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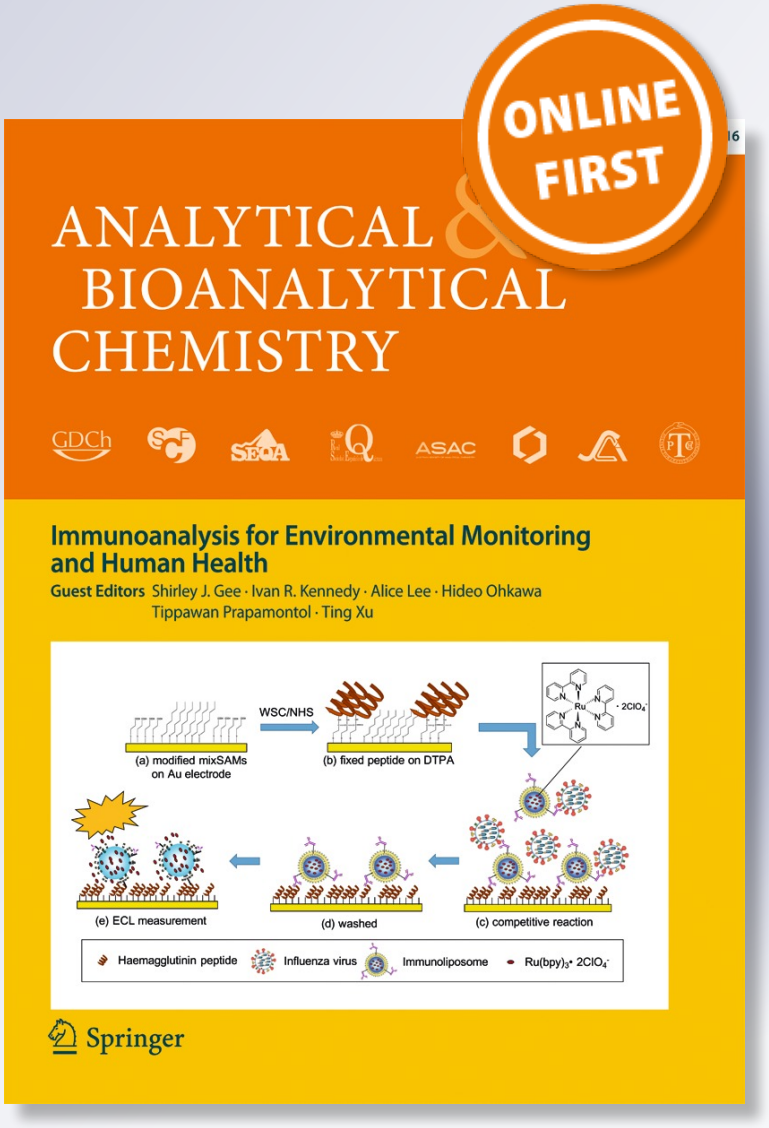

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REVIEW

Microchip electrophoresis for wine analysis

Federico J. V. Gomez[1] · **M. Fernanda Silva**[2]

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Abstract The present critical review provides a summary of representative articles describing the analysis of wine by microchip electrophoresis. Special emphasis has been given to those compounds able to provide background information to achieve the differentiation of wines according to botanical origin, provenance, vintage and quality or assure wine authentication. This review focuses on capillary electrophoresis (CE) microchips dedicated to the analysis of wine covering all the contributions concerning this area. The most relevant compounds in wine analysis such as phenols, organic acids, inorganic species, aldehydes, sugars, alcohols, and neuroactive amines were considered. Moreover, a special section is dedicated to the potential of CE microchip for wine classification. Indeed, potential directions for the future are discussed.

Keywords Bioanalytical methods · Microchip electrophoresis · Wine · Phenolic compounds · Organic acids

Abbreviations

C^4D	Capacitively coupled contactless conductivity detection
CC	Column-coupling
CE	Capillary electrophoresis
ITP	Isotachophoresis
PDMS	Polydimethylsiloxane
PMMA	Poly(methyl methacrylate)
SDS	Sodium dodecyl sulphate
ZE	Zone electrophoresis
μTAS	Micrototal analysis system

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Introduction

Wine analytical characterization for traceability, authentication, or classification purposes is a key target for wine producing countries. Wine consumers worldwide are continuously evolving; they demand information concerning composition, nutritional/health properties, and, more recently, grape growing region (terroir). This beverage has an enormous value-added and it is one of functional food with the highest economic value. With the aim to avoid adulteration and to preserve wine quality in international commerce, robust and reliable analytical methods are of upmost importance not only for producers and administrative authorities but also for customers. The correlation between the composition and organoleptic properties, and oenological and viticultural practices, represents vital information to long-term quality of wines [1].

Wine is the combination of art, science, and nature. Its composition is determined by viticultural and oenological inputs. The chemical profile of a wine is affected by grape, fermentation processes, aging, and storage conditions. Routine wine chemical monitoring includes the analysis polyphenols, anthocyanins, organic acids, volatile compounds, amino acids, sugars, metals, and isotopic analysis. Figure 1 shows all of the components that make up wine.

