Trends in Analytical Chemistry 161 (2023) 116974

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Capillary electrophoresis in phytochemical analysis: Advances and applications in the period 2018–2021

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ARTICLE INFO

Article history:
Received 4 November 2022
Received in revised form
1 February 2023
Accepted 7 February 2023
Available online 13 February 2023

Keywords:
Capillary zone electrophoresis
Micellar electrokinetic capillary chromatography
Capillary electrochromatography
Mass spectrometry
Natural compounds

ABSTRACT

The present article revisits the recent articles where capillary electromigration techniques have been applied to study numerous phytochemicals between the years 2018 and 2021. The manuscript has been divided according to the different separation methods used, and the main aspects of each application have been briefly discussed, including sample preparation, advantages and disadvantages of the developed methods, problems encountered during the process and ways to solve them.

1. Introduction

Phytochemicals refer to a wide variety of compounds naturally occurring in plants which confer them with color, aroma, flavor and protection against infections and predators. In addition to their original characteristics, these compounds may also exert protective or disease-preventing effects in humans, and therefore much research effort has been focused on their analysis during the last decades [1]. Generally, phytochemical compounds include carotenoids, phenols, nitrogen compounds, organic sulfur compounds and plant sterols and other terpenoid structures. In addition, the chemical variability within each category is very significant with thousands of diverse compounds identified in nature. The importance of these compounds has led to the development of different analytical methods directed to their characterization in different matrices, such as plants or foods. Most of these methods are based on chromatographic techniques, such as one- and two-dimensional paper chromatography, one- and two-dimensional thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and concurrent chromatography (CCC). However, the application of capillary electromigration techniques is not an exception in this field, as they can outperform the aforementioned analytical techniques. Capillary electrophoresis (CE) modes has been largely applied for the separation of an extent variety of compounds due to its low reagent and sample requirements, versatility, comprehensiveness and simple operation, and the development of new methodologies and instrumentation is an active research field [2]. This is also confirmed by the information given in Table 1, where selected review papers published on the phytochemical analysis in the period covered by the current

Abbreviations: ACN, acetonitrile; BGE, background electrolyte; CCC, concurrent chromatography; CE, capillary electrophoresis; CMC, critical micellar concentration; CTAB, cetyltrimethyl ammonium bromide; CZE, capillary zone electrophoresis; DAD, diode array detector; EO, essential oil; EOF, electroosmotic flow; FASI, field amplified sample injection; GC, gas chromatography; HPLC, high performance liquid chromatography; i.d., internal diameter; LIF, laser-induced fluorescence; LINF, laser-induced native fluorescence; LOD, limits of detection; LOQ, limits of quantitation; MEKC, micellar electrokinetic capillary chromatography; MS, mass spectrometry; PCA, principal component analysis; PEEK, poly(ether ether ketone); PLS, partial least squares; PMP, 1-phenyl-3-methyl-5-pyrazolone; PSP, pseudostationary phase; RP-LC, reverse phase-liquid chromatography; SC, sodium cholate; SDS, sodium dodecyl sulphate; TLC, two-dimensional thin-layer chromatography; TRIS, tris(hydroxymethyl)aminomethane; VP, voltage program.

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https://doi.org/10.1016/j.trac.2023.116974

Table 1
Some representative review papers published on the use of capillary electrophoresis modes for phytochemicals analysis in the period 2018–2021.

Topic or Matrix	Year	Ref
Phytochemical analysis (2014–2017)	2018	[3]
Bioactive compounds from natural products	2018	[4]
Phenolic compounds in plants and plant-derived food	2019	[5]
Analysis of curcuminoids in turmeric	2019	[6]
Screening active natural components using bioaffinity techniques	2020	[7]
Phytochemicals in vegetables oils and foods	2020	[8]
Analysis of plant phloem and xylem saps composition	2020	[9]
Fatty acids determination in vegetable and marine oils	2021	[10]
Plant alkaloids by CZE and MEKC	2021	[11]
Phytochemicals in Traditional Chinese Medicine	2021	[12]

work (2018–2021) are summarized [3–12].

Due to the high amount of CE applications developed in this period, and to facilitate the organization and ease of reading, these applications have been grouped according to the electroseparation method used, being capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MEKC) and capillary electrochromatography (CEC) the most used techniques. In the following sections, the separation modes are briefly reviewed, and the sample preparation methods (extraction and pre-concentration) and the applications found are discussed.

2. Capillary zone electrophoresis (CZE) mode

In this section, some aspects regarding the applications found involving the CZE mode (also called free solution capillary electrophoresis, FSCE) in phytochemical compounds, are discussed. The applications are the most diverse, as studies are found with fruit pulp, plants, algae and grains, among others. The objectives of the analysis ranged from the characterization of the sample, the study of the profile of the compounds for association with the species, the region of cultivation, the detection of possible adulterations and the study of the regulation of the plant's defensive response, as some examples. The applications found are limited to the use of three detection systems hyphenated to CZE, namely, diode array detector (DAD), the laser-induced fluorescence (LIF) and mass spectrometry (MS). Representative applications are compiled in Table 2, where analytes, sample, detector, and composition of the background electrolyte (BGE) are highlighted.

CZE is the most used electrophoretic separation mode among all electromigration techniques, where the separation of analytes occurs due to the difference in mobility of positively or negatively charged species. The separation of analytes takes place inside a capillary tube, normally made up of bare fused silica. The capillary is filled with a BGE, which at pH usually higher than 3 or 4 brings about the ionization of the silanol groups of the inner capillary wall and under the application of an electric field, an electroosmotic flow (EOF) is generated inside the capillary that sends the analytes from the anode to the cathode [3–12].

The BGE can be aqueous, can have an organic solvent fraction, or can be an organic solution. In the latter case, some authors considered this as a new mode of CE, called non-aqueous capillary electrophoresis (NACE) [13], although the International Union of Pure and Applied Chemistry (IUPAC) recommends that this term be no longer used [14]. The capillaries most used in CZE have an internal diameter (i.d.) of 50 μm or 75 μm, however, the use of thinner capillaries (e.g., 20 μm i.d.), can be interesting to minimize the effect of the current when BGE are very concentrated, improving the resolution of the compounds in the baseline [15]. Capillary length can vary by each method, but capillaries with an effective length of 50 cm (to the detector) are more common when using CE-DAD or CE-LIF systems, and capillaries around 90 cm in length when MS detection is selected.

The most used CE detector is the DAD, which is usually applied to the detection of compounds that can absorb energy in the ultraviolet–visible (UV–Vis) region. When passing through the detector, the compound causes a positive change in the observed signal and therefore the detector registers a positive peak, which is called direct detection.

It is also possible to detect compounds with low or no energy absorption capacity in the UV–Vis region by including chromophore species in the electrolyte solution, that is, a compound with higher molar absorptivity at the working wavelength, such as sodium dodecylbenzenesulphonate or adenosine monophosphate [16]. In this way, the baseline signal has high absorption, and when passing through the detector, the compound causes a negative variation in the observed signal, which the detector records as a negative peak called indirect detection [17–19].

As an alternative to UV-DAD detectors, sensitive LIF detection systems can be used. The main advantages of LIF are the good monochromaticity and high intensity of the laser technology, with an excellent excitation light source for on-column capillary detections. Although different derivatization reactions are routinely employed to convert the target analytes into their fluorescence sensitive derivatives, label-free laser-induced native fluorescence (LINF) detection greatly simplifies the CE-LIF analysis.

It is also possible to couple different MS analyzers to CE systems, such as single quadrupole, triple quadrupole (QQQ), time-of-flight (TOF) and hyphenated quadrupole time-of-flight (Q-TOF) [20]. CE analysis with MS detection has been widespread used due to the development of commercial equipment. By CE-MS hyphenated techniques, the compounds can be separated by their electrophoretic mobility and by their mass-charge ratio (m/z), which allows the separation of the compounds even when the peaks are not completely resolved in the CE system. Another advantage of using MS detection is that it is considered a universal detector, which detects any analyte with a m/z within a defined range, as well as a selective detector, detecting only analytes of a specific molecular weight.

For applications involving the separation of organic acids, it is possible to work in different pH ranges. For instance, Angonese et al. [17] and Schulz et al. [19] applied a method previously developed by the group [21] considering the effective mobility curve versus the pH and pKa of the analytes and defining, with the help of the Peakmaster® software, the best separation condition (pH 3.3) at which all organic acids are partially ionized. The compounds were extracted from the fresh pulp with water in an ultrasonic bath, the material was centrifuged, and the supernatant diluted in two concentrations, considering the different concentrations of the analytes in the samples, and injected into the equipment using a capillary with total length of 60.5 cm, effective length of 52 cm, and 75 μm i.d.. Pinsorn et al. [22] were interested in comparing the profile of the metabolites of the durian samples, which contained 6 amino acids and 4 organic acids, therefore, it was chosen to work at pH 10 and guarantee the maximum number of ionized species. The running electrolyte consisted of 20 mmol L^{-1} ammonium formate, much simpler than the electrolyte used by Angonese et al. and Schultz et al., which consisted of phthalic acid 20.0 mmol L^{-1}, tris(hydroxymethyl)aminomethane (TRIS) 14.0 mmol L^{-1}, cetyltrimethyl ammonium bromide (CTAB) 1.6 mmol L^{-1} and $CaCl_2$ 1.0 mmol L^{-1}. However, both Angonese et al. and Schultz et al. quantified the analytes using DAD indirect detection where the separation of the analytes in base line must be achieved, whereas Pinsorn et al. used the MS detector, which helps in the separation from the m/z ratio of the compounds and is not compatible with complex BGE.

