Capillary Electrophoresis in Determination of Low Molecular Mass Organic Acids

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***Abstract*—This paper presents overviews the capabilities of capillary electrophoretic techniques to determine low molecular mass organic acids in a variety of aqueous, gas and solid samples. It mainly focuses on short - chain carboxylic acids containing one or more carboxylic groups and possibly some other functional groups (hydroxyl, keto-, amino- etc.). Finally the procedures applied to the determine the acids in different matrices are shortly characterized.**

The principles of capillary electrophoresis its advantages over gas and liquid chromatographic separation and detection modes are briefly discussed. Finally, the brief overview of the technique is followed by characterization of CE based procedures for the analysis of a variety of samples such as food, environmental, biological etc. for the content of organic acids, mainly those with carboxylic groups.

***Index Terms*—Capillary electrophoresis, low molecular mass organic acids.**

I. Introduction

Short-chain carboxylic acids (SCCAs) are a group of chemicals containing one or more carboxylate functions (-COOH) in their structure and a short hydrocarbon group which can be aromatic, aliphatic, saturated or unsaturated, straight chain or branched, and substituted with hydroxyl- or keto-, or some other groups. They can occur in different environmental compartments due to natural processes and as a result of human activity. Many of them are formed in the degradation processes of larger biological molecules. For example, short chain alkane monocarboxylic acids, often termed volatile fatty acids (VFAs), are produced in anaerobic biodegradation of proteins, carbohydrates and fats. Some SCCAs are formed in animal farms, municipal and diary wastewater, municipal solid waste leachate, etc. [1]. Some of them play an important role in biological wastewater treatment, since they can be a source of easily assimilable carbon for microorganisms. Volatile fatty acids (mainly propionic and butyric) are responsible for the unpleasant smell in areas around wastewater treatment plants and municipal solid waste landfills. SCCAs can increase the mobility of heavy metals and radionuclides in the environment. Therefore, the concentration of individual SCCAs should be monitored in many media.

The most common technique for the determination of short –chain carboxylic acids is chromatography, especially gas chromatography, whose main limitation is that only volatile acids can be determined in an original form. Capillary electrophoresis has been investigated as an alternative separation technique. For simultaneous determination of monocarboxylic acids, dicarboxylic acids, hydroxylic acids, and also those substituted with other groups, liquid chromatography, mainly ion chromatography, has often been used [2]. However, capillary electrophoresis has the potential to provide far more rapid results and can often be better applied to volatile and non-volatile than GC.

In fact, the term ‘capillary electrophoresis’ describes a family of related techniques in which separations are carried out in a narrow bore capillary due to an electric field applied between its ends [3]. The separations performed with the use of capillary electrophoresis are generally highly efficient and rapid. In some cases both charged and neutral species can be simultaneously separated. Capillary electrophoresis has already been used in many areas, including pharmaceuticals, food and beverages, environmental and clinical analysis.

The potential of this technique for carboxylic acids determination was demonstrated before 1990. In one of the first papers on the topic Tran, Blanc and Leoplod described the separation of amino acid enantiomers with high-performance capillary electrophoresis (HPCE), after derivatization with Marfey's reagent (1-Fluoro-2,4 dinitrophenyl-5-L-alanine amide, FDAA) [4]. Since then, capillary electrophoresis has been widely applied in routine analysis and also a number of reviews have been published [1]-[5]. The exceptional power of separation, short analysis time, low detection limits, simple reagents and minimum sample requirements have made capillary electrophoresis an attractive methodology for the simultaneous determination of a variety of carboxylic acids, such as monocarboxylic, dicarboxylic, hydroxyl-, keto- and amino- acids in one analysis. The recent application of capillary electrophoresis for the determination of short-chain carboxylic acids in 1) aqueous, 2) gas and 3) solid samples has been reviewed.

II. Fundamentals of Capillary Electrophoresis

Electrophoresis can be defined as the differential migration of charged species (ions) in an electric field, and was first described as a separation technique by Tiselius in 1937 [5].

It is used to separate substances in an external electric field. In capillary electrophoresis, separation takes place inside a thin capillary tube with a diameter of several tens of micrometers.

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As a result of a dissociation of silanol groups, the inner wall of the fused silica capillary is negatively charged and an electrical double layer with a specific electrokinetic potential is formed. The layer consists of two parts: one directly adjacent to the capillary and the diffusion part, with a redundant positive charge. If such a system is in an electric field, hydrated cations move towards the cathode, causing a flow of liquid into the capillary [6]. These two effects allow for the simultaneous analysis of both ions and molecules, which have no charge. Based on the simultaneous effect of electromigration and electroosmotic flow (for principle, see Fig. 1) this approach offers a wide range of conditions under which successful separations and quantitations can be obtained.