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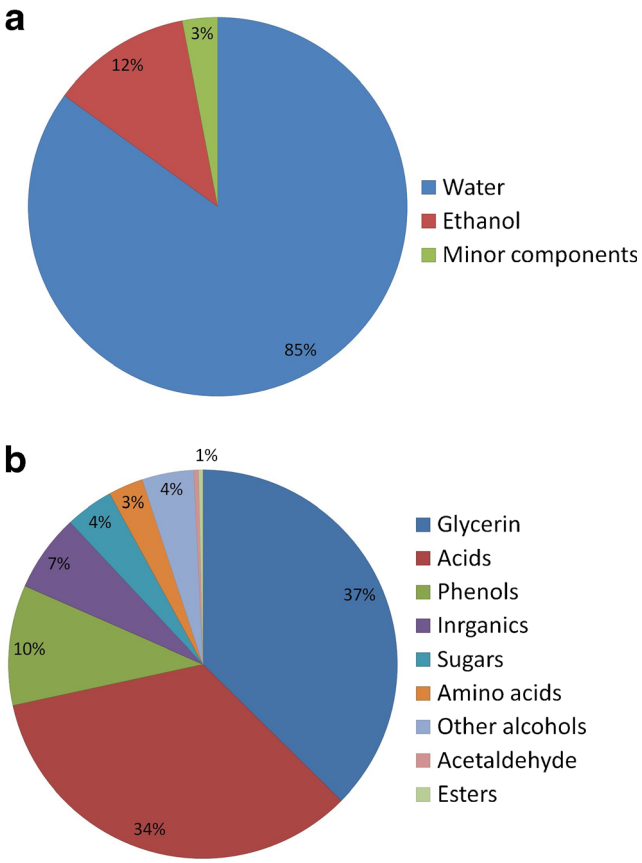


Fig. 1 Major (**a**) and minor (**b**) components of wine

Capillary electrophoresis (CE) is a versatile, robust, sustainable, and low cost technique that has been extensively applied in food analysis and Foodomics. CE tools can be applied for targeted or nontargeted analysis. The first approach is mostly performed for quality and safety purposes. The second one is performed for profiling; with the inherent ability for the simultaneous determination of a vast amount of analytes [2].

During the last decade, miniaturization has been a trend in science and technology. In this context, CE fully adapts to this tendency. Microfluidic chips are excellent examples on the way that miniaturization could provide new analytical technologies. Samples can be processed in a few seconds, with negligible sample and reagent consumption, almost without waste production [3]. Microchip electrophoresis has found mounting interest in biotechnology, environmental monitoring, and food control.

The development of point-of-analysis (in-situ testing) and point-of-care (bedside testing) analysis systems using microchips represents an area of research of great potential and interest [4]. Traditional chemical analyses have been performed in dedicated labs while, at the present time, some studies require on-field analysis. In this sense, microsystems play an essential role since smaller, easy to use, and minimal energy consumption equipment is needed. One fundamental approach for miniaturization is the concept of micrototal analysis system (μTAS) also called "lab-on-a-chip," presented by Manz et al. in 1990 [5]. A microfluidic chip is a small structure of a few cm^2 that contains an array of microchannels used to transport liquids within the chip [6].

Figure 2 shows an overview of the materials for the manufacture of microchip layouts (blue), detection systems (green), and the analytes determined (red) in analysis of wine. As can be seen, CE microchips for wine analysis are manufactured in both glass and polymeric materials, such as polydimethylsiloxane (PDMS) and poly(methyl methacrylate) (PMMA). The most common materials by far are PMMA and glass. This is because they exhibit excellent electroosmotic and electrokinetic properties [7]. The detection route preferred is electrochemical due to its inherent sensitivity, ease of miniaturization, and high technological compatibility with the manufacture of CE microchips. Phenolic compounds and organic and inorganic ions have been the selected compounds studied in wine by CE microchips, being predominantly inorganic species.

The analysis of published works using microchip electrophoresis (1992–2016) indicated that only 12 % of the contributions involve the analysis of food matrices. Most of the approaches were developed for the separation of target analytes in standard solutions. Undoubtedly this can be explained considering the complexity of real-world samples, which cause depletion of the separation efficiency and sensitivity. Thus, a sample preparation step would be mandatory when food matrices are involved.

This critical review focuses on CE microchips regarding the analysis of wine covering all the contributions in this area. The most relevant compounds in wine analysis such as phenols, organic acids, inorganic species, aldehydes, sugars, alcohols, and neuroactive amines were considered. Moreover, a special section is dedicated to the potential of CE-microchip for wine classification. Indeed, future perspectives are discussed.

Phenolic compounds

Phenolic compounds are a family of natural antioxidants with crucial biological roles in plants, foods, and humans (Fig. 3). They are known for their anticancer and antiaging properties, and they represent a most vital, abundant, and ubiquitous chemical family in the plant domain, being synthesized by plants during normal development and in response to different stress situations [8]. The content of phenolic compounds is a significant indicator of wine quality because they have a great influence on sensory attributes and color evolution during storage [1, 9]. On the other hand, these secondary metabolites are important markers for wine classification.