Table 2
Representative applications on the use of CZE for phytochemicals analysis in the period 2018–2021.

Analyte	Sample	CE mode and detector	CE separation conditions	Ref.
maleic, malonic, fumaric, tartaric, formic, citric, malic, glycolic, lactic, gluconic, succinic, glutaric, and acetic acids	organic dragon fruits	CZE-UV-DAD, indirect detection, 230 nm	phthalic acid 20.0 mmol L^{-1}, tris 14.0 mmol L^{-1}, CTAB 1.6 mmol.L-1 and CaCl2 1.0 mmol.L-1, pH 3.3	[17]
glutathione, γ-glutamylcysteine, glutamate, cysteine, alanine, aspartate, leucine, malate, citrate, ophthalmate	two durian cultivars	CZE-TOF	ammonium formate 20 mmol L^{-1}, pH 10.0	[22]
hormone−systemin	tomato plant	CZE- UV-DAD, direct detection, 214 nm	phosphate 25 mmol L^{-1}, pH 2.5	[23]
3,5-diiodo-L-thyronine, 3,5-diiodo-L-tyrosine, and L-tyrosine	kelp and seaweed	CZE- UV-DAD, direct detection, 210 nm	sodium borax solution 30 mmol L^{-1}, pH 10.5	[34]
derivatives of bergenins and catechins	*Rourea minor*	CZE- UV-DAD, direct detection, 205 nm	sodium borax solution 30 mmol L^{-1}, pH 10.5	[35]
sinapic, ferulic, syringic, p-coumaric, vanillic and p-hydroxybenzoic acid	wheat	CZE- UV-DAD, direct detection, 200, 220 and 300 nm	sodium phosphate 50 mmol L^{-1}, water:methanol 80:20, pH 6.0	[29]
hordeins and fragments	barley grain and malt	CZE- UV-DAD 200, 214 and 254 nm	acetic acid 1 mmol L^{-1}, pH 2.3	[24]
vitamin B2	salvia	CZE-LIF, excitation was performed by a 488 Ar-ion laser and emission at 520 nm.	borate 20 mmol L^{-1}, of pH 9.5	[28]
fucose, galactose, arabinose, glucose, rhamnose, xylose, mannose, fructose and ribose	coffee	CZE-QQQ	triethylamine 500 mmol L^{-1}, pH 12.3	[15]
Proteins	quinoa	CZE- UV-DAD, scanning from 190 to 300 nm	boric acid 60 mmol L^{-1}, pH 9.0	[25]
Alkaloids	*Sophora flavescens*	CZE- UV-DAD, direct detection, 210 nm	sodium tetraborate solution 50 mmol L^{-1}, boric acid 500 mmol.$L^{-1}$and citric acid 1.2 mmol L^{-1}	[39]
pyrogallol, homogentisic acid, ferulic acid, cinnamic acid, gentisic acid, p-coumaric acid, chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, gallic acid, 3,4 -dihydroxybenzoic acid.	edible mushrooms	CZE- UV-DAD, direct detection, 210 nm	boric acid 175 mmol L^{-1}, pH 8.5	[31]
peimine and peiminine	*Bulbus Fritillariae thunbergii*	CZE-MS	15% water, 60 mmol.L^{-1}ammonium acetate and 1% acetic acid in methanol	[38]
fructose, glucose, sucrose	*Myrtaceae fruits*	CZE- UV-DAD, indirect detection, 254 nm	sorbic acid 20 mmol L^{-1}, CTAB 0.2 mmol.L-1, 40 mmol L-1 NaOH, pH 12.2	[18]
fructose, glucose, citric acid, malic acid, malonic acid	*Euterpe edulis martius*	CZE- UV-DAD, indirect detection, 230 nm to aliphatic organic acids and 254 nm to sugar	aliphatic organic acids:phthalic acid 20.0 mmol L^{-1}, tris 14.0 mmol L^{-1}, CTAB 1.6 mmol.$L^{-1}$and $CaCl_2$ 1.0 mmol L^{-1}, pH 3.3; sugar: sorbic acid 20 mmol L^{-1}, CTAB 0.2 mmol L^{-1}, 40 mmol.L^{-1}NaOH, pH 12.2	[19]
gibberellic acid, abscisic acid, 3-indole acetic acid, 3-indolepropionic acid, 3-indolebutyric acid, 2,4-dichlorophenoxyacetic acid, and 2-methyl-4-chlorophenoxyacetic acid	grape, orange, pear, kiwifruit and watermelon	CZE- UV-DAD 200, 220 and 252 nm	borax 25 mmol L^{-1}, 29% ethanol, pH 10.5	[43]
hesperidin, chrysin, epicatechin, epigallocatechin gallate, and morin	orange juice, black and green tea	CZE- UV-DAD, direct detection, 214 nm	borate 25 mmol L^{-1}, pH 9.0	[32]
oleanolic acid, ursolic acid and betulinic acid	*Forsythiae fructus*	CZE- UV-DAD, direct detection, 200 nm	borax 50.0 mmol L^{-1}, 0.5 mmol.L^{-1}β-CD, pH 9.5	[42]
isorhamnetin, rutin, kaempferol, taxifolin, luteolin and quercetin hydrate	goji berry	CZE- UV-DAD, direct detection, 214 nm	borax 30 mmol L^{-1}, 10% methanol, pH 9.0	[33]
punicalagin	*Combretum aculeatum*	CZE- UV-DAD 200, 254; 280 and 366 nm	phosphate 25 mmol L^{-1}, pH 7.4	[36]
sinapic acid; ferulic acid; syringic acid; ellagic acid; p-coumaric acid; vanillic acid; caffeic acid; gallic acid; p-hydroxybenzoic acid	rapeseed plants	CZE- UV-DAD, direct detection, 254 nm	sodium tetraborate 70 mmol L^{-1}, pH 9.35	[30]
peimine, peiminine, and peimisine	*Fritillariae thunbergii bulbus*	CZE-Q-TOF	ammonium acetate 20 mmol L^{-1}	[37]
glucose, man, rhamnose, fucose, galactose, and xylose	*Ginkgo biloba*	CZE- UV-DAD, direct detection, 245 nm	borax 30 mmol L^{-1}, pH 9.5	[27]
nucleotides, vitamins, phos- phorylated sugars, amines, peptides, and coenzymes	tobacco leaf	CZE-TOF	Positive mode: formic acid 1 mol L^{-1}, pH 2.0; Negative mode: ammonium acetate 50 mmol L^{-1}, pH 8.5	[44]

In the same run with the same analytical conditions, including sample preparation, Pinsorn et al. also evaluated the profile of some amino acids present in the durian samples, which were pulped and frozen with liquid nitrogen, lyophilized, crushed, the metabolites were extracted with solvents, filtered, resuspended in water, and injected into the CE equipment. Mucha et al. [23] evaluated the presence of three peptides related to the defense system in the exudate of the stem and leaves of the tomato plant. Three systemin peptides (a plant peptide hormone) were injected into the stem and leaves of mature tomato plant and its transportation throughout the plant tissue was traced by CE.

A simple BGE, 25 mmol L^{-1} phosphate buffer, was also used, with good separation of the compounds, especially considering that the sample preparation consisted only of filtering the exudate with subsequent injection into the CE-DAD equipment.

In addition to amino acids and peptides, applications of CZE in phytochemicals have also been found for protein analysis. The limitation of analysing proteins by CE is their adsorption on the

capillary surface, negatively charged. This problem can be partially resolved with the use of coated capillaries, high ionic strength in the BGE, and working pH high or below 3.0. In this last point, the works found followed opposite strategies: Pont et al. [24] worked with BGE at pH 2.3 constituted by 1 mol L^{-1} of acetic acid (HAc), and Galindo-Luján et al. [25] worked with BGE constituted by 60 mmol L^{-1} of H_3BO_3 in pH 9.0 adjusted with NaOH. Both studies are from the same research group, CE-DAD equipment was used for their protein analysis and the sample profile obtained by CZE was corroborated with results of matrix-assisted laser desorption ionization (MALDI)-TOF-MS [24] and Kjeldahl [25]. Sample preparation was similar for samples of both studies, namely barley grain and malt from Pont et al. and quinoa from Galindo-Luján et al., where samples were dried, ground, extracted (different solutions were used), centrifuged, filtered, and injected in a CE-DAD equipment.

