND
HW 120	120	152	290	355	36.3	84.0	20.5	<LOQ	136	78.8	ND	ND
HW 180	180	179	375	445	43.6	97.8	<LOQ	<LOQ	205	93.8	ND	ND
HW 240	240	19 1	402	477	45.0	101	<LOQ	<LOQ	225	105	<LOD	<LOQ

ND: not detected, LOD = 1 mg L^{-1}, LOQ = 2 mg L^{-1}.

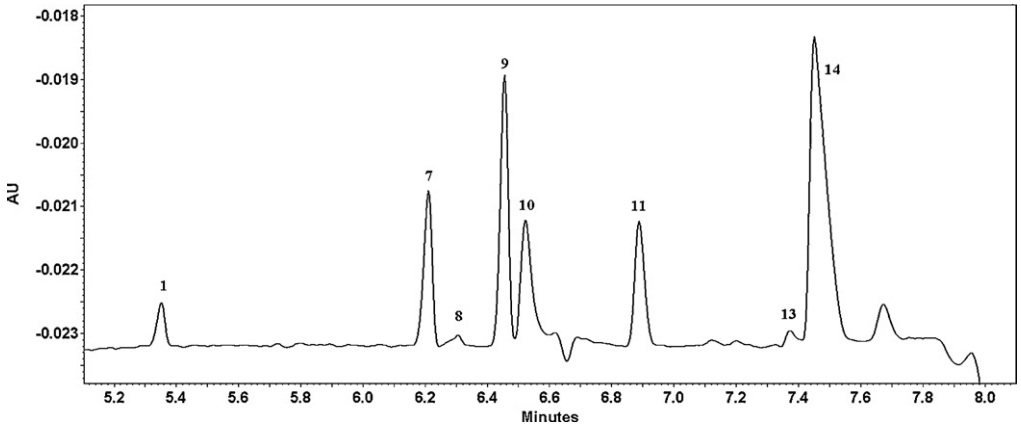

Fig. 4. Wine sample "Patagonia", diluted to 1:20 (v/v). The electropherogram is typical to wines, only concentrations varied. Peak identification as in Fig. 1.

Table 3
Quantitative results of wine samples.

Analyte	Wine Patagonia (mg L^{-1})	±RSD%	Wine Switzerland (mg L^{-1})	±RSD%
HPO_4^{2-}	843	3.4	687	3
SO_4^{2-}	268	4.3	229	1.6
Acetate	529	2.8	611	1.9
Citrate	82	12.9	ND[a]	–
Lactate	1602	2.7	3418	1.4
Malate	79	2.1	ND	–
Succinate	830	3.1	341	8.1
Tartarate	1528	3.4	1092	5

[a] ND: not detected.

Tartaric, citric, and malic acids come from the grape, whereas lactic, succinic, and acetic acids originate from the fermentation process [48]. Lactic acid is a major organic acid in wine and it is thought to have a great influence on the taste [2]. In addition, tartaric acid gives a sharp and hard taste to wine therefore high quality wines contain less of this acid. For example, botrytised wines contain more than 2 g L^{-1} tartaric acid [32]. The quantification of acids allows controlling the acidity during the different steps of the winemaking process, such as alcoholic fermentation, malolactic fermentation, and aging [48]. The source of sulphate may be the sulphite which is added to wines to prevent oxidation and to stabilize the chemical composition. Phosphate concentration reflects the amount of soil phosphates. Its concentration depends on the amount and type of fertilizers used on the vineyard [49].

4. Concluding remarks

A simple, fast, and repeatable method was developed for the analysis of low molecular weight carboxylic acids. The BGE has a significant effect on the separation efficiency of analytes. The addition of a complex forming cation to the BGE solution clearly enhances the selectivity of closely related acids. Moreover, by including urea in the electrolyte, it is possible to even further improve the resolution. The buffering of the electrolyte with the zwitterionic tricine decreases the separation current and thus reduces the Joule heating. In addition, the selected detection wavelength allows the monitoring of the heterocyclic carboxylic acid together with aliphatic ones with great sensitivity. The method is linear over the range from 2 to 150 mg L^{-1}. The optimized method is suitable for reliable analysis of carboxylic acids in various samples. Only minimal sample preparation is needed prior to analyses. The presented method was applied to the determination of low molecular weight carboxylic acid contents of two different types of samples where the acid profile has great importance. Wine is an example of food samples while the lignin degradation samples represent a sample type found in pulp and paper related research. Both sample types are originally natural products which are subjected to various biochemical or chemical processes. Therefore it is not surprising that the same carboxylic acids can be found in different concentration ratios in both sample groups. The ability of simultaneous monitoring 2-furancarboxylic acid together with aliphatic acids is beneficial in the analysis of major anions in lignin degradation processes.

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