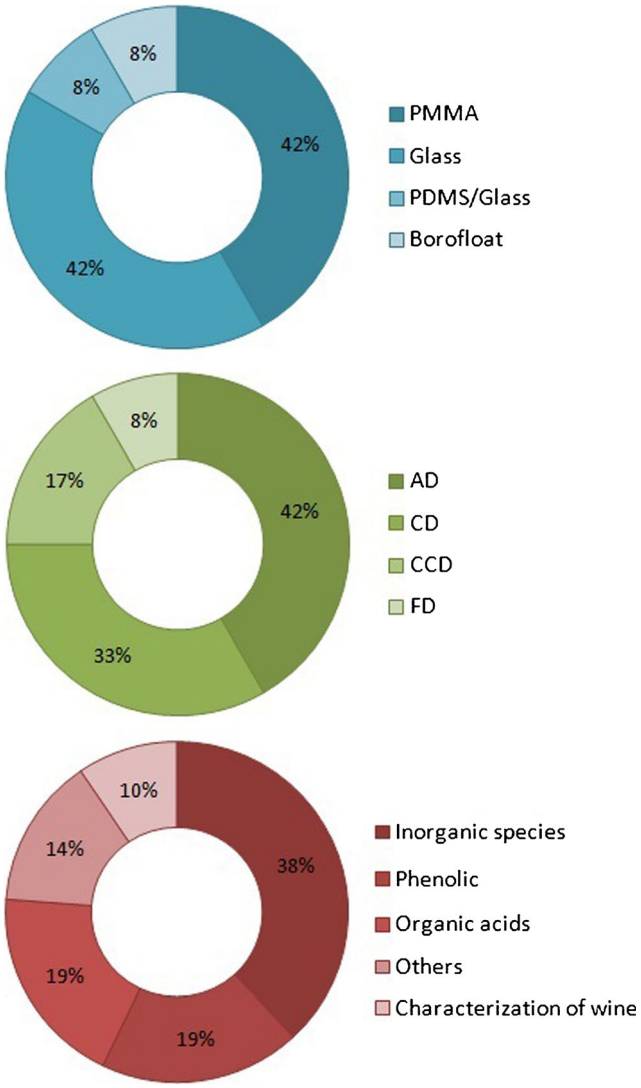
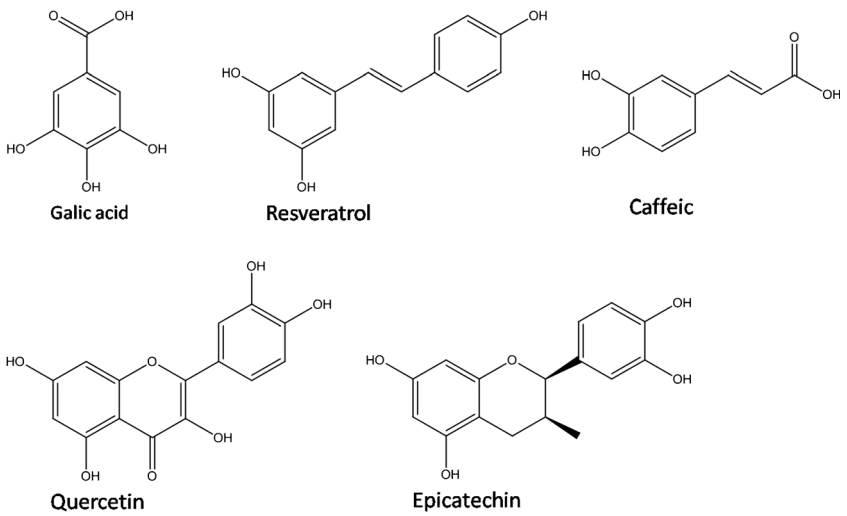


Fig. 2 Overview of the materials for the manufacture of microchips layouts (blue), detection systems (green), and the analytes determined (red)

The determination of phenolic compounds is a challenging analytical task because of the importance of these compounds in vegetal food and beverages. Traditionally, two general approaches of phenol determination have been applied. The first is a total index determination by spectrophotometric detection (Folin-Ciocalteu method), but this universal approach is neither selective nor robust, being highly affected by matrix effects [10–12]. The second strategy involves the separation of the target analytes by HPLC or CE prior to spectroscopy detection. Traditional approaches take long analysis times, while liquid chromatography uses toxic organic solvents..

Microfluidic chips could be a smart alternative to traditional techniques. This technology offers rapid analysis and inherent portability, which also indicate possible future commercialization [3]. In this sense, using a single-cross glass michochip, Kovachev et al. [3] have developed a new microchip approach with amperometric detection in which different pH values were selected in the same device according to pKa values of the phenolic compounds involved. They presented the class-selective electrochemical index determination (CSEID) and individual antioxidant determination (IAD). These methods have been applied to complex food samples, including apple and pear skins and pulps, red and white wines, and green tea tablets. The first methodology (CSEID) classifies by means of a rapid analysis the different phenolic structures according to their antioxidant structure. First, pH of both sample and background electrolyte are established so that flavonoids are neutral species whereas phenolic acids are single ionized species (Fig. 4A). The second approach (IAD) is devoted to separation and determination of individual antioxidants of samples, allowing the separation of nine representative natural phenolic compounds according to their q/m ratio. Catechin, rutin, ferulic acid, chlorogenic acid, vanillic acid, quercetin, caffeic acid, gallic acid, and protocatechuic acid have been separated in borate buffer at pH 9 in only 260 s (Fig. 4B). Samples of red and white wines were diluted before