To separate carbohydrates by CZE, a strongly alkaline BGE should be used, with a pH greater than 12, because in this condition it is possible to ensure that the analytes are ionized and will be analyzed as anions, since the pKa of the sugar molecules is normally greater than 11.9. Another point is that for analysis of sugars in CE-DAD equipment, derivatization reactions should be considered since such compounds do not present enough molar absorptivity in the UV−Vis region for their identification to happen by direct detection. An alternative can be the addition of a chromophore species to the BGE, in this way an indirect detection can be performed.

Seraglio et al. [18] evaluated three types of Myrtaceae fruits in two stages of maturation by characterizing the sample by several tests with different techniques, and the sugar content was evaluated by CE-DAD. Schulz et al. [19] studied seven maturation stages of *Euterpe edulis* Martius through the variation of the content of organic acids and sugars by CE-DAD. The results were evaluated by principal component analysis (PCA) suggesting the best time to harvest the fruits according to the higher amounts of sugars. Seraglio et al. [18] and Schulz et al. [19] used the same method for sugar analysis by CZE-DAD, previously developed by the group [26]. The BGE was composed of 20 mmol L^{-1} sorbic acid, 0.2 mmol L^{-1} CTAB, and 40 mmol L^{-1} NaOH to pH 12.2. The sample preparation was simple: the samples were triturated with water, vortexed, centrifuged and the supernatant was injected in the CE-DAD system for indirect detection of the compounds, where the chromophore added to the BGE was CTAB (also used in the indirect determination of organic acids by Angonese et al. and Schultz et al. [17,19]) as above mentioned.

Liu et al. [27] optimized a method for analysing 6 monosaccharides in *Ginkgo biloba* leaves by CE-DAD with direct detection. Before to be analyzed, samples were washed, oven dried, ground with liquid nitrogen, dried, dissolved in water, derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) in alkaline medium, the solution was neutralized with HCl, purified, and injected into the CE-DAD system. The BGE was composed of 30 mmol L^{-1} borax and the pH was tested in a range of 8−10, and the best condition, considering peak resolution and analysis time, was pH 9.5. A fused silica capillary with 51.5 cm effective length and 50 μm i.d. was used. The derivatization step was adapted from the process for analysis of the same analytes by HPLC, with the advantage of requiring less sample and reagent, low consumption of organic solvent and shorter analysis time.

Daniel et al. [15] chose to work at a very alkaline pH to ensure the ionization of the analyzed sugars, the BGE used was composed of a solution of triethylamine at 500 mmol L^{-1}, pH 12.3 and used capillaries with 20 μm i.d. The samples were submitted to acid hydrolysis with H_2SO_4, filtered, neutralized with $Ba(OH)_2$ to eliminate the sulphate ion, centrifuged, filtered again, diluted and injected into the CE-MS equipment. Typical electropherograms obtained for the analysis of monosaccharides in coffee and in the possible adulterant plant matrices tested, namely soybean and corn, are shown in Fig. 1. Mixtures of plant matrices were analyzed by CE-MS and the profiles obtained were evaluated by PCA, with more than 99% of the variance explained by principal components 1 and 2. This study demonstrates an interesting application of the CZE technique, using the profile of the sugars present in the sample to explore possible adulterations in plant matrices of great economic interest. Gezek et al. [28] characterized 14 species of Turkish salvia based on mineral content, total phenolics, antioxidant capacity and vitamin B2 content, the latter being evaluated by CZE-LIF. The BGE was composed of 20 mmol L^{-1} borate at pH 9.5, excitation was performed by a 488 nm Ar-ion laser and emission at 520 nm, and a simple sample preparation was used where dry salvia leaves were ground, extracted with boiling water, the supernatant was filtered and injected into the CE-LIF system. Data from vitamin B2 analysis were correlated with the other results and evaluated by PCA, where it was possible to verify the differentiation between the salvia species studied.

The family of phenolic compounds including phenolic acids, lignans and flavonoids, are compounds synthesized by the plants that can act in the metabolic defense of the plant in response to external stresses or form polymers to confer resistance and mechanical protection. These compounds generally have antioxidant activity, and may be directly related to the color, aroma and flavor of plants. The phenolic compounds can be free or glycate in the vegetables, and to analyze the free portion, protocols of liquid-liquid extraction with hydrolysis under mild conditions are applied. For the remaining portion, acid hydrolysis and alkaline solutions with more concentrated reagents and for a long time are necessary. For separation of these compounds by CZE, generally a pH around 9.0 is chosen, because phenolic acids (pka1~4 and pKa2~8), catechins (pkã8) and flavonoids (pKã7) are ionized. It is also possible to separate some of these compounds at pH < 3, as they would be protonated.

Gotti et al. [29] differentiated wheat varieties based on the composition of phenolic compounds, more specifically, the phenolic acids present in the samples. The BGE consisted of sodium phosphate solution (50 mmol L^{-1}) in water/methanol 80:20 (v/v) at pH 6.0, in this case, performing the separation with the partially ionized analytes. The free portion was extracted with solvents, and from the solid residue the bound portion could be extracted. Seven phenolic acids were separated and identified in less than 12 min, the results obtained were used in a PCA, which separated the evaluated wheat varieties from the profile of the analyzed compounds.

Huang et al. [30] evaluated the profile of 11 free phenolic acids in seeds, leaves, and flowers by CZE and compared the results with HPLC and TLC. A BGE composed of 70 mmol L^{-1} sodium tetraborate at pH 9.35 was used to separate the compounds, and sample preparation consisted of weighing the sample, extracting it with methanol in an ultrasonic bath, centrifuging, drying, and resuspending the material in methanol to further analysis. The analysis time by CZE was about three times shorter than the analysis time by HPLC, and the number of compounds identified by CZE was greater than that found for HPLC and TLC. Junior et al. [31] also analyzed 11 phenolic acids in mushroom samples in 20 min by using a BGE consisted of 175 mmol L^{-1} aqueous boric acid solution at pH 8.5. Sample preparation was optimized in order to reduce extraction time and not including organic solvents during the process. The optimized sample preparation showed three times higher extraction yield and about 50 times shorter extraction time than the compared method.

A separation of five flavonoids in juice and tea samples in less than 8 min was optimized by Memon et al. [32] using a BGE with

25 mmol L^{-1} borate buffer of pH 9.0 and 17.5 mmol L^{-1} of 1-butyl-3-methyl imidazolium hexafluorophosphate, which is an ionic liquid (IL) whose addition to BGE helped to improve the resolution between analyte peaks. In the optimization of the analytical conditions, different pHs were evaluated as well as the contribution of the BGE concentration in the separation of the compounds. Wang et al. [33] evaluated the profile of 6 flavonoids in goji berry samples by CZE using field amplified sample stacking also called field amplified sample injection (FASI). FASI is a pre-concentration strategy that consists of injecting the sample prepared in a solution with low molar conductivity into the capillary previously filled with BGE. When applying the electric field, the ions present in the region of low conductivity undergo greater action by the field, and migrate quickly to the region of high conductivity, when their speeds decrease, and stacking occurs. An optimized solution containing 30 mmol L^{-1} borax in pH 9.0 with 10% (v/v) methanol was used as the BGE. FASI has allowed low limits of detection (LOD) and quantitation (LOQ) to be achieved, which can facilitate the analysis of compounds at trace levels. Another interesting application for IL involving CE was presented by Wang et al. [34], who developed an in-line solid-phase extraction (SPE) CE method to determine three species of organic iodine in seaweed samples in less than 10 min. Several experimental conditions were evaluated, the final BGE was 5 mmol L^{-1} sodium tetraborate and for the pre-treatment of the samples, ultrasonic extraction in aqueous solution and injection in into a SPE-CE-DAD equipment. Although the LOD and LOQ obtained were lower than those observed in previous works for the same compounds, in the order of nanograms per liter, only one of the evaluated compounds was identified in the tested samples.

Determination of seven bergenin and catechin derivatives in *Rourea minor* stems was performed using the CZE technique by Ngoc et al. [35], using 30 mmol L^{-1} sodium tetraborate solution with pH 10.5 as BGE. Separation took place in less than 12 min with baseline-resolved peaks. The sample preparation process consisted of sonicating the dried and finely ground plant material with methanol, centrifuging, drying the supernatant, resuspending in methanol, filtering and injecting it into the CE. Additional analysis from nuclear magnetic resonance (NMR) and MS were performed, and in total almost 30 phytochemicals were identified, two of which were described for the first time.

Diop et al. [36] developed a CZE method for the analysis of punicalagin in *Combretum aculeatum*. The target analyte is a polyphenol with biological activity against *Mycobacterium tuberculosis*, which causes tuberculosis. The method used BGE constituted by 25 mmol L^{-1} phosphate at pH 7.4 and was applied in the evaluation of extracts from different parts of the plant, different times of extraction by decoction and different harvest periods. Punicalagin was obtained in higher concentration in the leaves of the plant by decoction for 30 min.