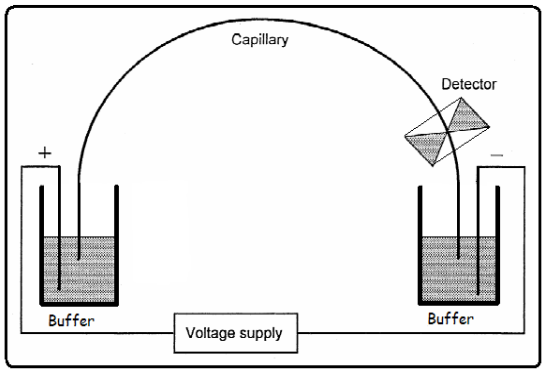

Fig. 1. Schematic drawing of capillary electrophoresis instrumentation.

In the early 1980s, Jorgenson and Lukacs [7]-[9] demonstrated that high-performance analytical electrophoretic separations in capillaries were possible. In their instrumentation, electro-osmosis was used to analyse negatively and positively charged species in a single run; separations were extremely rapid.

Electrophoretic separation is based on the difference in migration velocities of analytes in a constant electric field. Movement of analytes inside the capillary is caused by the flow of the buffer (electroosmotic flow) and due to the voltage applied (ions - electrophoretic flow) [1]. The buffer solution flows from an anode to cathode. Upon applying voltage along the capillary, anions move in the direction of the anode, while cations to the cathode. The electroosmotic flow moves the whole medium present in the capillary from the anode to cathode (in most cases). Consequently, cations move ahead of the electroosmotic flow, while anions are behind it and are eluted last. Neutral species move with the same speed as the buffer. The electroosmotic flow can sometimes be so strong that even ions with a high negative charge will travel to the cathode.

For the determination of anions by capillary electrophoresis, reversal of electroosmotic flow is often applied. A reversed flow effect is essential to achieve rapid separation and to reverse the elution order of the ions. In order to decrease or even reverse the electroosmotic flow direction, electroosmotic flow modifiers are used. Their operation consists of blocking the negative charge formed on the inner wall of the capillary. Reversal of electroosmotic flow can be achieved by proper selection of the modifier concentration.

Capillary electrophoresis is an instrumental development of traditional slab gel electrophoretic techniques [10]. Since its introduction, capillary electrophoresis has shown great potential in the determination of ionic species in aqueous, gas and solid samples.

However, as a result of the great popularity of HPLC as an analytical tool, few companies offer capillary electrophoresis instruments. Comprehensive reviews on capillary electrophoresis as an analytical technique from its beginning until now include papers by Weinberger [11] and Issaq [12].

A. Modes of CE Operations

The simplest variety of the capillary electrophoresis separation of charged analytes results from the differences in their electrophoretic mobilities [13].

The key factors determining selectivity of separation when using *capillary zone electrophoresis* (*CZE*) are charge to size ratio and pH. The latter determines the degree of ionization of moderate and weakly basic, or moderate and weakly acidic analytes. The background electrolyte should be characterized by a good buffering capacity at a chosen pH for reproducible separations, and sufficiently low conductivity not to generate a high current, which could lead to excessive Joule heating. The most commonly employed background electrolytes have been derived from the large body of work with gel electrophoresis, and include phosphate, borate, phosphate/borate, and citrate buffers [14]. Separation takes place inside a quartz capillary tube filled with a suitable electrolyte under the influence of applied voltage. This causes the sample components to separate by moving at different speeds in the direction of the detector window. This method allows the separation of substances of both small- and macromolecules such as carbohydrates, vitamins, peptides, proteins, organic acids and ions.

A commonly encountered mode of capillary electrophoresis is *micellar electrokinetic chromatography* (*MEKC*). This combination of electrophoresis and chromatography allows for the separation of both neutral and charged solutes [15]. It is achieved by the addition of surfactants to the background electrolyte at concentrations greater than the critical micelle concentration (CMC). Separation is based upon interaction of the analytes with the micelles, which can be considered a “pseudostationary phase” [16]. The nature of the interaction between the solute and micelle can be altered by using different types of surfactants. The most commonly employed surfactants are sodium dodecyl sulphate (SDS), bile salts, and quaternary ammonium salts [14]. The presence of organic solvents, such as methanol and acetonitrile may be used as organic modifiers to alter the selectivity of a run, similar to reversed-phase chromatography [14]-[16]. Micellar electrokinetic chromatography is mainly used for separating the components of mixtures, which are endowed with charged molecules, as well as electrically neutral. Separation occurs as a result of the partition of the analyzed components between micelles (hydrophobic environment) and the buffer (hydrophilic environment) - non-polar molecules penetrate the micelles, while polar remain in the buffer. Using this technique, substances such as carbohydrates, vitamins, pharmaceuticals, amino acids, peptides, oligonucleotides can be separated.