Fig. 3 Chemical structure of most common phenolic compounds present in wine



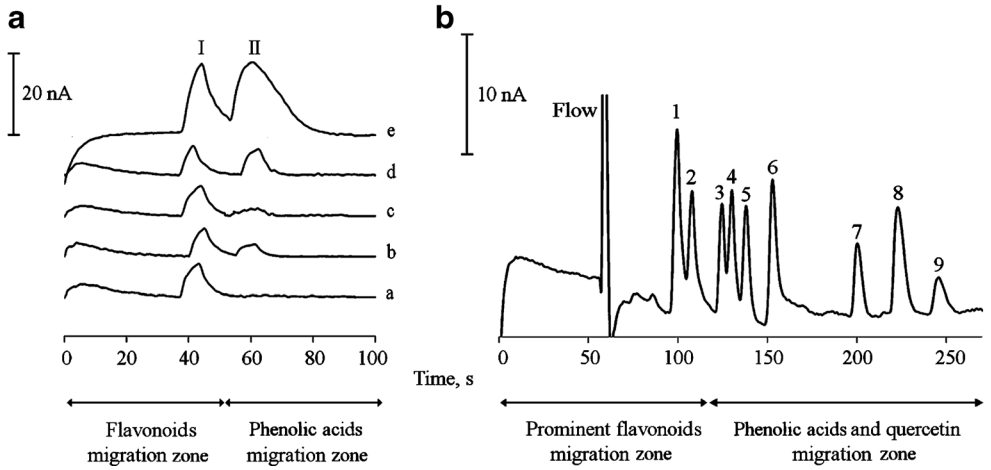


Fig. 4 Analytical approaches visualization. (**A**) class-selective electrochemical index determination (MES, pH) 5, 10 mM); (**I**) total flavonoids peak, (**II**) total phenolic acids peak (analyte concentration of the different compounds in all cases: 50 μM each). Mixtures' composition (**a**) (+)-catechin; (**b**) (+)-catechin + gallic acid; (**c**) (+)-catechin + rutin + ferulic acid; (**d**) (+)-catechin + ferulic acid + gallic acid; (**e**) all nine analytes. (**B**) Individual antioxidant determination (borate, pH) 9, 10 mM); (1) (+)-catechin 80 μM, (2) rutin 80 μM, (3) ferulic acid 80 μM, (4) chlorogenic acid 80 μM, (5) vanillic acid 80 μM, (6) quercetin 80 μM, (7) caffeic acid 200 μM, (8) gallic acid 80 μM, and (9) protocatechuic acid 200 μM. In all cases, $t_{injection}$ = 3 s, $V_{separation}$ = 2 kV, and E = +1 V. Reprinted with permission from [3]. Copyright (2016) American Chemical Society

analysis. The good resolving power of this approach has been demonstrated and could be the basis for future developments.

In other work, Scampicchio et al. [13] have developed a microchip CE procedure for measurements of phenolic acids present in food (chlorogenic, gentisic, ferulic, and vanillic acids) with end-column amperometric detection. The glass simple-cross microchip (88 mm × 16 mm) consisted of a four-way injection cross. The original waste reservoir was cut off, leaving the channel outlet at the end side of the chip in order to allow end column amperometric detection. The working screen-printed carbon electrodes applied in this work were printed using carbon ink. The suitability of the CE microchip for measuring μmolar concentrations of phenolic acids was demonstrated by analyzing wine samples spiked with chlorogenic acid, gentisic acid, ferulic acid, and vanillic acid. Under optimum conditions, the analytes could be determined, utilizing borate buffer with MeOH, within 300 s using a separation voltage of 2000 V and a detection voltage of +1.0 V. Due to the small sample amounts involved, no typical surface fouling was observed. It has to be pointed out that the approach could be applied to the fast analysis of several foods with low cost.

In a related work but using a lab-fabricated microchip based on PDMS and glass, Nikovaev et al. [14] have developed a CE microchip with electrochemical detection for determination of polyphenols in red wine. The manufacturing of the chip included the following steps: manufacturing a silicon matrix for molding a polymer part of the chip; molding the polymer part of the chip of PDMS; making gold electrodes for an object glass; and joining of the polymer and glass parts. PDMS is a hydrophobic material, channels are poorly wetted by aqueous solutions, hydrophobic substances could be adsorbed on the walls, and bubbles are easily formed even in very dilute buffer solutions. In this study, the authors examined two options of chip treatment: (1) oxidation of the PDMS surface in a gas discharge, and (2) dynamic modification with surfactants (SDS, sodium deoxycholate, and 1-dodecyl-3-methylimidazolium chloride). The reproducibility of the electroosmotic flow (EOF) and efficiency for each of the components at different methods of surface modification were assessed. After plasma treatment of the surface, a higher efficiency and the most complete separation of phenolic components were achieved compared with anionic detergents. After modification with an ionic liquid, the efficiency was higher than in the case of oxidation in the discharge. In using plasma and SDS, the sample components and the EOF traveled in different directions, which led to an increase in the migration times and the broadening of the analyte zones due to diffusion. Finally, SDS was selected as a modifier because the chips remained separable (treatment in the discharge resulted in an irreversible adhesion of the parts), and in the case of clogging of the channel, the polymer part can be easily replaced. After the selection of conditions for the separation of catechols and the determination of the analytical parameters, the electrophoretic analysis of red wine samples was performed in less than 240 s, in which the concentration of polyphenols reached hundreds of micrograms per milliliter.

Organic acids

Organic acids (Fig. 5) are important constituents of wine that affect wine sensory attributes, influencing the taste and mouth-feel, color stability, avoid oxidation, and contribute to

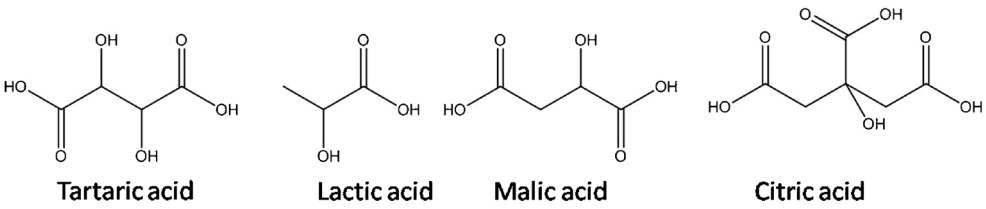


Fig. 5 Chemical structure of main organic acids present in wine

microbial and physicochemical stability [15]. They are metabolites of sugar oxidation (tartaric, citric, and malic acid) or of alcoholic fermentation during wine production (succinic, acetic, and lactic acid).

