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Review

Recent trends in the determination of polyphenols by electromigration methods

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Abstract

An overview mapping recent trends in the determination of polyphenols of natural origin (mostly flavonoids) and their synthetic derivatives by electromigration methods is presented. The overview (covering the period of the recent 5 years and comprising 61 references) is focused on capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) with various detection methods. Techniques comprising on-line pre-separation such as isotachophoresis (ITP)-CZE and flow-injection-CZE, chiral separations and CZE evaluation of antioxidation activity are also discussed.

Keywords: Electromigration methods; Polyphenols; Chiral separations; Detection techniques; Antioxidants; Review

Contents

Abbreviations: AOT, bis(2-ethylhexyl)sodium sulfosuccinate; BGE, background electrolyte; BHA, butylated hydroxyanisole; BHQ, butylated hydroquinone; BHT, butylated hydroxytoluene; CD, cyclodextrin; CHAPS, 3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propansulfonate; CS, catechin sulfate; ECS, epicatechin sulfate; ED, electrochemical detection; EOF, electroosmotic flow; FEP, fluorinated ethylene–propylene copolymer; FI, flow injection; 6G-β-CD, 6-*O*-α-D-glucosyl-β-cyclodextrin; HEC, 2-hydroxyethylcellulose; HP-CD, hydroxypropyl cyclodextrin; HPLC, high performance liquid chromatography; ITP, isotachophoresis; LE, leading electrolyte; NACE, non-aqueous capillary electrophoresis; SC, sodium cholate; S/N, signal to noise ratio; TE, terminating (trailing) electrolyte

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1. Introduction

Antioxidants are both natural and synthetic compounds possessing ability to scavenge free radicals and to inhibit oxidation processes. Polyphenols (flavonoids and polyphenolic acids) together with ascorbic acid, vitamin E and carotenoids are the most important natural reducing agents occurring in diet that are believed to protect biological tissues from oxidative stress. As evidenced by results published by some authors, a diet rich in these substances can reduce the incidence of coronary heart disease, some kinds of cancer and inflammation processes [1,2].

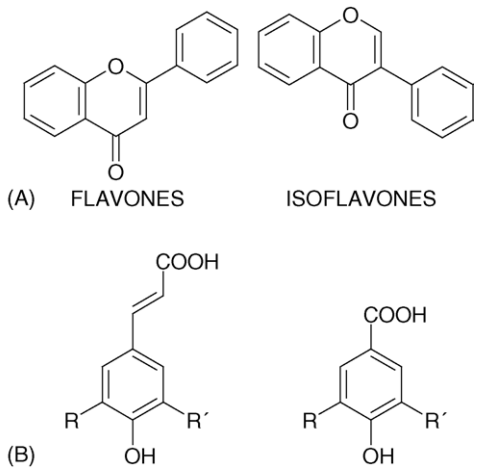

Fig. 1. The basic skeleton of flavonoids (A) and hydroxyphenylcarboxylic acids (B).

The main structural types of polyphenols are represented by hydroxyphenylcarboxylic acids and flavonoids (see Fig. 1). Their content was determined in many herbs and natural products such as olive oil, wines and propolis. It is assumed that the consumption and use of these nutritional products in France (red wine) and Mediterranean countries (Greece, Spain) is associated with lower incidence of heart diseases and cancer.

The number of papers dealing with the assay of antioxidants in various analytical matrices is increasing in accordance with growing interest in the investigation of their pharmacological and biological effects. Due to complex composition of plant materials, the separation methods play the most important role in the assay of antioxidants. In addition to chromatographic methods (LC and GC) that still occupy the leading position in pharmaceutical and phytochemical analysis, numerous papers dealing with the determination of antioxidants by means of electromigration methods have been published. High separation efficiency as well as short analysis time and low consumption of solvents and samples are characteristic features of electrophoretic separation techniques. On the other hand their drawbacks are generally lower sensitivity and worse reproducibility compared to HPLC.

Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are the two “classical” modes of electromigration methods that are most frequently employed for the determination of various antioxidants in different herb materials.