Alkaloids are cyclic amines that may have nitrogen-containing heterocyclic rings. They are compounds produced by animals, bacteria, fungi, and plants and usually have low concentration, so the sample preparation for analysis of these compounds usually considers pre-concentration steps [11]. The separation of alkaloids by the CZE method can be carried out under acidic or alkaline conditions, depending on the chemical structure of the compound. The quantification of three alkaloids in *Fritillariae Thunbergii Bulbus* by CE-MS was performed by Wang et al. [37] using solid phase dispersion microextraction as sample preparation, where the sample was macerated with a solvent, eluted by a commercial cartridge, centrifuged, filtered, and injected into the CE-MS equipment. The BGE consisted of a 20 mmol L^{-1} ammonium acetate solution. The peaks showed the same migration time by CZE, not being separated by electromigration, only by MS.

The addition of organic solvents to BGE can improve many aspects in the quality of the CZE separation, however, the most traditionally used capillaries, with an external polyimide coating, do not present good resistance under these conditions. Over time, the capillary tip coating dissolves and causes several problems such as loss of sensitivity in the analysis, drop in current and eventual capillary clogging. Although eliminating the capillary tip coating by burning it (as it is done for creating the detection window in CE-DAD equipment) is a simple solution, it leaves the silica exposed and with low mechanical strength, which often leads to the breakage of the capillary at that point. Zhou et al. [38] tested a polyether-ether-ketone (PEEK) capillary for analysis of alkaloids from BGE composed of methanol with 15% water, 60 mmol L^{-1} ammonium acetate and 1% acetic acid. The CZE-MS method was applied for the determination of peimine and peiminine in samples of *Bulbus Fritillariae thunbergii* herb. The results showed more symmetrical peaks, good resolution between them, and good capillary durability, as all experiments were performed with only one PEEK capillary. The main disadvantage of this capillary is that it is not CE-DAD or CE-LIF compatible due to its poor optical permeability.

Hou et al. [39] developed a method of analyzing alkaloids by CZE to obtain as many peaks as possible and better separation in a relatively short time. Eleven compounds were separated and the electropherograms were related to the values of total antioxidant capacity obtained for each sample (Fig. 2). From this matrix, a partial least squares (PLS) method was developed to holistically monitor the consistency of the quality of *Sophora flavescens* extracts and characterize the connection between fingerprints and *in vitro* antioxidant activities. Several experimental conditions were tested and the BGE considered optimal was constituted by 50 mmol L^{-1} sodium tetraborate solution, 500 mmol L^{-1} boric acid and 1.2 mmol L^{-1} citric acid, sonication was applied for extraction before CE-DAD analysis. Assays involving the chemometric modeling of UV−Vis, infrared and Raman spectroscopic data, as well as data from MS-associated separation techniques, such as HPLC-MS, GC-MS and CE-MS, are already well studied and established in the literature, so, it is interesting to see that CE data modeling associated with other detection methods has been explored in recent years [25,40,41].

Another class of compounds that is usually present in low concentration in samples is the diverse class of terpenes, which can be produced by some animals and many plants, usually related to ecological factors, such as fungal protection or to ward off herbivorous animals. In this class are pentacyclic triterpenic acids, minor components present in *Forsythiae Fructus*, which show evidence of good anti-inflammatory activity. Three terpenic pentacyclic acids were evaluated by CZE-DAD with a BGE containing 50.0 mmol L^{-1} borax and 0.5 mmol L^{-1} beta-cyclodextrin (β-CD) at pH 9.5 [42]. Experimental parameters were evaluated during the optimization of the method and interesting information was discussed, such as the fact that the increase in borax concentration generates an increase in ionic strength, which helps in the separation in this case, as the analytes migrate in the opposite direction to the EOF. Although the contents of the compounds were higher in green fruits, the preference of the population for consumption is for ripe fruits.

Phytohormones are plant hormones responsible for regulating plant growth. Chen et al. [43] developed a CZE-DAD method for evaluating 7 phytohormones in fruit samples. Several tests were performed to adjust the analytical conditions and the BGE considered optimal was constituted by borax buffer pH 10.5 containing 29% of ethanol. The samples were ultrasonically extracted, dried, purified with modified QuEChERS, dried again, redissolved in methanol-water solution, and injected into the CE-DAD equipment. Only two phytohormones, gibberellic acid and abscisic acid, were identified in the analyzed fruits.

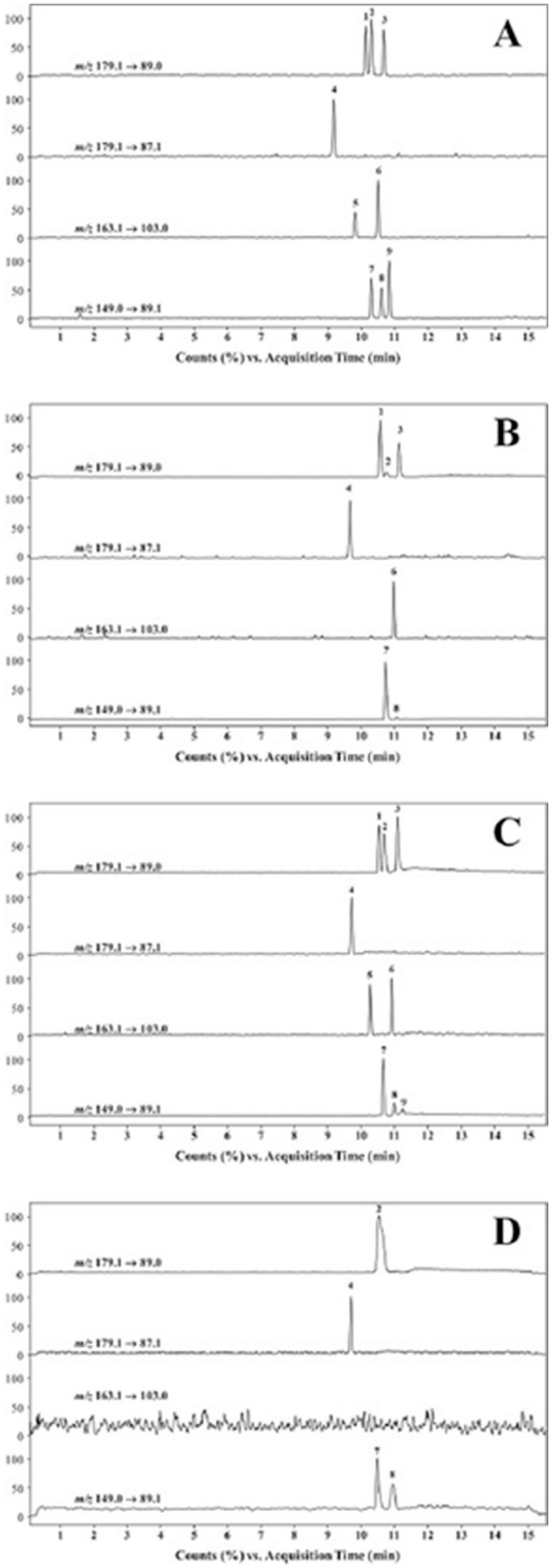

Fig. 1. Normalized electropherograms of monosaccharides: (A) Standard, (B) Coffee, (C) Soybean, (D) Corn. Peaks: (1) Galactose, (2) Glucose, (3) Mannose + Fructose, (4) Inositol (IS), (5) Fucose, (6) Rhamnose, (7) Arabinose, (8) Xylose, and (9) Ribose. Reprinted from Ref. [15] with permission from Elsevier.

Zhao et al. [44] performed a cross-platform metabolomics study to assess the effect of topping on tobacco plant metabolism. Topping in this case means cutting off the bloom as soon as it appears, to preserve the quality of the tobacco leaves. As the objective was to assess the broadest possible profile of the metabolites present, the samples were evaluated in positive and negative mode in the MS detector. There was a difference in the metabolic profile of the plants between the two stages evaluated, and it was found that the coverage seemed to delay the senescence of the upper leaves and increased the accumulation of secondary metabolites, such as nicotine.

3. Micellar electrokinetic Capillary Chromatography (MEKC) mode

MEKC is considered a hybrid technique where electrophoretic and chromatographic phenomena are involved in the separation. This is mainly due to the addition of surfactants, such as sodium dodecyl sulphate (SDS) or sodium cholate (SC), to the separation buffer at a concentration above its critical micellar concentration (CMC), which enables the separation of neutral compounds based on their differential partitioning between the aqueous phase and the micellar pseudostationary phase (PSP) [45]. Compared to conventional CE methods, where only ionic compounds can be separated based on the different mobilities of the charged analytes - according to their charge to mass ratio under the application of an electric field -, MEKC can separate both ionic and neutral compounds in short analysis times, while it makes also possible the separation of chiral solutes if adequate enantioselective protocols are used (e.g., cyclodextrin derivatives added to the pseudostationary phase) [46]. In addition, as many laboratories embrace the concept of “green analytical chemistry”, it is now worth reconsidering the replacement of reverse phase-liquid chromatography (RP-LC) methods with MEKC-based approaches for certain applications, which means replacing larger volumes of harmful organic solvents by smaller volumes of BGE, bio-derived solvents or micellar solutions [47].