Their analysis in wine is usually carried out with the aim to monitor fermentation processes, product stability, and organoleptic properties. In this sense, α-hydroxy acids (tartaric, malic, lactic, and citric acids), are mainly responsible for total acidity of wines, and they are routinely determined in wines and its precursors. Nevertheless, organic acids constitute potential markers for wine discrimination. The content of organic acids is higher in young wines [16]. Chemical reactions during wine production (fermentation and aging) produce the above-mentioned effect, attributable to precipitation of acid salts, acid decarboxylation, as well as acid-microorganism interactions.

On the other hand, it has recently been demonstrated that organic acids relative concentrations are also interesting tools for varietal discrimination [17]. Vintage-based classification is also possible, oxalic, tartaric, malic, and fumaric acids being the classification markers.

For years, organic acids have been determined by UV-Vis spectrometry, enzymatic methods, and chromatography. Taking into account the ionizable nature of the acid functional group, capillary electrophoresis is ideal for their analysis. Optical detection technique, in particular the most sensitive laser-induced fluorescence (LIF), is not adequate for the analysis of small ionic compounds considering poor signal to noise ratios. Indeed, this technique is not easily integrated into lab-on-a-chip devices. Derivatization is required, and the lack of suitable fluorescent moieties is a restrictive factor. Hence, amperometric and conductometric detection techniques have been used in microchip format, conductometry being the choice for the detection of charged species. Table 1 presents the most representative works for the analysis of organic acids in wines by microchip electrophoresis.

A pioneer work for the analysis of organic acids in wines by chip-based CE was presented by Masár et al in 2001 [18]. They developed a PMMA planar chip with column-coupling (CC) configuration of the separation channels and on-column conductivity. ITP separation mode was used (pH range of 2.9–6.0), utilizing methylhydroxyethylcellulose as suppressor of the electroosmotic flow. Red and white wines were diluted 20–100 times prior to injection. Chloride was used as leading ion, β-alanine as counter-ion, and glutamate or capronate as terminating ions. Reproducibility was better than 2 % for wine samples. Nevertheless, the major drawback of this approach is that analysis times (more than 10 min) are comparable to conventional capillary zone electrophoresis.

The same group [19] presented a zone electrophoresis (ZE) approach for the determination of organic acids in wine on a PMMA CE microchip with integrated conductivity detection. They studied the effect of the different separation mechanisms involved (pK values, host–guest complexations, and ionic strength) to separate 22 organic acids in wines. Polyvinylalcohol was used as EOF suppressor and α- and β-cyclodextrin as complexing agents. pH was set at 5.90 in order to prevent anionic migration of amino acids, phenolic constituents, and other very weak acids present in wine. Commercial as well as reference wine samples were analyzed. Samples were diluted (100–400 times) in sodium fluoride solution (ITP stacker) and *N*-acetylserine (internal standard). Recoveries within the range of 90 to 106 % were obtained for the most representative acids with satisfactory reproducibilities (lower than 2 and 5.3 % for migration times and peak areas, respectively). Nevertheless, separation in a 115 mm length channel took more than 10 mins.

In 2005, Kubáň and Hauser [20] developed a PMMA CE microchip in a cross injector configuration with capacitively coupled contactless conductivity detection (C^4D). Two electrodes with antiparallel orientation were separated by a 0.5 mm gap, which determines the detection volume of the C^4D cell. The electrodes were prepared by lithographic etching. The excitation and output voltages of the C^4D cell were monitored using a dual-channel oscilloscope. The electrolyte solutions contained L-histidine and L-glutamic acid (pH 5.75). Wine was diluted (1:20) with an electrolyte solution before injection onto the microchip. Tartrate, malate, citrate, succinate, acetate, and lactate were found in red wine, tartaric acid being the most abundant. RSDs of migration time were better than 0.4 % for all cases. The approach presented by Kubáň and Hauser is an excellent alternative to traditional systems, considering its robustness and total analysis time (70–90 s for inorganic and organic anions). Additionally, its versatility should be considered; the same microchip configuration is suitable for the analysis of inorganic cations (see Inorganic species section).