Capillary gel electrophoresis (*CGE*) is still undergoing instrumental development. CGE may contain chemically cross-linked gels or non-cross-linked linear polymer matrices. Although they have a higher efficiency and resolving power, capillaries filled with cross-linked gels are very delicate and prone to clogging. An alternative is represented by non-cross-linked sieving gels, generated by the physical entanglement of linear polymers (alkylated celluloses or linear polyacrylamide) dissolved at suitable concentrations in the running buffer. In effect, the non-cross-linked polymers exert a sieving mechanism similar to traditional crosslinked gels, but remain fluid and can be replaced by refilling the capillary by pressure application.

The gel capillary electrophoretic separation of macromolecular substances is due to the difference in their size. This process takes place in a capillary filled with a gel of a suitable pore diameter. With CGE, nucleic acids, oligonucleotides, peptides and proteins can be split.

The same principles can be applied to *capillary isoelectric focusing* (*CIEF*), with the notable differences being that separation is carried out in free solution, and that, after focusing, the various isoelectric bands have to be mobilized to the detector using hydrodynamic or electroosmotic methods. The focused mixture components are separated on the basis of the differences in isoelectric points (pI). In the capillary, the pH gradient is generated, from the lowest to the highest at the inlet and at the outlet of the capillary, respectively. When a voltage is applied, the components of the sample move until the pH zone corresponding to the pI of the solute is reached. With CIEF, mainly substances (amino acids, peptides, proteins) having amphoteric properties can be separated.

Capillary isotachophoresis (*CITP*) is a 'moving boundary' electrophoretic technique, where a combination of two background electrolytes is applied, wherein these zones move at the same velocity [16]. The sample is introduced into the capillary between the two buffers – the "leading" and "terminating" – whose ion mobilities are greater and smaller than those of the sample ions, respectively. When a voltage is applied, the ions move behind "the leading" buffer as their mobility decreases. In cITP, only analytes of the same charge are separated in a given run. cITP principles may be applied not only for separation, but also for sample pretreatment, before CZE, achieving concentration factors in excess of 100 times.

In *capillary electrochromatography* **(**CEC**)**, a chromatographic stationary phase is contained in the capillary (packed or wall-immobilized) interacting with the solutes according to the usual chromatographic separation mechanisms. The mobile CEC phase is driven through the capillary by electro-osmosis, not by pressure, as occurs in chromatography. This allows high resolving power and efficiency.

CEC is still at an early stage of development but looks particularly promising for separation of neutral hydrophobic molecules and in view of its coupling with MS.

Currently, there are a variety of techniques that can be used to analyse various samples, e. g. food, environmental, wastewaters from animal farms, etc.

The ''classic'' version is limited to charged water soluble analytes only, while other types of electrophoresis have many benefits:

1) only minute sample quantities are required;
2) samples do not need to be pure, provided CE can separate the analyte of interest from the impurities;
3) radiolabeling of molecules is not necessary;
4) automated CE instrumentation is available, and;
5) molecular interactions can be characterized in a free solution.

B. Separation Conditions and Background Electrolyte Gradients

Often cationic surfactants are added to the electrolyte to reverse the electroendosmotic flow (EOF) by forming a double layer and a positive charge on the capillary surface. The EOF moves in the same direction as the negatively charged acids and gives good peak shape. Typically, the cationic surfactant used [17] is tetradecyltrimethylammonium bromide (TTAB) with concentrations in the region of 0.5 mM.

Selectivity can be optimized through pH adjustment of the electrolyte because the acids have multiple pKa values. A number of separations can be performed by applying pH or flow gradients in capillary electrophoresis or organic modifier gradients in the micellar electrokinetic separation [18]. The electrophoretic mobility of SCCAs is strongly dependent on the pH of the running buffer. However, most previous research on gradient separation in capillary electrophoresis dealt with pH gradients. The same results were observed by Chen et al. [19] in the determination of C2 –C18 carboxylic acids. The analysis lasted less than 12 min. The migration time increased as the pH decreased; the mobilities of SCCAs increased with increases in pH between 6.5 and 9.0. According to the dissociation constants (pKa) of background electrolyte and analytes published in the CRC Handbook of Physics and Chemistry (57th edition 1), all of the SCCAs studied (pKa in the range of 4.81–4.87) and p-anisic acid (pKa 4.47) have very similar pKa values. When the desired pH (6.5–9.0) was applied, they were all in their anionic forms and, thus, the decrease in migration time could not have been due to the ionization of analytes. However, at a high pH, more silanol groups in the capillary wall undergo ionization; this results in a higher zeta potential, and hence the increase in electroosmotic velocity and smaller migration times. However, when more p-anisic acid was added to lower pH, the ionic strength of the electrolyte also increased. Galli, Olmo and Barbas [2] used a similar approach to generate dynamic pH gradients in a range from 5.5 to 7.5. The separation buffers were prepared with 0.5 M H3PO4, 0.5 mM cetyltrimethylammonium bromide (CTAB), as a cationic surfactant to avoid electroosmothic flow. In practice, the buffers generally used are based on Tris.