In the last years, MEKC methods have found great applicability to the analysis of a broad variety of neutral phytochemicals in different matrices such as foods, dietary supplements, plants, natural extracts, cosmetics, and pharmaceutical preparations. Table 3 summarizes representative applications on the use of MEKC for phytochemicals analysis in the period 2018–2021. A critical revision of these application will be carried out focusing the discussion on the use of single or mixed micellar systems or the addition of chiral selectors in the BGE, as well as on the effect of different additives (e.g. cyclodextrins) or organic modifiers present in the separation medium. Indirect methods based on the derivatization of chiral analytes and the use of different injection systems (e.g., short-end injection, field-amplified sample injection, sweeping injection) will be also considered.

Most of recent MEKC methods for phytochemical analysis are mainly coupled to UV/DAD detectors, using mostly uncoated fused-silica capillary of different effective length (90–7.5 cm) and i.d. (75-50 μm). These methods are mainly based on protocols using minimal and environmentally friendly sample preparation procedures, compared to alternative LC-based methods that often require laborious sample preparation procedures (SPE, ultrasound-assisted extraction or as microextraction techniques) to achieve sufficient sensitivity and remove matrix interferences. In this line, Waraksa et al. [48] developed and validated a MEKC-DAD-based method for

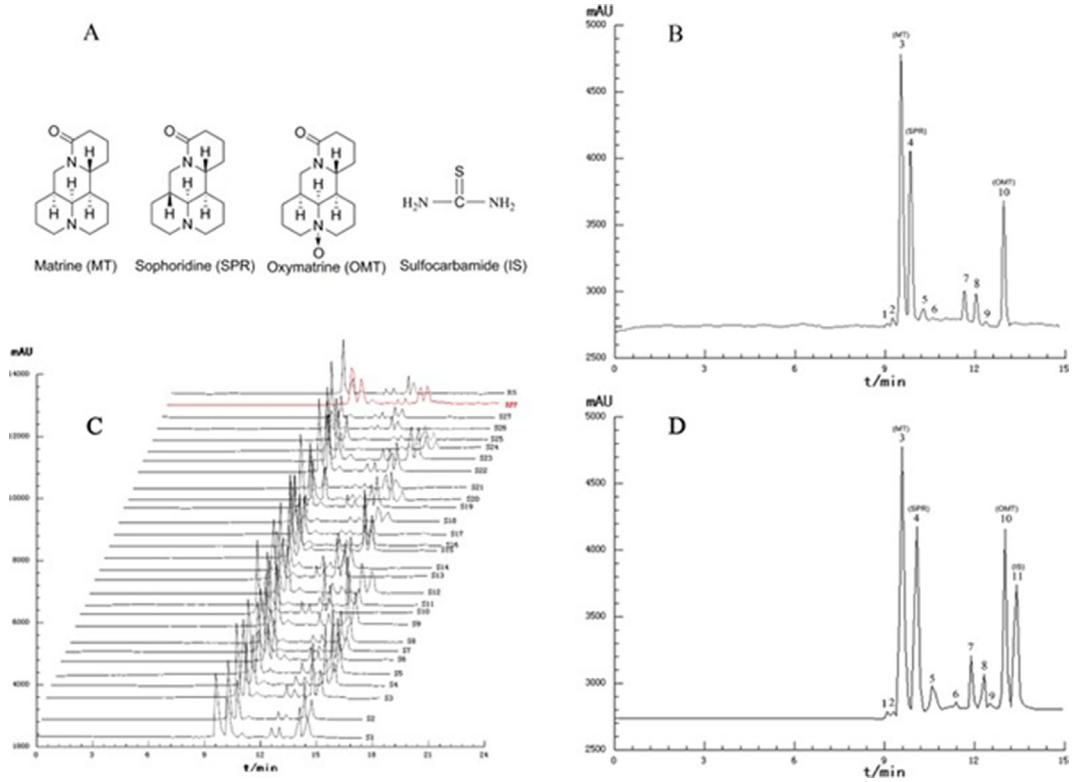

Fig. 2. (A) The structures of the three marker compounds and sulfocarbamide. (B) Typical electropherogram without internal standard peak. (C) CE fingerprint profilings of 27 batches of *Sophora flavescens* samples and one reference sample (RS). (D) Typical electropherogram with internal standard peak. Reprinted from Ref. [39], with permission from Elsevier.

the determination of caffeine and the detection of vitamin B3 and B6 in pre-workout supplements. Separation was performed in an uncoated fused-silica capillary (50 μm i.d.; 64.5 cm effective length), using a borate buffer with SDS (see details of MEKC separation conditions in Table 3). The analysis was carried out after simple sample dilution and syringe filtration with the use of only 1 mL of water as solvent. Gonzalez-Jimenez et al. [49], carried out the phytochemical characterization of Hawthorn (*Crataegus pubescens*) fruit. The profile of the polyphenolic compounds was studied using MEKC with a photodiode array detector, and fused silica capillary (50 cm effective length 75 μm i.d.). The authors demonstrated that MEKC is a time and reagents saving alternative to the use of HPLC for the qualitative and quantitative analysis of phenolic compounds such as catechin, epicatechin, chlorogenic acid and procyanidins in food matrices, without affecting the resolution of the analyzed compounds. Other works have also provided evidences that MEKC can be an interesting alternative to established HPLC-DAD and HPLC-DAD-MS approaches for the analysis of phytochemicals like iridoids, phenylpropanoids and flavonoids in natural extracts (e.g. in *Lippia alba*) [50]. For flavonoids analysis borax buffers usually provide good separations, since borate ions form complexes with flavonoids facilitating their separation. The addition of organic modifiers like isopropanol in the buffer was essential for the separation of all the iridoids. Although MEKC and HPLC both fulfill essential validation requirements, it was not possible to separate the isomers acteoside and isoacteoside by MEKC, which can be resolved by conventional RP-HPLC.

Terpenoids-like phytochemicals such as steroidal saponins (e.g., diosgenin) from *Trigonella foenum-graecum* seeds [51] or bioactive di- and sesquiterpenoids (e.g., triptolide, wilfortrine, wilfordine, wilforgine, wilforine, triptophenolide, and triptonide) from the root extracts of *Tripterygium wilfordii* were selectively analyzed by MEKC method using a borate buffer with SDS and methanol as organic modifier in the BGE [52]. Other triterpenoid saponins including picfeltarraenins IA and IB, picfeltarraenin IV, and a phenylethanoid glycoside (acteoside) from *Picriae Herba* were separated by a solvent-modified MEKC method using the anionic surfactant SC in combination with methanol and isopropanol as modifiers [53]. Combinations of SDS and SC as mixed micelles in borate buffer with acetonitrile were used for the simultaneous separation and determination of highly hydrophobic and neutral compounds like coumarins (e.g. praeruptorin A, B and C) in *Peucedani Radix* [54].

Although various types of surfactants forming micelles as PSP are employed in MEKC, the only use of the surfactant as additive may not be effective for resolution of polymeric molecules. Thus, for electrophoretic analysis of molecules with high dispersion in molecular weight, such as the hyaluronic acid, a modified MEKC approach, combining SDS and a linear low molecular-weight hydrophilic polymer (polyethylene glycol - PEG) was shown to be a successful strategy for the separation of highly dispersed hyaluronic acid (HA) [55]. This combination allowed the formation of the SDS micelles with a sieving matrix of PEG to resolve the mixture of HA with low molecular weight (380 kD) and high molecular weight (2180 kD) in the normal polarity.

The separation of chiral analytes very often requires derivatization with a chiral reagent to generate the corresponding stable diastereomers for subsequent separation under achiral conditions. Although different chiral derivatizing reagents can be used, (±)-1-(9-fluorenyl) ethyl chloroformate (FLEC) is one of the most widely used due to the simple, fast, and quantitative reaction with primary and secondary amines, excellent enantioselectivity and sensitivity with UV and fluorescence detectors [45]. This derivatization procedure was applied by Perez-Miguez et al. [56], for the enantioselective separation of selenomethionine (SeMet) in food

Table 3
Some representative applications on the use of MEKC for phytochemicals analysis in the period 2018–2021.