CZE separation is based on different migration of charged solutes (caused by the differences in their charge to mass ratio) in a conductive liquid placed in a capillary under the influence of a high-voltage electric field. The movement of solutes in a silica capillary is also affected by the electroosmotic flow (EOF) that originates thanks to negatively charged silanoate groups of the capillary walls. Cations of the electrolyte are attracted by the negatively charged wall to form a fixed layer; other cations form a mobile layer which migrates toward the cathode while the bulk of the buffer solution co-migrates with it and gives rise to the EOF. Since the EOF is greater than electrophoretic mobility of the negatively charged solutes (originally they tend to migrate toward the anode) both negatively and positively charged solutes can be analyzed within one run. On the other hand all neutral analytes migrate with the rate of the EOF and remain unseparated [3].

Neutral analytes can be separated by another electromigration technique called micellar electrokinetic chromatography (MEKC). In MEKC the BGE contains a charged surfactant (often sodium dodecyl sulfate, SDS) at a concentration level exceeding its critical micellar concentration (CMC). The micelles formed serve as a “pseudostationary phase” and the analytes undergo partitioning between the micelles (hydrophobic phase) and BGE (hydrophilic phase). Here the mechanism of separation is also based on the differences in the lipophilicity of analytes [3].

In isotachophoresis (ITP) a zone containing a mixture of analytes (cations or anions) is introduced between two different buffers. One buffer called “leading electrolyte” (LE) contains a leading ion (cation if the analytes are cations) that has higher mobility than that of any of the analytes and the other buffer called “terminating (or trailing) electrolyte” (TE) contains a terminating ion that has mobility lower than that of any of the analytes. When an electric field is applied to the capillary the analytes are stacked into zones according to their mobilities and in equilibrium state these distinct zones migrate at the same velocity. The analyte zones closely follow one another (with sharp boundaries, no gap between them) arranged according to their mobilities, with the fastest analyte ion moving behind the leading electrolyte, etc. In the steady state each ITP zone contains only one kind of analyte and common counter-ion. Due to the existence of the “Kohlrausch regulating function” the concentration effect exists; it means that the concentration of analyte in its zone is spontaneously adjusted to the concentration of the LE. In one run either cations or anions can be separated but not both. In comparison with CZE and MEKC the zone dispersion in ITP is significantly decreased [3].

Several reviews concerning the assay of polyphenols by electrophoretic methods were published earlier. Boyce [4] surveyed the use of CZE for the determination of additives involving synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (BHQ) and gallic acid esters in food. Another review published by Klampfl et al. [5] focused on the CZE assay of polyphenols, low-molecular acids, amino acids and fatty acids in foods. Gu et al. [6] reviewed the CZE methods employed for the assay of resveratrol and some other flavonoids in wine. Recently a critical review [7] evaluating the merits and drawbacks of using CZE and HPLC in the analysis of phytochemical substances including flavonoids and phenolic acids has appeared. The author came to the conclusion that CZE will not eventually replace HPLC in the phytochemical analysis but it can be an alternative where analysis requires higher efficiency or resolution than HPLC, especially in cases of phenolic polymers, bioflavonoids and alkaloids. Two overviews recounting the determination of tea components were published. Dalluge et al. [8] reviewed the most important separation methods such

as HPLC, CZE, GC and TLC used for the determination of tea catechins that belong to the group of polyphenols possessing strong antioxidant and anticarcinogenic activity. Horie et al. [9] summarized the information about the analysis of tea components (amino acids, polyphenols, purine alkaloids and vitamins) by HPLC and CZE. Other reviews focused on the determination of active components in *Rhododendron dauricum* [10], *Scutelarrie baicalensis* [11] and *Hippophae rhamnoides* [12] by various separation methods including also the CE. A comprehensive overview devoted to separation methods currently in use to determine flavonoids in various matrices was published recently [13] but only nine out of 133 references appearing in the review are related to electromigration methods.