C. Detection

Although CE nowadays is a versatile separation technique, next to GC and LC, and well suited for the determination of a wide variety of compounds in various matrices, the limited detection sensitivity and selectivity still is a problem. This is due to the fact that the small internal diameter of the capillary offers, on one hand, relatively low mass detection limits and a

low sample volume, but on the other hand, the small detection volume results in relatively high concentration detection limits. In combination with CE mainly on-column detection techniques have been applied. So far the most frequently applied detection techniques are ultraviolet (UV) absorbance, diode-array (DA), fluorescence (CIF), laser-induced fluorescence (LIF), electrochemical (AD), and mass spectrometric (MS) detection. A short description of these detection modes will be given, including a comparison with respect to sensitivity and selectivity of these detectors.

The most widely used detection method technique is UV detection because it is a relatively straightforward and universal technique. An optical window is formed by burning off a section of the polyimide coating of the capillary. This type of detector is routinely used in commercial instruments, which can also be fitted with a diode array detector for the simultaneous acquisition of spectra [20]. However, the small internal diameter of a capillary being a path length limits the sensitivity of the detection system, which is generally 1–2 orders of magnitude lower than that found in HPLC [21]; consequently, detection limits for UV–VIS absorption are usually in the micromolar range. One way to improve the sensitivity in capillary electrophoresis is to increase the path length by using a Z-shaped capillary, in which detection is conducted by axial illumination [14].

The other too alternative described approaches are a rectangular capillary extended in the direction of the light path with a reduction in height, this to keep the volume to minimum or a capillary having a locally increased diameter in the detection region, which is called a 'bubble cell'. Wherein the path length is also increased, improving the sensitivity of the system 3–5 times [13]. At the moment the Z-shaped capillaries provide the best compromise between gain in sensitivity and robustness of the system. The major disadvantage of UV detection is the limited sensitivity because of the short optical path lengths that can be obtained. The cross-column pathlengths, which are in the order of 100 mm, are the reason that the concentration limits of detection obtained are normally in the μM range, which is in many cases not sufficient for the analysis of samples.

Other modes of detection include fluorescence and laser-induced fluorescence [22]. On-column fluorescence detection encounters sensitivity limitations, due to the small path lengths provided by the capillaries [14]. Thus, this detection mode is advantageous if selectivity is required, since only a few molecules can produce significant fluorescence. Laser-induced fluorescence (LIF) is a very sensitive detection system, but is instrumentally complex, requiring appropriate laser sources.

As aliphatic carboxylates lack any suitable chromophores or fluorpohores, the sensitive detection of these compounds requires a derivatization. Moreover, the selective derivatization of a particular functional group of a given class of analytes provides high selectivity and the possibility of enrichment of analytes in the process of derivatization. The labelling of analytes with a fluorophore is preferable due to the possibility of highly sensitive detection with laser-inducted fluorescence [22].

Capillary electrophoresis–mass spectrometry (CE–MS) provides an orthogonal approach to analysis in a single analytical run. CE–MS combines the advantage of both techniques, so that quantitative and qualitative information based on migration time, in combination with molecular masses and/or fragmentation patterns can be obtained in one analysis. Currently, electro-spray ionization (ESI) serves as the most common interface between capillary electrophoresis and MS, as it can produce ions directly from liquids at atmospheric pressure, and provide sensitivity and selectivity for a wide range of analytes [16], [23].

In addition to the above mentioned, a variety of the other detection techniques were employed. They were based on such phenomena as chemiluminescence [24], conductivity [25], electrochemical behaviour [26] etc. Conductivity can provide a "universal" detection system. However, the selectivity of such a system could be too low for many analytical tasks [26].

The detection based on any of the above phenomena requires modification of the typical capillary electrophoresis apparatus. Currently, corresponding detectors can be considered research tools rather than routine detection instruments.

D. Detection Mode

Indirect UV Detection is the most frequently used detection mode. An additive is included in the electrolyte which migrates at the same speed as the acids and provides the background signal for indirect detection. It is important that the additive moves at a similar rate to obtain good peak symmetry and improved detedtion limits. Examples that are frequently used are phthalate [27] and PDC (2,6 – pyridinedicarboxylic) [28]. Additive concentrations are typically in the order of 5 – 10 mM to give optimum sensitivity. Choosing the buffer very important is to find the one which provide good buffering capacity with low operating currents. High currents lead to high temperatures within the capillary which generates refractive index changes and poor baseline noise.