Analyte	Sample	CE mode and detector	CE separation conditions	Ref
selenomethionine enantiomers	food supplements	MEKC- UV-DAD	100 mmol L^{-1} APFO at pH 8.5	[56]
Caffeine	preworkout supplements	MEKC-UV-DAD	Borate buffer (20 mmol L^{-1}; pH 9.3) with SDS (30 mmol L^{-1})	[48]
polyphenolic compounds	tejocote (*Crataegus pubescens*) fruit extracts	MEKC-PDA	28 mmol L^{-1} Na2B4O7, 3.2 mmol L^{-1} KH2PO4, and 24 mmol L^{-1} SDS, pH 8.8	[49]
steroidal saponins	fenugreek seeds (*Trigonella foenum-graecum* L.)	MEKC-UV-DAD	Na2B4O7 (20 mmol L-1) containing SDS (50 mmol L-1) at pH 9.5	[51]
triptolide, wilfortrine, wilfordine, wilforgine, wilforine, triptophenolide, and triptonide	root extracts of *Tripterygium wilfordii* Hook. F. and tripterygium preparations	MEKC-UV-DAD	10 mmol L^{-1} sodium tetraborate, 30 mmol L^{-1} SDS, and 30% v/v methanol	[52]
Iridoids, phenylpropanoids and flavonoid	*Lippia alba* extracts	MEKC-UV-DAD	50 mmol L^{-1} borax buffer containing 75 mmol L^{-1} SDS and 5% isopropanol	[50]
praeruptorin A, B and C	peucedani radix and its medicinal preparations	MEKC-UV-DAD	20 mmol L^{-1} borate buffer containing 40 mmol L^{-1} sodium cholate (SC), 22 mmol L^{-1} sodium dodecyl sulphate (SDS) and 25% acetonitrile (pH 10.00)	[54]
hyaluronic acids	cosmetic and pharmaceutical preparations	MEKC-UV-DAD	25 mmol L^{-1} borate buffer (pH 9.75) containing 30 mmol L^{-1} SDS and 10% polyethylene glycol (MW: 8000)	[55]
acteoside and pic- feltarraenins IA, IB and IV	picria fel-terrae lour.	MEKC-UV-DAD	20 mmol L^{-1} sodium tetraborate, 40 mmol L^{-1} sodium cholate, 10% methanol and 10% isopropanol (pH 9.76)	[53]
Coumarin	curry samples	Sweeping-MEKC-UV-DAD	150 mmol L^{-1} SDS and 25% MeOH in 25 mmol L^{-1} H3PO4 (pH 2.5)	[47]
schizandrin, schisandrol B, schisantherin B, schisantherin A, schisanhenol, deoxyschizandrin, schisandrin B.	*Schisandra chinensis*	ABTS + -sweeping MEKC-DAD	35 mmol L^{-1} phosphate (pH 8.0) with 10 mmol L^{-1} beta-cyclodextrin, 30 mmol L^{-1} sodium dodecyl sulphate (SDS) and 10% ACN.	[58]
indole-3-acetic acid and indole-3-butyric acid	bean sprout, tomato, potato, cucumber, and wheat grain	FASI-MEKC-UV-DAD	25 mmol L^{-1} borate buffer (pH 9.2) with 12% ethanol and 15 mmol.L^{-1}SDS	[59]
erianin, dendrophenol, naringenin and scoparone	Dendrobium officinale Kimura et Migo (D. officinale)	CD assisted sweeping-MEKC-UV-DAD	50 mmol L^{-1} phosphoric acid (PA) containing 100 mmol L^{-1} SDS and 30% methanol.	[57]
tryptophan enantiomers	dietary supplements	CD-MEKC-UV-DAD	100 mmol L^{-1} formate buffer (pH 2.2) containing 1.25% sulfated-γ-cyclo- dextrin	[62]
theanine and catechin isomers	green tea	CD-MEKC-UV/DAD	65 mmol L^{-1} SDS and 28 mmol L^{-1} cyclodextrin in acidic buffer (pH 2.5)	[63]
alpha and beta-acids homologues and isomers	*Humulus lupulus* L.	CD-MEKC-UV-DAD	30 mmol.L^{-1}sodium tetraborate solution, 45 mmol.L^{-1}sodium dodecyl sulphate, 20 mmol.L^{-1}beta-cyclodextrin and 10% acetonitrile	[61]
huperzine alkaloids	huperzia serrata	CD-MEKC-UV-DAD	10 mmol L^{-1} sodium tetraborate solution, 40 mmol L^{-1} sodium dodecyl sulphate (SDS), 50 mmol L^{-1} sodium cholate (SC) and 3.0 mmol L^{-1} mono-(6-ethylenediamine-6-deoxy)-β-cyclodextrin (ED-β-CD) at pH 9.3	[60]
curcumin, demethoxy curcumin (DMC) and bisdemethoxy curcumin (BDMC)	–	MEKC-LIF	20 mmol L^{-1} Triton X-100, 20 mmol L^{-1} SDS, 30% methanol in 10 mmol L^{-1} borax solution at pH 10.0.	[64]
capsaicin and dihydrocapsaicin	gochujang, local snack (swingchip®), black pepper, garlic, and ginger	MEKC-LIF/UV	0.05% Tween 20, 15 mmol L^{-1} sodium borate, and 1.8 mmol L^{-1} SDS with 1 mol L^{-1} NaOH to adjust the pH to 10	[65]
artemisinin and its analogs	*Artemisia annua* leaves	MEKC-MS	40 mmol L^{-1} ammonium perfluorooctanoate (pH 9.5) with 2% isopropanol (IPA)	[66]

supplements using ammonium perfluorooctanoate as a volatile PSP. This surfactant is fully compatible with more sensitive approaches based on ESI-MS detection, particularly interesting for complex food samples analysis.

Preconcentration strategies FASI or sweeping to increase MEKC methods sensitive for separation of neutral and hydrophobic phytochemicals have also been implemented. In sweeping-MEKC, sample is prepared in a solution with conductivity lower, similar, or higher to the BGE which contains a PSP. The analytes are accumulated by the PSP that penetrates the sample zone when the voltage is applied [57]. This stacking technique was recently implemented for the analysis of coumarin in curry samples at levels relevant to the EU limit [47]. Compared to RP-LC, sweeping-MEKC provides better accuracy, since interferences in RP-LC lead to significant overestimation of coumarin levels in some of the analyzed samples. Another sweeping method was proposed to screen and quantify trace antioxidants from *Schisandra chinensis,* combining in-capillary ABTS+ with sweeping MEKC-DAD [58]. This approach allows measuring the total antioxidant activity and simultaneous screening and quantification of the antioxidants in the sample. The obtained sensitivity was improved compared to other CE methods and it was even higher than the obtained by HPLC. An alternative on-line preconcentration technology – FASI – is based on the conductivity of sample and electrolyte solutions, which require low conductance sample. Therefore, in FASI- MEKC methods, a sample pretreatment step is normally required. Zheng et al. [59] proposed an activated Carbon SPE procedure prior to FASI- MEKC separation of indole-3-acetic acid and indole-3-butyric acid in several food samples (e.g., bean sprout, tomato, potato, cucumber, and wheat grain) improving the sensitivity by 30 times.

Cyclodextrins (CD) are frequently used as additive in BGE for CE separation due to their capacity to form inclusion complexes in their hydrophobic cavity via noncovalent interaction with a broad range of guest compounds, improving their chemical stability and aqueous solubility. Peng et al. [57] developed a CD assisted sweeping-MEKC method for the separation of four phytochemicals (i.e., erianin, dendrophenol, naringenin, and scoparone) from *Dendrobium officinale*, by dissolving the target analytes in CD solution. The separation was carried out under suppressed cathodic EOF conditions by employing a BGE containing SDS with methanol

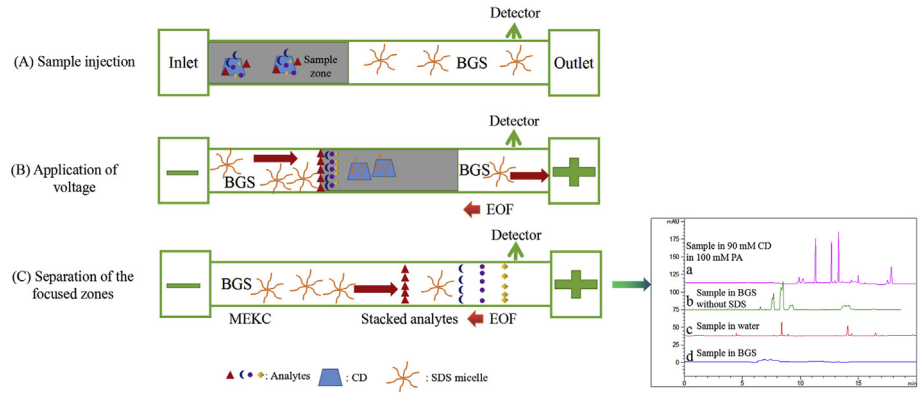

Fig. 3. Schematic diagrams of the CD assisted sweeping-MEKC model. (A) Injection of a sample plug of a low-pH matrix with CD into a capillary filled with low-pH BGE. (B) Application of voltage and stacking of analytes. (C) Separation of the stacked analytes by MEKC. And the chromatograms of target analytes obtained from the CD assisted sweeping-MEKC (a), conventional sweeping-MEKC (b, c) and MEKC (d). Reprinted from Ref. [57], with permission from Elsevier.

at low pH. Fig. 3 shows the different steps of the developed MEKC model, where the target analytes are injected in combination with CD. After negative voltage application, the SDS micelles penetrates the sample zone, and the analytes are released from CD. Further transport and partitioning processes with SDS micelles led to the enrichment of the target analytes. CD-modified MEKC methods also showed excellent separation capacity of isomers and other compounds exhibiting very close structural similarity. This is the case of lycopodium alkaloids Huperzine A, B and C from *Huperzia serrata*, that cannot be completely separated by HPLC. Yang et al. achieved complete separation of the three Huperzine alkaloids using a borate buffer in a BGE containing 40 mmol L^{-1} SDS, 50 mmol L^{-1} of SC, and 3.0 mmol L^{-1} mono-(6-ethylenediamine-6-deoxy)-β-cyclodextrin (ED-β-CD) as additive [60]. Using CD-modified MEKC separation strategies, higher resolution at shorter separation times (up to 8 min of analysis) can be achieved for structurally related phytochemicals such as humulones and lupulones homologues and their isomers in hop [61]. This was possible due to the introduction of 20 mmol L^{-1} of beta-cyclodextrin and 10% (v/v) acetonitrile as modifiers in the borate buffer with 45 mmol L^{-1} SDS.