Table 1 Analysis of organic acids in wines by microchip electrophoresis

Anion	Microchip	Detection	Technique	Wine	Conc. (mg L^{-1})	Ref.
Tartrate	PMMA simple cross	C^4D	ZE	Red	2040 ± 16.5	[20]
				White	NI	
	PMMA CC configuration	On-column CD	ITP	Red	1243.6	[18]
				White	1072.7	
	PMMA CC configuration	CD	ZE	Red 1*	1210	[19]
				Red 2*	1500	
				Red 3*	1050	
				White 1*	2470	
				White 2*	1630	
Citrate	PMMA simple cross	C^4D	ZE	Red	158 ± 2.2	[20]
				White	NI	
	PMMA CC configuration	On-column CD	ITP	Red	821.3	[18]
				White	488.2	
	PMMA CC configuration	CD	ZE	Red 1*	100	[19]
				Red 2*	90	
				Red 3*	140	
				White 1*	850	
				White 2*	180	
Malate	PMMA simple cross	C^4D	ZE	Red	171.4 ± 5.1	[20]
				White	NI	
	PMMA CC configuration	On-column CD	ITP	Red	3976.8	[18]
				White	3576.9	
	PMMA CC configuration	CD	ZE	Red 1*	100	[19]
				Red 2*	750	
				Red 3*	2490	
				White 1*	3250	
				White 2*	4060	
Lactate	PMMA simple cross	C^4D	ZE	Red	1035 ± 23.3	[20]
				White	NI	
	PMMA CC configuration	On-column CD	ITP	Red	814.9	[18]
				White	547.4	
	PMMA CC configuration	CD	ZE	Red 1*	850	[19]
				Red 2*	1080	
				Red 3*	810	
				White 1*	2130	
				White 2*	230	
Succinate	PMMA simple cross	C^4D	ZE	Red	480 ± 6.7	[20]
				White	NI	
	PMMA CC configuration	CD	ZE	Red 1*	710	[19]
				Red 2*	660	
				Red 3*	700	
				White 1*	470	
				White 2*	290	
Acetate	PMMA simple cross	C^4D	ZE	Red	250 ± 11.1	[20]
				White	NI	
	PMMA CC configuration	CD	ZE	Red 1*	210	[19]
				Red 2*	330	
				Red 3*	510	
				White 1*	840	
				White 2*	700	
Oxalate	PMMA simple cross	C^4D	ZE	Red	ND	[20]
				White	NI	

ND: Not detected

NI: Not informed

*: Reference wines

Inorganic species

The elemental profile of wines is the result of the contribution of two primary sources: (1) natural or endogenous sources (minerals from soil); (2) exogenous sources (fertilizers, inorganic pesticides/herbicides, pollution, additives during winemaking, etc.) [21].

The content of in inorganic species in wine is mostly determined by flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS),

inductively coupled plasma mass spectrometry (ICP-MS), or inductively coupled plasma optical emission spectrometry [22], and its characterization significantly contributes to the "terroir" (grape-growing region) correlation [23].

Kubáň and Hauser [20] also presented the analysis of inorganic cations by using the PMMA microchip CE mentioned in the Organic acids section. A BGE containing histidine, acetic acid, and 18-crown-6 at pH 4.1 was used for the determination of NH_4^{+1}, K^{+1}, Ca^{+2}, Na^{+1}, and Mg^{+2} in less than 35 s (Fig. 6). The major inorganic cations present in wines are potassium, calcium, sodium, and magnesium. Limits of detection within the range 90–200 mg L^{-1} were obtained, K^{+1} being the most abundant in red wine (1100 mg L^{-1}). The same work presented also the analysis of inorganic anions (same BGE mentioned in the Organic acids section) in red wine, and they reported the contents of chloride (9.1 mg L^{-1}), sulfate (351.0 mg L^{-1}), and phosphate (166.7 mg L^{-1}) in red wine.

In 2006, a remarkable CE biochip was presented [24], incorporating a contactless conductivity detector in a microfluidic biochip. Semicircular detection electrodes were developed incorporating two buried shielding electrodes located on either side of the channel; a field effect is generated between the electrodes and the analytes are identified by changes in capacitance as the sample flows through the detection region between the electrodes. Samples were electrokinetically driven. The CE biochip allowed the determination of Na^{+1}, Ca^{+2}, K^{+1}, and Mg^{+2} in red wine with limits of detection comparable to LIF detection, providing sample separation and detection simultaneously, representing a viable basis for μ-TAS devices.

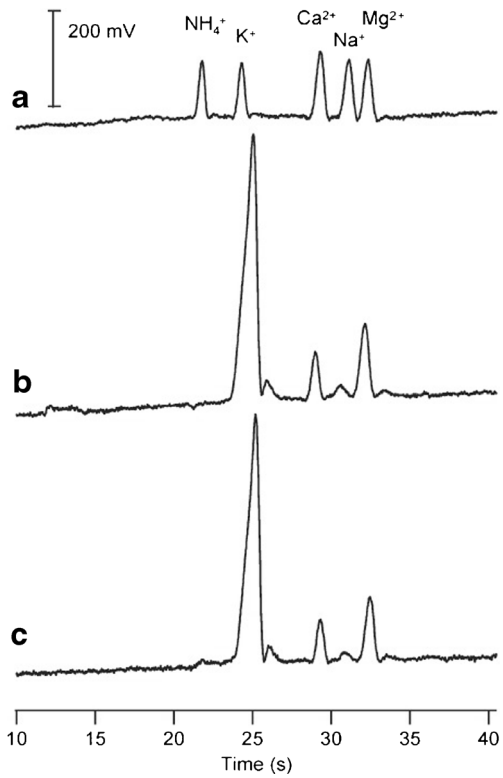


Fig. 6 EPG of determination of inorganic cations in wine samples. Electrophoretic conditions: microchip, 85/75 mm total/effective length, background electrolyte, 10.5 mM His, 50 mM acetic acid, 2 mM 18-crown-6 (pH 4.10); separation voltage 4 kV, injection 1 kV for 2 s. Reprinted with permission from [19]. Copyright (2016) John Wiley and Sons

Sulfiting compounds during winemaking are of upmost importance to prevent microbial growth and stabilize flavor [25]. However, detrimental effects of the sulfiting agents have been reported, and daily intake of sulfite is regulated; its concentration in beverages should be lower than 10 mg L^{-1} (EU regulation 1169/2011). In 2004, Masár et al. [26] presented an interesting zone electrophoresis (ZE) with on-line isotachophoresis (ITP) sample pretreatment on a column-coupling (CC) PMMA chip with conductivity detection system for the determination of free sulfite in wine and a pre-column conversion of sulfite to hydroxymethanesulfonate to minimize oxidation. Methylhydroxyethylcellulose was the EOF suppressor. The ITP–ZE approach is highly selective and robust, but the analysis time is rather long and several steps are involved in the procedure.