Direct UV Detection at low wavelengths is possible because of a limited amount of conjugation of organic acids. This is achieved using inorganic buffers such as phosphate or borate that have no residual UV absorbance. Enhanced detection is possible [29] using wavelengths as low as 185 nm. In some cases [30] to enhance sensitivity and eliminate interference from co-migration spacies, for example 200 nm is used as the primary wavelength with 260 nm as the reference wavelength.

III. Selected Aplications

Short-chain carboxylic acids are determined either as concentrations of individual acids in the sample, or as their total content. Capillary electrophoresis is used to determine short-chain carboxylic acids in liquids, solids or gases. While water samples usually do not require pre-treatment, both solid and gas samples must first be subjected to extraction. A variety of approaches to determine SCCAs in different matrices have been reported, including classical capillary electrophoresis [1], [31]-[34], capillary zone electrophoresis [27], [31], [35]-[41], isotachophoresis [42], [43], and capillary electrochromatography [44]. Each of these versions

of electrophoresis (EC) is discussed in detail.

The choice of mode of CE operations depends largely on the sample type and chemical structure of the target analytes. A typical detection system used in CE is a UV-Vis detector characterized by rather low sensitivity. Combined with a small sample, this makes the limits of quantification (LOQ) rather high. Carboxylic acids are characterized by low light absorption coefficients and generally, determination is carried out using indirect methods of analysis. The detection limit for UV detection reached up to now has been of 2.0×10^{-6} mol/L for acids including oxalic, citric, acetic, tartaric, malic, succinic, lactic, aspartic, glutamic and gluconic in foods and beverages [45]. The same method was applied for the analysis of wastewater samples collected in a dumping area. Nine organic (formate, acetate, butyrate, oxalate, malonate, succinate, phthalate and maleate) and seven inorganic anions were separated by capillary electrophoresis and the measurement of indirect UV absorption was used for detection because the absorbances for most analytes were very low at 254 nm. The detection limit was below 0.5 g/L for all acids [46].

CE with derivatization in an aqueous solution and laser-inducted fluorescence detection was used to determine carboxylic acids in atmospheric air. The labeling of the acids is based on the reaction of 4-aminofluorescein activated with dicyclohexylcarbodiimide (DCC). The reaction procedure includes results with an enrichment factor of the analyte of up to 10. The limits of detection of carboxylic acids are in the range of 3 nmol/L for caprylic acid and 150 nmol/L for acetic acid. The reproducibility of migration times is <1% R.S.D. Using the misting chamber as a sampling system, this method allows the measurement of diurnal profiles of monocarboxylic acids (C5-C9) in ambient air with a time resolution of 1 h for the first time.

Organic acids, which may occur in such beverages as wine, beer, fruit and vegetable juices, were well separated and identified using the CE technique with a photodiode detector after transformation of acids into hydrazides [35]. Capillary zone electrophoresis (CZE) with photodiode array detection (PDA) was applied to simultaneously determine eleven organic acids after derivatisation with 2-nitrophenylhydrazine (2-NPH) in the presence of N-(3-dimethylaminopropyl)-N0-ethylcarbodiimide hydrochloride (EDC HCl). Optimum conditions at 25 ℃ were: 30mmol/L borate buffer, pH 10.0, containing 100ml/L acetonitrile, at 20 kV, sample injection at 0.5 psi for 5 s, with direct detection at 230 nm. Separation of eleven organic acids was achieved within 12min. Linear calibration curves with good fit were obtained in the range of 10.0–100.0mg l/L. Limits of detection ranged from 2.0 to 10.0mg/L. Intra-day precision of a RSD≤4.0% for migration time and ≤5.0% for peak area, and inter-day precision of a RSD≤6.0% and ≤9.0% for migration time and peak area, were obtained. Recovery for all beverage samples was 97 ±15%.

Isotachophoresis was used for the determination of carboxylated oligo(ethylene glycol)s and their degradation products in biodegradation tests of PEG 250 DA [a mixture of dicarboxylated oligo(ethylene glycol)s]. Two tests were performed in the studies: the Organization for Economic Cooperation and Development (OECD) screening test and the river water die-away test. Both biodegradation tests proved relatively fast biodegradation of the studied compounds. In the OECD screening test, the biodegradation was faster than in the river water die-away test which can be ascribed to a higher concentration of bacteria in the biodegradation liquor. Minimal sample pretreatment and relatively low cost of analysis made the isotachophoretic method a good alternative to existing methods of carboxylic acids analysis [47].

This information shows that capillary electrophoresis can be applied to analyse a variety of real samples for the content of saturated and unsaturated mono-and dicarboxylic acids. Detection limits can be reduced by converting the analytes into derivatives, to which the detectors are more sensitive and selective, especially through the transformation of these analytes into derivatives exhibiting fluorescence [22].