The aim of the present compilation is to indicate new trends in the use of electrophoretic methods that have been utilized for the determination of polyphenols during the last 5 years. The list of separation conditions, analytical matrices, and analytes are presented in the form of tables (see Tables 1–3). Other details, where appropriate, are discussed in the text.

2. General characteristics of the electrophoretic methods reviewed

On the basis of the overview of 47 original papers concerning electrophoretic analysis of polyphenols it is clearly seen that the most exploited mode is conventional CZE (32 papers) followed by MEKC (11 papers). Isotachophoresis (ITP) was employed three times [30–32] but solely as a pre-concentration technique coupled to CZE (see Section 5). Merely two papers dealing with the separation of polyphenols are based on novel techniques of non-aqueous capillary electrophoresis (NACE) [14] and microchip-CE [15].

2.1. Background electrolytes (BGEs)

It is well known that the separation mechanism in CZE is based on the differences between the charge/mass ratio of the solutes and that the degree of ionization of polyphenols (the pK_a values of –OH groups ranging between ≈7 and 12, depending on the structure of the polyphenol molecule, see, e.g., [21]) can be simply manipulated by using BGEs comprising alkaline buffers. As follows from the data collected in Table 1, for conventional CZE the pH of the most frequently exploited BGEs range between 9 and 10. Such BGEs are typically based on tetraborate buffers; here the ability of borate to form negatively charged complexes with vicinal –OH groups of polyhydric phenols is utilized as well. It must be noted that until now practically no attention has been paid to possible oxidation of rather reactive polyphenols with oxygen in alkaline BGEs. A tendency of using organic solvents (such as methanol and acetonitrile) as modifiers for improving efficiency in both CZE and MEKC is also notable [14,17,18,20,21,25,29–32,44,52–54,60]. Furthermore various types of cyclodextrins (CDs) were employed as components of the BGEs acting as chiral selectors or just improving the efficiency of separation of non-chiral analytes [16].

3. Micellar electrokinetic capillary chromatography (comparison with CZE and HPLC)

Even though it is generally believed that MEKC possesses higher separation efficiency than CZE the utilization of MEKC in the determination of flavonoids was about three times less frequent than that of CZE in recent 5 years. It seems that the use of borate-based buffers in conventional CZE allows sufficient resolution of polyphenols in relatively complicated mixtures most probably thanks to the above-mentioned complex-formation effects of borate. On the other hand in the case of compounds with similar structure but different lipophilicity, the use of micelles is advantageous since the separation process is affected by more factors compared to CZE (e.g., unsaturation of C-ring lowers the migration time; methylation of hydroxyl group increases the hydrophobicity of analyte and consequently its affinity to the micelles which results in increase in migration times; glycosylation and higher number of –OH groups increases hydrophilicity and therefore migration times are decreased) [21].

In the MEKC of polyphenols sodium dodecylsulphate is the most widely used surfactant as can be traced in the data of Table 1 [17,19,21,22,26,29,49,54,59,60]. Sodium cholate (SC) was reported as an auxiliary additive to SDS-containing BGE in the so called “mixed MEKC”. This separation technique utilizes the formation of mixed micelles formed from both surfactants to improve the resolution of either polar or non-polar analytes that could not be separated by conventional MEKC [17,18].

Favorable properties of BGEs containing mixed micelles were reported by Gotti et al. [19] who added sodium taurodeoxycholate and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) to the SDS-containing BGE. The separation efficiency of these buffers in “mixed MEKC” was compared with the CD-modified-MEKC when determining catechins and xanthines in chocolate and cocoa. The best results were obtained with the addition of hydoxypropyl-β-cyclodextrin (HP-β-CD) to the SDS-containing buffer but satisfactory results were attained also with the above-mentioned mixed-micelles systems based on taurodeoxycholate and CHAPS.

Sodium bis(2-ethylhexyl) sulphosuccinate (AOT) as the vesicles-forming surfactant was employed in the assay of BHQ, BHA, BHT, propyl-, octyl-, dodecyl-gallate and vitamin E in olive oil [20].