The analysis of inorganic species by microchip electrophoresis is far from being mature and future developments are required in order to apply this technology for regulatory purposes.

Others (aldehydes, sugars, alcohols, neuroactive amines, etc.)

Tyramine and histamine, produced by the decarboxylation of tyrosine and histidine, are dangerous biogenic amines found in fermented beverages. Ingestion of foods with high contents of tyramine can induce hypertension and panic attacks [27]. Histamine in wine can induce headaches in patients prone to alterations of diamine oxidase activity. CE offers interesting possibilities for biogenic amine analysis because of its high sample throughput and miniaturization compatibility [28–33]. Jayarajah et al. [4] used a portable microchip CE instrument for the determination of neurologically active biogenic amines, tyramine and histamine, in fermented beverages [Mars Organic Analyzer (MOA)], (Fig. 7), which includes laser excitation, optical detection system, and electrophoresis power supplies. The analytes were derivatized with fluorescamine, and the samples were directly analyzed in only 120 s. Results show that tyramine, the principal amine in red wine (<1–3.4 mg L^{-1}), biosynthesis occurs during malolactic fermentation. Although histamine is produced during yeast fermentation (1.8–19 mg L^{-1}), its content increments during secondary fermentation and storage. The authors proposed that using the MOA, winemakers can monitor key amines in situ, an important parameter for regulatory purposes. The

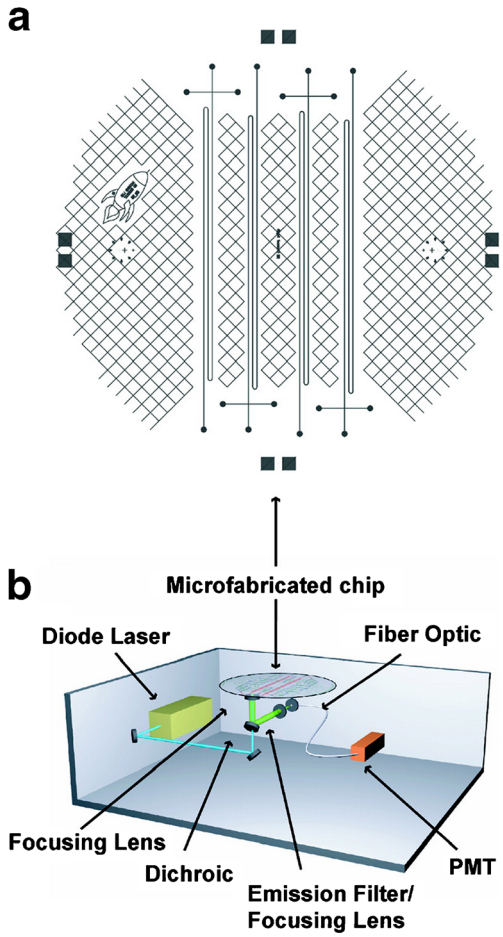


Fig. 7 Schematic of the adapted microchip capillary electrophoresis chip and instrument. (**a**) The microfabricated chip consists of four folded separation channels 21.4 cm in length, each with a 0.6-cm-long, 70-μm-wide injection channel placed 0.6 cm from the anode reservoir. The crosshatch marks are included in the chip design for improved bonding. (**b**) This microchip is placed against the planar face of a composite objective through which the confocal excitation and fluorescence detection is performed. Electrophoresis power supplies, a thermoelectric cooler for temperature control, and pneumatic valve actuators are also contained within the instrument. The 11-kg instrument measures 10-12-4 in. Reprinted with permission from [4]. Copyright (2016) American Chemical Society

work [4] has the potential to be applied for correlating the contents of tyramine and histamine with different genotypes, and thus consumers could take advantage of this knowledge to prevent health risks.

The ability to determine quickly and simultaneously ethanol and sugars is of upmost importance for alcoholic beverages. Determination of alcohols and sugars in wines using a simple cross glass microchip with end-column electrochemical detection has been reported [34]. The nickel detector used in this work was set on the channel outlet by an electroless deposition procedure. This easy and low-cost electroless preparation route can be developed in any wet-chemistry laboratory and avoids the need for photolithographic electrode fabrication or careful channel/electrode alignment. The on-chip nickel detector displays well-defined concentration dependence and offers high sensitivity and robustness. A series of eight repetitive injections of a mixture of ethanol and glucose resulted in low RSDs for the current response and migration times. Wines were analyzed without any sample preparation, and only 100-fold dilutions were necessary. Well-defined and baseline-resolved signals were obtained in 200 s.

Wine characterization

The approaches employed for authentication and quality control of wine protect consumers from illegal adulteration practices [35, 36]. During the past few years, innovative analytical approaches concerning trace analysis and/or simultaneous determination of a wide number of analytes have been coupled to powerful data analysis systems. Consequently, wine traceability, discrimination, and classification of grapes and wines (mainly in terms of grape varieties, geographical origin, and wine-making technologies) have become possible [36].