The basic parameters of the apparatus used in the determination of short-chain carboxylic acids which have to be optimized for good separation are shown in Table I. The proposed optimization was achieved by Fung-Hwa Chi, Pearl Hsiu-Ping Lin, Min-Her Leu [1].

TABLE I: CAPILLARY ELECTROPHORESIS PARAMETERS FOR DETERMINATION OF SCCAS

PARAMETER	CHARACTERISTICS
Pretreatment	Filtration / dilution
Type of capillary	fused-silica capillary, effective length 40 cm, 50 μm I.D.
Background electrolytes (BGEs)	10 mM p-anisate
Electroosmothic flow modifiers (EOF)	20 mM TRIS (pH 8.0)
Voltage	30 kV
Temperature	25 ℃
Concentration level	ppm level with minimum sample needed (nanoliter)
Time for one analysis	could be completed within 10 min.

The choice of final determination technique depends principally on the properties of the analytes. Determination of SCCAs in various samples depends on the type and the composition of the matrix. For the determination of short-chain carboxylic acids, gas chromatography is most frequently used because of hogh selectivity and low detection limit. High performance liquid chromatography or capillary electrophoresis are the more appropriate separation techniques to determine many other compounds, which can be present in the sample studied e.g. nonvolatile organic acids. If carboxylic acids are in ionized form (pH is sufficiently high) they can be separated as carboxylate anions by anion exchange chromatography (AEC) as well as by electrophoretic techniques. Ion exclusion chromatography (IEC) with cation exchange columns can also be used to separate some carboxylic acids.

The analysis of carboxylic acids in livestock waste, beverages, landfill leachates has been extensively studied. Short-chain carboxylic acids are often determined by well-established chromatographic techniques, such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and ion chromatography (IC). The

methods used are accurate, but there is still a demand for techniques, especially those that avoid time-consuming derivatization. The volatility of many important carboxylic acids is too low for direct GC analysis. The use of HPLC can also be problematic. The detection limit of underivatized carboxylated acids with the most popular UV absorbance detector is often too low due to a lack of proper chromophores in their structure. Derivatization can make the determination of lower analyte concentrations with UV or fluorescent detection possible. However, the approach often requires a complicated and time-consuming isolation and derivatization procedure. Capillary electrophoresis can be a promising alternative for the simultaneous determination of different acids in a single sample. It is a highly specific, quantitative analytical method. The advantages of CE in comparison to HPLC are presented in Table II. Also the comparision several parameters of CE and IC technique are presented in Table III. The measured concentrations of SCCAs in various samples identified by various modes of CE are presented in Table IV.

TABLE II: COMPARISON OF SEPARATION OF AMINO ACIDS USING HPLC (4 MM COLUMN) WITH CONVENTIONAL CAPILLARY ELECTROPHORESIS (CE) ON 75 MM CAPILLARY [35]

	HPLC	CE
Analysis time	30 min	20 min
Cycle time	45 min	25 min
Flow rate	1.5 ml/min	350 nl/min
Mobile phase consumption	68 ml	9 µl

TABLE III: TYPICAL OPERATIONAL PARAMETERS OF IC AND CE [48]

	IC	CE
Sample volume	1–50 ml	< 1–5 nl
Flow-rate	0.1–2.0 ml/min	0.1–2.0 ml /min
Peak volume	ca. 500 ml	1–10 nl
Peak elution time	ca. 30 s	1–5 s
Time of analysis	10–40 min	5–15 min
LOD (UV detection)	ca. 10-7 to 10-9 mol/ l	10-5 to 10-7 mol/ l
Reproducibility of the retention parameters	< 2.0%	< 5%
Precision and accuracy for quantitation	ca. 2.0	ca. 2.0 – 5%