In a number of papers the merits and drawbacks of HPLC and the electromigration methods as utilized in routine analysis of polyphenols were discussed [17,22–24].

Bonoli et al. [22] validated RP-HPLC and MEKC methods employed for the determination of tea catechins. In this study the MEKC surpassed HPLC by higher sensitivity (the LODs for CE ranged from 0.0013 to 0.0051 μg/ml whereas for HPLC the LOD values varied from 0.0250 to 0.385 μg/ml at S/N ratio = 3), resolution and migration time repeatability (R.S.D. of retention times in HPLC ranged from 0.2 to 4.14% whereas the R.S.D. of migration times in MEKC did not exceed 1.78%). The MEKC lost to HPLC in the repeatability of the quantification of the overall content of catechins (the R.S.D. values ranged from 0.77 to 1.72% and from 1.01 to 5.54% for HPLC and MEKC, respectively).

Table 1
General characteristics of the reviewed methods

Analytes/matrix	BGE	Capillary/voltage	Detection	Reference
Catechin, epicatechin, myricetin, quercetin, *trans*-resveratrol/wine	5 mM malonate, 9.6 mM TBAOH in 100% MeOH	poly (GMA-co-NVP), 58.5 cm (8.5 cm) × 50 μm/30 kV	UV 230 nm	[14]
Chlorogenic, ferulic, gentisic and vanilic acid/wine	15 mM borate (pH 9.5)	Microchip CE/2000 V	ED-carbon working electrode, +1.0 V	[15]
Isorhamnetin, kaempferol, quercetin/*Hippophae rhamnoides*	20 mM borate, 5 mg/ml DM-β-CD (pH 10.00)	35 cm (30 cm) × 50 μm/15 kV	UV 270 nm	[16]
Ascorbic acid, butylated hydroxyanisole, *tert*-butylhydroquinone, butylated hydroxytoluene, propyl-, octyl-, dodecyl-gallate, isoascorbic acid, tocopherol/sesame oil, wine	10 mM borate, 40 mM SC, 15 mM SDS, 10% MeOH (pH 9.3)	60 cm (52 cm) × 75 μm/18 kV	UV 254 nm, 214 nm	[17]
Tert-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, propyl-, octyl-, dodecyl-gallate/jam	35 mM SC, 15 mM SDS, 20 mM borate, 10% MeOH (pH 9.3)	60 cm (52 cm) × 75 μm/18 kV	UV 214 nm	[18]
Caffeine, catechin, cAMP, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin, theobromine, theophylline/chocolate, cocoa	Detailed in text	38.5 cm (8.5 cm) × 50 μm/15 kV	UV 220 nm	[19]
Tert-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, propyl-, octyl-, dodecyl-gallate, vitamin E/olive oil	20 mM borate, 20% ACN, 20 mM AOT (pH 9.4)	57 cm (50 cm) × 75 μm/24 kV	UV 280 nm	[20]
Catechin, epicatechin, hesperidin, icariin, ikarisoside, kaempferol, kaempferol-3-*O*-rhamnoside, naringin, quercetin, 2″-*O*-rhamnosylicariside, tiliroside, wogonin/-	10 mM H_2PO_4, 5 mM $Na_2B_4O_7$, 90 mM SDS, 10% ACN (pH 7.3)	50 cm (42.4 cm) × 75 μm/18 kV	UV 214 nm	[21]
Gallic acid, catechin, catechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, epicatechingallate, gallocatechin, gallocatechingallate	20 mM KH_2PO_4, 50 mM $Na_2B_4O_7$, 200 mM SDS (3:1:2) (pH 7.0)	47 cm (40 cm) × 50 μm/30 kV	UV 200 nm	[22]
Chlorogenic acid, hyperoside, rutin/*Fagopyrum esculentum*	60 mM borate (pH 10.0)	64.5 cm (56 cm) × 50 μm/30 kV	UV 206 nm	[23]
Apigenin, baicalein, caffeic acid, galangin, hesperetin, kaempferol, luteolin, myricetine, naringenin, quercetin/wine grape	35 mM borate (pH 8.9)	70 cm (45 cm) × 75 μm/16.8 kV	UV 250 nm	[24]
Medicarpin, vestitone	25 mM borate, 2 mM HP-β-CD, 20 mM HP-γ-CD, 10% MeOH (pH 10.0)	80 cm (50 cm) × 50 μm/15 kV	UV 210 nm	[25]
Caffeine, catechin, epicatechin, catechin gallate, epicatechin gallate, epigallocatechin, epigallocatechin gallate/tea beverage	200 mM borate, 20 mM phosphate, 240 mM SDS, 25 mM 6-*O*-α-D-glucosyl-β -CD (pH 6.4)	64.5 cm (56 cm) × 50 μm/25 kV	UV 210 nm	[26]
Apigenin sulfate, catechin sulfate, epicatechin sulfate, 6,2′, 3′-flavonoid sulfate, quercetin sulfate	20 mM phosphate buffer, 15% β-CD (pH 3.5)	32.5 cm × 50 μm/−5, −10 kV	UV 230, 280 nm	[27]
Cis/*trans*-resveratrol/wine	40 mM $Na_2B_4O_7$ (pH 9.5)	25 cm × 75 μm/5 kV	UV 320 nm	[28]
Cis/*trans*-resveratrol/wine	75 mM SDS, 30 mM H_3BO_3, 30 mM Na_2HPO_4, 15% ACN (pH 9.2)	37 cm (30 cm) × 50 μm/25 kV	UV 314 nm	[29]
Caffeic, chlorogenic, cinnamic, ferulic, isoferulic, pivalic and *p*-coumaric acid, hyperosid, isoquercitrin, quercetin, rutin, vitexin, vitexin-2-*O*″-rhamnoside/*Sambucus*, *Crataegus*	25 mM MOPSO, 50 mM TRIS, 10 mM H_3BO_3, 0.2% HEC, 20% MeOH (pH 9.0)	FEP 16 cm × 0.3 mm	UV 254 nm, conductivity	[30]
Chlorogenic acid, hyperoside, isoquercitrin, quercetin, quercitrin, rutin/*Hypericum perforatum*	25 mM MOPSO (TAPS), 50 mM TRIS, 55 mM H_3BO_3, 0.2 % HEC, 20% MeOH (pH 8.3; 8.75)	FEP 16 cm × 0.3 mm	UV 254 nm, conductivity	[31]