Among all the chiral selectors that can be used in MEKC, CD continue to be nowadays the most widely used. Thus, a sulfated-γ-CD was employed as chiral selector for the enantioselective analysis of tryptophan in food supplements, using short-end injection to reduce the analysis time [62]. Using a formate buffer (pH 2.2) containing 1.25% sulfated-γ-CD, this CD-assisted MEKC procedure does not require any derivatization step. Another CD - heptakis (2,6-di-O- methyl)-β-CD - was used as chiral additive for the separation of six major green tea catechins, including enantioresolution of (±)-catechin, and D/L-theanine enantiomers [63]. The poor detectability of theanine required the previous derivatization with *o*-phthaldialdehyde in the presence of n-acetyl-L-cysteine (OPA/NAC), yielding two isoindole derivatives diastereomers. The electropherogram of a green tea sample before and after derivatization using OPA/NAC are shown in Fig. 4, illustrating the enantioselective separation of D/L-theanine and (±)-catechin derivatives.

LIF is also applied in MEKC methods and phytochemicals like curcuminoids can be detected with high sensitivity and specificity by LINF due to their native fluorescence after excitation by violet-blue light. To sensitize the native fluorescence of curcuminoids and enhance their separation efficiency, Wu et al. used mixtures of 20 mmol L^{-1} Triton X-100 and 20 mmol L^{-1} SDS in the BGE [64]. Using a commercial 445 nm laser diode as an excitation source, the resulting Triton-X 100/SDS mixed micelles induced fluorescence

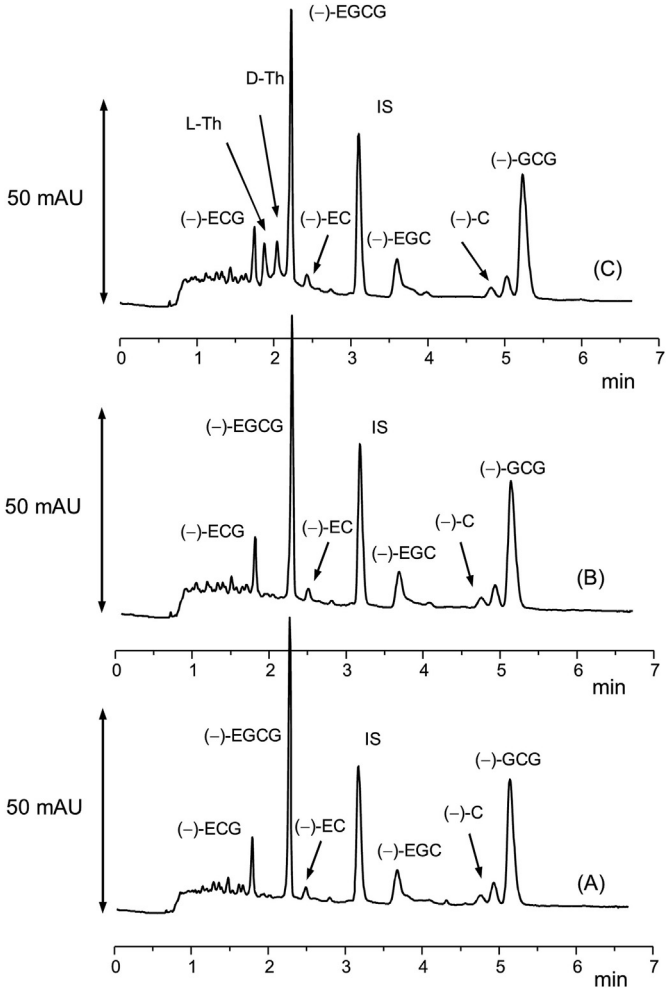

Fig. 4. Electropherograms of Bancha Hojicha tea sample by CD-MEKC; A) analysis before derivatization; B) analysis after derivatization using OPA/NAC; C) analysis of the sample spiked with D/L-Th and derivatized using OPA/NAC. Reprinted from Ref. [63], with permission from Elsevier.

synergism could enhance the signals of curcumin up to 77-fold, effectively avoiding the interference of other components in the samples. Another mixed micelle-induced fluorescence method, this time combining the nonionic tween 20 and anionic SDS surfactants

in a sodium borate running buffer, allowed the sensitive determination of the oleoresin capsaicin and dihydrocapsaicin in foods [65]. To obtain rapid detection with a short migration time, a voltage program (VP) has been used without loss of resolving power. The VP- MEKC-LIF method showed enhanced detection sensitivity compared to conventional UV absorption.

MS-based detectors have also been coupled to MEKC methods, although in a lesser extent, mainly due to their higher cost and specialized interfaces, which are not beneficial for their wide use in CE analysis. However, MEKC-MS hyphenations are required when dealing with phytochemicals like the sesquiterpene lactone artemisinin and its analogs (e.g. ascaridole, artemisia ketone, casticin, deoxyartemisinin, arteannuic acid, artemetin, dihydroartemisinic acid), due to their low concentration, and lack of chromophore/fluorescent characteristics or functional groups suitable for quantitative derivatization [66]. However, MEKC-MS methods requires the application of "MS-friendly" surfactants, like perfluorooctanoic acid (PFOA) to avoid signal suppression and contamination of the ion source.

4. Capillary electrochromatography (CEC) mode

Capillary electrochromatography (CEC) is a hybrid of CE and LC, where CE capillaries are coated or packed with different stationary phases, and the mobile phase liquid passes over the capillary wall and the stationary phase using EOF instead of pressure. CEC offers the loadability and selectivity of LC and the high efficiency of CE. One of the most important aspects in CEC is the selection and preparation of the stationary phase, as it has a substantial effect on the separation efficiency and loading capacity. There are mainly three types of column technologies (packed columns, monolithic columns, and open-tubular columns), and the interest on the development of novel stationary phases in this field has increased in the last years [67]. These technologies have been also applied to the analysis of different phytochemicals from complex matrices, or by using standard compounds to test the capabilities of the developed technologies. Table 4 summarizes the applications of the use of CEC for phytochemicals analysis in the period 2018–2021, highlighting the technology and the stationary phase used.

In the case of packed columns, the packing material for CEC that has been mostly utilized is similar to the used in HPLC, being reversed-phase on spherical particles the most popular. For instance, fused silica capillary (32 cm total length x 100 μm i.d.) were packed with LiChrospher 100 RP-18 endcapped particles (5 μm particle size, 100 Å pore size) to successfully analyzed and quantified ρ-cymene, α-terpinene, γ-terpinene, β-myrcene, limonene, β-pinene, thymol, carvacrol, linalool, α-terpineol, borneol and β-cariophyllene in *Thymus vulgaris* L. solid essential oil (EO) as well as from liquid EO [68]. The electrochromatogram of the analysis of *Thymus vulgaris* EO sample under optimized conditions is shown in Fig. 5.

The sample preparation for EO was quite straightforward, as it only consists of the dilution of the sample in the mobile phase and filtering it through a 0.2 μm nylon syringe filter. In the case of formulations containing *Thymus* EO, a previous extraction step of the solid material was required. This was performed by diluting 100 mg in 10 mL of mobile phase by vortex agitation for 30 s and ultrasonic bath for 15 min. The suspension was then centrifuged at 4500 RPM for 10 min, the supernatant was collected and injected in the system. The optimum mobile phase was a mixture of 50 mmol L^{-1}, pH 5.5 ammonium acetate solution and 15% acetonitrile (ACN) (v/v), and the detector selected was DAD. The described method provides good sensitivity (LOD of 2–5 μg mL^{-1}) and linearity ($r2 \geq 0.9991$) over the 5–200 μg mL^{-1} range for all the analytes.

Nanoparticles have also made a significant contribution to the development of stationary phases in CEC, as they possess large surface-to-volume ratios and can improve the separation selectivity, the column efficiency, and the chemical stability of CEC [69]. The two main immobilization strategies are physical adsorption to the inner face of the capillary through electrostatic interactions, or by covalent bonding. In the field of phytochemical analysis, the first strategy was used by Polikarpova et al. [70] to immobilize nano-sized anion-exchanger (NSAE) on fused silica capillary (60 cm total length x 50 μm i.d.). The used material was anionite of the AB-17 type with particle size of 50–250 nm, and it was applied to separate carboxylic acids (oxalic, formic, tartaric, malic, citric, succinic, lactic and acetic acids) in wine samples after on-line preconcentration [70]. The authors showed a good stability of the nano-particles at a wide range of pH (2–10), with high separation efficiency (N = 148000–732000 TP/m) and good selectivity (Rs = 1.2–5.7) for carboxylic acids. Due to the aqueous nature of the wine samples, a simple preparation method (dilution of the wine samples with water followed by centrifugation and supernatant collection) was required. However, the mayor drawback of the method is that the NSAE UV-adsorption was in the range of the targeted analytes, which decreased the LOD and required the use of preconcentration steps.