TABLE IV: PRACTICAL APPLICATIONS OF VARIOUS MODES OF CE FOR THE DETERMINATION OF SCCAS IN AQUEOUS, SOLID AND AIR SAMPLES

Sample	Other information	Final determination technique	Detection	LOD Concentration	Ref.
Plant and soil extracts	Separation was carried out on fused-silica capillaries of 75 cm (70.4 cm effective length)350 mm I.D. The UV detector was set for the rapid scanning of an absorbance range between 190 and 230 nm to obtain the optimal wavelength.	Capillary Zone Electrophoresis (CZE)	UV	[µM] Oxalic 8.0; Fumaric 5.0; Tartaric 1.0; Malonic 1.0; Malic 2.0; Citric 1.0; Maleic 1.0; Phtalic 3.0; Acetic 2.0; Benzoic 2.5; Salicylic 3.0; p-Hydroxybenzoic 4.0; p-Coumaric 4.0; Ferulic 4.0; Sinapinic 7.0	[31] 2002
Silage	Samples were extracted with water, purified through a cartridge column to remove interferences, diluted with water, filtered with a centrifugal filter and applied on the CZE.	CZE	Photodiode-array detector (PDA)	[mg/L] Acetic 5.0; Lactic 5.0; Suberic 5.0; Glutaric 2.0; Succinic 2.0; Malic 3.0; Malonic 5.0; Tartaric 3.0; Oxalic 4.0; Citric 3.0; t-Aconitic 10.0	[35] 2007
Silage	The separation was carried out with a fused-silica capillary column at 30kV. A wavelength of 350nm was selected for indirect UV detection and a wavelength of 275nm was selected as a reference. A30mM2,6-pyridinedicarboxylic acid (PDC) solutionwasused as a background electrolyte (BGE) at pH 12 with 0.5mMof cetyltrimethylammonium hydroxide (CTAH)	CZE	PDA	[g/kg] Formate 0–2.6; Acetate 0.7–5.5; Butyrate 0–3.4; iso-valerate 0–2.4; citrate 0–1.8; lactate 0–12.3	[20] 2010
Beverages (wine, beer, vegetable and fruit juices)	The samples were filtered through a 0.45 µm membrane filter and were diluted with water to the appropriate values in order to ensure that the results obtained were in the range of the calibration graph. The diluted samples were then derivatised and analysed by CZE. All analyses were performed in triplicate.	CZE	PDA	[mg/L] Oxalic 0.01; Formic 0.05; Tartaric 0.38; Malic 0.05; Succinic 0.05; Maleic 0.06; Glutaric 0.53; Pyruvic 0.17; Acetic 0.29; Lactic 0.10; Citric 0.23; Butyric 0.52; Benzoic 0.01; Sorbic 0.25; Gluconic 0.94	[36] 2006

Traditional Chinese medicines (Portulaca Oleracea L, Crataegus Pinnatifida, Aloe Vera L)	2.00 g powder of P. oleracea L., C. pinnatifida and A. vera L. was soaked with 20 × 2 mL ethanol in an ultrasonic bath for 30 min, respectively. After ultracentrifugation, the upper clear solution was diluted and metered volume to 50 mL volumetric flask. The solution was kept in a refrigerator. The sample extraction was diluted to proper concentration with ultrapure water (containing 40% (v/v) ethanol) and filtered through a 0.45 _m nylon filter before injected directly into the CE system. Peak identification was performed by standard addition methods.	CZE	UV	[µg/mL] Linolenic 0.09; Lauric 3.44; p-Coumaric 0.08; Ascorbic 0.20; Benzoic 0.02; Caffeic 0.04; Succinic 0.23; Fumaric 0.02	[37] 2012
Coffe	The extraction process consisted in mechanical shaking of 1 g of coffee with particle size under 0.4mm with 10ml of purified water for 10min. Then, samples were centrifuged at 2700 rpm for 5min. All sample solutions were filtered through 0.22mm nylon filters prior to analysis.	CZE	UV	[mM] Acetate 17.59; Formate 156.01; Citrate 0.41; Glycolate 3.58; Lactate 2.01; Malate 1.49; Maleate 0.003; Succinate 1.21	[38] 2004
Atmospheric particulate matter	Aqueous extraction of filters; 2,3-Pyrazinedicarboxylic acid (PZDA) solutions were studied as background electrolytes (BGEs);	CZE	UV	[mg/L] Oxalic 3.104; Malonic 0.930; Succinic 1.533; Glutaric 2.632; Adipic 2.660	[39] 2003
Beer	Degassing, dilution	CZE	UV	Not available. Analyzed µM concentration	[40] 2003
Soil solutions, root extracts	Aqueous extraction, centrifugation, filtration	CZE	UV	[µM/L] Formic 0.49; Acetic 3.96; Lactic 2.01; Tartaric 1.30; Malic 6.00; Citric 3.21; Succinic 2.03	[41] 2003
Biodegradation samples	Equipped with a column coupling system consisting of two capillaries made of fluorinated ethylene-propylene copolymer. The first, preseparation cap- illary (90mm×0.8mm I.D.) was connected to the analytical capillary (160mm×0.3mm I.D.) via the bifurcation block.	Isotachophoresis (ITP)	Conductivity Detection (CD)	[mM/L] Glycolic 0.003; Oxalic 0.001; Acetic 0.003; Formic 0.001; Dicarboxylated; tetraethylene glycol 0.002	[42] 2005
Tobacco	Tobacco (2 g) was extracted with a sulfuric acid solution (100 ml, 5 mM/L) by vigorous shaking for 3 h. The extract was filtered through a 0.45 mm membrane (Millipore) and diluted 25-fold with double distilled water. A 30-ml volume of solution was injected.	ITP	CD	[mM/L] Perchloric 0.006; Oxalic 0.004; Malonic 0.004; Phosphoric 0.002; Formic 0.004; Citric 0.004; Pyroglutamic 0.003; Malic 0.003; Lactic 0.004; Succinic 0.004; Acetic 0.004	[43] 2003
Grape red wines	Prior to chemical analyses, the wines were diluted to the appropriate level with ultra pure water. For the cation and carboxylic acid analyses, samples were diluted 1:20 and 1:50 v/v with water, whereas for the determination of carbohydrate content the samples were analysed both as undiluted and using the dilution of 1:3 (v/v). For quantitation, all the standards were analysed twice in triplicate and samples in triplicate.	capillary electrophoresis (CE)	PDA UV/Vis	LOD of all quantified carboxylic acids was 1 mg/L	[32] 2011
Urine	All the urine samples were obtained from healthy adult volunteers. The sample of pathological urine (methylmalonic aciduria) was kindly provided by the Institute of Inherited Metabolic Disorders (1st Faculty of Medicine, Charles University, Czech Republic). The urine samples were frozen immediately to −20 ◦C and maintained at this temperature until the analysis. Prior to the measurement, the samples were filtered through aDuraporemem-brane (pore size 0.45µm, centrifugal filter devices, Millipore) and diluted ten- or five hundred-times with deionized water. The individual OAs were identified in electropherograms by step-by-step spiking of biological samples with standard solutions of OAs.	CE	CD	[µM] Oxalic 0.6; Fumaric 0.9; Formic 1.6; Tartaric 1.2; Malonic 1.0; Malic 1.1; Citric 4.5; Succinic 1.2; Methylmalonic 1.1; Glutaric 1.1; Pyruvic 6.8; Glycolic 3.1; Acetic 1.9; Acetoacetic 2.4; Lactic 2.9; Glyceric 2.9; Phosphoric 2.4; Propionic 2.5; Pyroglutamic 4.6; Orotic 4.0 3-Hydroxybutyric 3.9 Aspartic 6.5 4-Hydroxyphenylacetic 33.3; Hippuric 50.0; Vanillic 27.3; Vanillylmandelic 7.7; Homovanillic 7.9; 5-hydroxy-3-indoleacetic 14.3; Uric 25.0	[31] 2011