Chemometrics analysis methods have been applied to analyze data obtained by ICP-AES [37–39] and ICP-MS [40], flame AAS [38], GC-MS [41], HPLC and CE [42]. Most of these methods are expensive, time-consuming, require sample preparation, and often make use of large amounts of toxic solvents/reagents. With the aim to solve this, Wang and coworkers [43] reported a CE glass microchip device with electrochemical detection for wine fingerprinting of different geographical regions by the use of multivariate data analysis. They applied high separation potentials to get a complete electropherogram in less than a minute. They reported marked effects of separation voltage on electrophoretic behavior. The obtained electropherogram was split into seven characteristic zones, arbitrarily chosen after a study of several replicates of each type of wine. Obtained data were turned into initial 14 variables, concerning peak's area and peak's height. Principal component analysis (PCA) was applied to analyze electrophoretic data. Thus, a complete classification of Italian, Australian, and New Mexico Cabernet Sauvignon red wines takes less than 1 min, with a 100 % correct classification in prediction. Although nonparametric methods (CART analysis) requires only 30 s, the predictive power is worse. A great advantage of microchip chemometric classification is that no sample prep is needed. The coupling of microchips with chemometric approaches demonstrated the possibility for wine classification, as well as endless possibilities for a wide range of applications

Wine is one of the most vulnerable products to food adulteration, even though there are strict international regulations to protect its authenticity. Nevertheless, powerful analytical methodologies are necessary to detect wine adulterations, including wrong statements of varietal and geographical origins.

Traditional traceability methods are expensive and involve long analysis times. In this regard, Recupero et al. [44] proposed the use of microchip electrophoresis for polymorphisms detection, to determine the presence of foreign grape in 'Nebbiolo'-musts produced in purity. The CE microchip used in this work was a commercial 2100 Bioanalyzer from Agilent Technologies (Agilent Technologies Ltd., South Queensferry, UK). Polymorphisms related to a specific locus are due to the variation in length of the microsatellite, which depends on the number of repetitions of the basic motif. They are common genetic markers, attributable to their codominant inheritance, great allelic diversity, high abundance, ease of assessing SSR size variation through PCR with pairs of flanking primers, and high reproducibility [45]. Although their approach presents some practical inconveniences, particularly concerning the yield of extraction (and the quality) of grape genomic DNA isolated from wines, the combined use of the end-point amplification and the Lab-on-chip capillary microelectrophoresis technique provides an objective and reproducible tool for the authentication of 'Nebbiolo'-based musts. Indeed, this technique reached a sensitivity/selectivity not achievable by traditional agarose gels, without using either polyacrylamide gels or expensive sequencing analysis.

As it has been stated that chip electrophoresis is safer and faster than classic electrophoresis, so it has been proposed to analyze the saliva precipitation index (SPI) of wines and grapes [46]. The SPI is an approach based on the precipitation of selected salivary proteins by polyphenols [47, 48]. The method involves a binding reaction between wine solutions with human saliva at 37 °C for 5 min that reproduces the conditions in mouth during tasting. Then the reaction mixture is centrifuged at 4 °C in order to separate polyphenol-bound proteins from the proteins that have not interacted. Satisfactory correlation of SPI and sensory analysis were found [48], concluding that SPI is a good astringency parameter. Chip electrophoresis in the same manner as for SDS-PAGE was proposed. A commercial Experion Pro260 (Bio-Rad, Milano, Italy) analysis kit and Experion system were used. Several wines (four Aglianico, five Merlot, and five Cabernet Sauvignon) and grapes (five Aglianico skin and seed extracts) were analyzed. Quantitative data were obtained by the interpolation of the relative decrease of fluorescence of selected protein bands versus the concentration of tannin B expressed as gallic acid equivalents. Chip electrophoresis is an interesting alternative of SDS-PAGE to obtain SPI, a crucial tool to evaluate wine astringency

Conclusions and future outlooks

The wine industry encompasses one of the most economically important worldwide commodities in the agro-food area. Wine is considered as one of the oldest beverages and it has become a sign of social status and increasingly marketable activity. Moderate consumption of wine is related to several health benefits, including reduction of the risks of coronary heart disease, cellular aging damage, cognitive function, and atherosclerosis.

During the last two decades, CE microchips have demonstrated their potential for in situ analysis and fast multiparameter analysis. The application of CE microchips in food analysis is expanding rapidly [23], considering the following advantages over traditional CE: insignificant consumption of reagents and samples, the capability of fast, in situ automated analysis, and the great potential of electrochemical detection. Achieving adequate sensitivity has been a major challenge in CE microchip. In this regard, LIF detection has been widely used because of its ease in focusing and high sensitivity. Electrochemical detection has gained importance considering that electrochemical systems are miniaturizable without loss of performance and are compatible with microfabrication technologies with intrinsic good sensitivity. Robustness of electrochemical detection is also a key parameter to consider; in most cases it is superior than for traditional applications of electrochemical detection in conventional CE. In this regard, alternative electrochemical detectors will keep growing technologically, particularly novel materials for electrode modifications and new techniques for electrode fabrication. That is why the use of green solvents such as natural deep eutectic solvents (NADES) [49] or novel nanomaterials [50] that could enhance electrochemical detection are promising areas of research.

The analysis of wine in lab-on-a-chip devices is on its first steps. Endless opportunities for the creativity of researchers appear in the near future. The development of a technology is evaluated by its real-life application. So, comprehensive studies that could allow wine classification by means of microsystems are needed, as well as their correlation with traditional technologies. Chemometric applications, which are capable of handling huge analytical wine data, constitute very promising tools for assessing wine classification. Nontargeted analysis in CE microchips for wine analysis is also a very promising area of research; such studies could provide an important advance for varietal discrimination, a topic of upmost importance for wine marketing. In the near future, the wine industry would benefit from robust, portable, and low-cost commercial microchip CE devices dedicated exclusively to the wine industry.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest

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