On the other hand, the research group of Zilin Chen have extensively worked on the functionalization of monolithic columns. For instance, methyl-vinylimidazole functionalization has been developed to analyze *Evodiae Fructus* phytochemicals (evodiamine, rutaecarpine and limonin) for authentication purposes [71]. The polymerization mixture was infused into a 50 cm capillary to a final effective length of 40 cm, and the optimized CEC-MS conditions were performed with a running buffer containing 1% ammonia aqueous solution in 30% ACN (v/v). In this case, an extensive sample preparation (sample pulverization, ultrasound extraction using 70% ethanol and sample filtering through a 0.22-μm nylon membrane) was required before sample analysis. The developed CEC-MS method provided good linearity ($R^2 > 0.99$) of 0.8–160 μg mL^{-1} with low limits of detection of 0.15–0.31 μg mL^{-1}, which means a 4–16 folds better LODs when compared with CEC-UV method.

Finally, open-tubular columns are extremely suitable for the introduction of innovative materials as the stationary phases, and they have the advantage of the ease of column preparation, low back pressure and simple instrumental handling. However, they are limited by the low phase ratio, low sample capacity and poor separation efficiency for some applications. In the case of phytochemical analysis, these columns have been used to develop a novel spherical vinyl-functionalized covalent-organic framework (COF–V) as stationary phase for the analysis of alkylbenzenes, chlorobenzenes and phenolic compounds by CEC-UV analysis [72]. The dimensions of the COF–V modified column were 40.5 cm total length/32 cm effective length (50 μm i.d.), and the mobile phase composition varied depending on the compounds to be analyzed (Table 4). The COF–V stationary phase showed a powerful separation selectivity to different compounds, and the RSD values of retention times for intra-day (n = 5), inter-day (n = 3) runs, and column-to-column (n = 3) were all less than 2.1%. Due to the good performance of the functionalized column, the same separation system was coupled to MS for the determination of limonin, evodiamine and rutaecarpine with successful results. A similar approach was applied by Li et al. [73] to develop a COF open tubular column composed of 4,4′,4″-(1,3,5-triazine-2,4,6-triyl)trianiline (Tz) and 2,5-dihydroxyterephthalaldehyde (Da). In this case, the analyzed compounds were chlorobenzenes, alkylbenzenes, phenols, anilines, vanillin and its analogs, amino acids and polypeptides, and the separation conditions were specific according to the different compound classes. It has to be noted that better

Table 4
Some representative applications on the use of CEC for phytochemicals analysis in the period 2018–2021.

Analyte	Sample	CE mode and detector	CE separation conditions	Stationary phase	Ref
ρ-Cymene, α-terpinene, γ-terpinene, β-myrcene, limonene, β-pinene, thymol, carvacrol, linalool, α-terpineol, borneol and β-cariophyllene	*Thymus vulgaris* L. solid essential oil	CEC-UV-DAD	$NH_4CH_3CO_2$ buffer (50 mmol L^{-1}, pH 5.5) in 15% ACN	LiChrospher 100 RP-18 endcapped particles (5 μm particle size, 100 Å pore size)	[68]
carboxylic acids	wine	CEC-UV	10 mmol L^{-1} benzoic acid and 9 mmol L^{-1} diethanolamine	Nano-sized anion-exchangers (NSAE)	[70]
evodiamine, rutaecarpine andlimonin	*Evodiae Fructus* herbs	CEC-UV/MS	1% ammonia aqueous solution in 30% ACN	Methyl-vinylimidazole func-tionalized organic polymer monolith	[71]
alkylbenzenes	standards	CEC-UV/MS	$NH_4CH_3CO_2$ buffer (20 mmol L^{-1}, pH 4.0) in 50% ACN	Spherical vinyl-functionalized covalent-organic framework (COF–V)	[72]
chlorobenzenes			$NH_4CH_3CO_2$ buffer (20 mmol L^{-1}, pH 5.0) in 50% ACN		
phenolic compounds			$NH_4CH_3CO_2$ buffer (20 mmol L^{-1}, pH 5.0) in 45% ACN		
active ingredients			$NH_4CH_3CO_2$ buffer (20 mmol L^{-1}, pH 4.0) in 40% ACN		
alkylbenzenes	standards	CEC-UV	Phosphate buffer (10 mmol L^{-1}, pH 9.0) in 25% ACN	Covalent-organic frameworks (COF) composed of 4,4′,4″-(1,3,5-triazine-2,4,6-triyl)trianiline (Tz) and 2,5-dihydroxyterephthalaldehyde (Da)	[73]
chlorobenzenes			Phosphate buffer (10 mmol L^{-1}, pH 7.0) in 30% ACN		
phenolic compounds			Phosphate buffer (30 mmol L^{-1}, pH 9.0) in 25% ACN		
food additives			Phosphate buffer (30 mmol L^{-1}, pH 9.0) in 20% methanol		
amino acids			Phosphate buffer (20 mmol L^{-1}, pH 8.0) in 40% methanol		

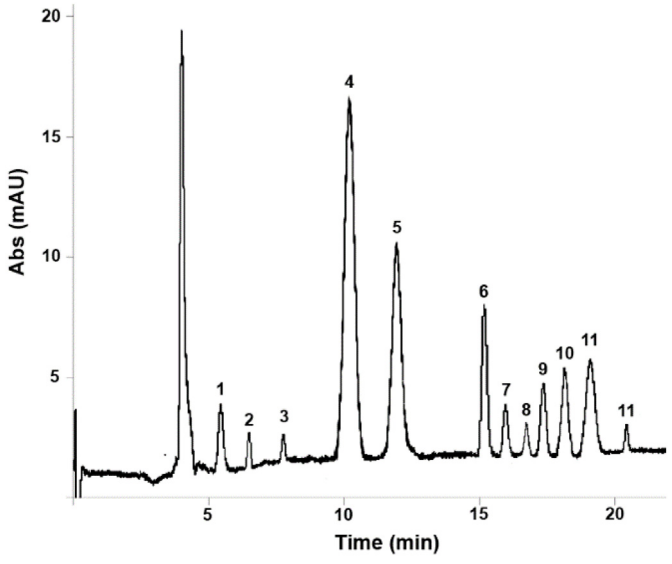

Fig. 5. Capillary electrochromatography coupled to diode array detection (CEC-DAD) electrochromatogram obtained from the analysis of a *Thymus vulgaris* L. EO sample under the optimized conditions: 1, borneol; 2, linalool; 3, α-terpineol; 4, thymol; 5, carvacrol; 6, p-cymene; 7, β-pinene; 8, α-terpinene; 9, β-myrcene; 10, β-Cariophyllene; 11, γ-terpinene; 12, limonene. Reprinted from Micucci et al. [68].

results than previous developments of COF-based stationary phases were obtained with the TzDa coated column, but its performance when analyzing more complex matrices, such as plant extracts, have not been evaluated.

5. Conclusions

The objective of this manuscript is to review the recent bibliography, between the years 2018 and 2021, applied to the analysis of phytochemical compounds by CE. It was noticed that many studies started to apply methods developed by CE as part of the characterization of some material, that is, the CE technique is more mature and well established in the field of analytical chemistry, where publications are no longer only focused on the development of the method. This fact may be related, in part, to the constant search for analytical methodologies that are in accordance with green chemistry, with less expenditure on organic solvents, shorter analysis times and, of course, lower costs. The development of pre-concentration techniques and the inclusion of new materials, such as ionic liquids or nanoparticles in the BGE or the stationary phases used in CEC, proved to be powerful allies to CE methods, as, in some cases, they contributed to achieve LOD and LOQ values in the order of ng mL^{-1}. Another interesting point is the association of CE with chemometric modeling techniques, either using the analysis results as discrete variables, or directly using electropherograms. The association of these tools allows the exploration of the CE technique in a more comprehensive way. For the near future, it is expected to find more classes and subclasses of compounds contemplated in phytochemical studies by CE, as well as a greater number of studies

that will correlate the electropherogram with PCA and PLS analyses, and more works focused on reducing or even eliminating the use of organic solvents from sample preparation to BGE.

Author contributions

Alberto Valdés - Writing - Review & Editing.
Alejandro Cifuentes – Editing & Supervision.
Brenda Lee Simas Porto - Writing - Review & Editing.
Gerardo Alvarez - Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

A. V. and G.A.-R. would like to acknowledge the Ministry of Science and Innovation (MICINN) for their "Juan de la Cierva-Incorporación" postdoctoral grants IJC2018-037560-I and IJC2019-041482-I, respectively. This work was supported by the Ministry of Science and Innovation of Spain (Grant No. PID2020-113050RB-I00 and Grant No. PDC2021-120814-I00).

B. L. S. P. would like to acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, for your CAPES Print funding as a visiting professor, process number 88887.569366/2020-00. This work was supported by the Ministry of Education of Brazil (Finance Code 001).

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