Plants samples Var splendens, which is a common vegetable in China	10-g sample material was weighted and placed into a 50-ml beaker, adding 10 ml deionized water, incubating and homogenizing for 30 min in a water-bath at about 50 ℃. After cooling, the slurry was transfer red quantitatively into a 25-ml volumetric flask.	CE	UV	[ug/mL] Formic 0.01; Tartaric 0.008; Malic 0.01; Citric 0.08; Succinic 0.01; Acetic 0.01; Glutaric 0.03; Lactic 0.01	[33] 2002
Soy sauce, seasoning sauce, mature vinegar	Real samples were directly diluted with double-distilled water to 1:10 (v/v) and filtered through a 0.45 lm filter membrane.	CE	variable wavelength detektor (CV)	[mg/L] Quinic 1.80; Anisic 1.21; Salicylic 2.07; Benzoic 2.19; Sorbic 0.44	[34] 2007

IV. Conclusion

CE is now considered to be a competitive method in the field of separation sciences. It should also be noted that separation mechanisms are fundamentally different from those in GC and HPLC. Nevertheless, real sample analysis of complex matrices requires high separation efficiency. Asides from the classic strategies that can be applied, this review describes a non-exhaustive presentation of recent original strategies for improving electrokinetic separation resolutions. High performance capillary electrophoresis technique is under continuous development.

This technique is often considered to be an alternative and complementary to the commonly used analytical methods, such as high performance liquid chromatography (HPLC) or gas chromatography (GC). The separations are performed on standard CE equipment and capillaries which may prevent the need to purchase specific equipment and consumables such as ion-exchange chromatography system and columns. The capillaries can be rinsed between samples which allow direct injection of liquid samples and can reduce the need for sample clean-up prior to analysis. Capillary electrophoresis allows accurate determination of compounds with similar structures. The method is increasingly used to control technological processes and the identification of many compounds in food products.

In order to determine volatile fatty acids (C3-C6) in liquid manure by this method, a sample can be separated in less than 10 min. Additionally, the main advantages offered by CE are low cost, easy automation, high and easy sample pre-treatment (filtration and dilution).

In the case of capillary electrophoresis, the sample may be of the order of μL/min or even nL or pL. Due to the consumption of reagents and solvents and small samples, capillary electrophoresis (CE) can be regarded as a green analytical technique.

It is clear from the body of work reviewed above that capillary electrophoresis offers significant operational advantages in terms of resolving power and analysis time.

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