Table 1 (*Continued*)

Analytes/matrix	BGE	Capillary/voltage	Detection	Reference
Caffeic, ferulic, gallic, *p*-coumaric, protocatechuic, syringic and vanillic acid, apigenin, catechin, epicatechin, quercetin, quercitrin, kaempferol, rutin, vitexin/red wine	25 mM MOPSO (TAPS), 50 mM TRIS, 15 (40) mM H_3BO_3, 0.2 % HEC, 5 mM β-CD, 20% MeOH (pH 8.5)	FEP 16 cm × 0.3 mm	UV 254 nm, conductivity	[32]
Caffeic, gallic, gentisic, *p*-coumaric acids, catechin, epicatechin, myricetin, quercetin, *trans*-resveratrol/wine	0.1 M $Na_2B_4O_7$ (pH 9.5)	67 cm × 75 μm/20 kV	UV 280 nm	[33]
Epicatechin, hyperoside, quercetin, rutin/*Fagopyrum esculentum*	50 mM borate (pH 8.7)	NA/NA	ED-carbon disc electrode, +0.9 V	[34]
Baicalein, baicalin, quercetin/*Scutellarie radix*	100 mM borate (pH 9.0)	40 cm × 25 μm/12 kV	ED-carbon disc electrode, +0.9 V	[36]
Daidzein, quercetin, rutin/*Cinnamomum camphora, Ligustrum lucidum, Flos sophorae*	100 mM borate (pH 9.0)	40 cm × 25 μm/12 kV	ED-carbon disc electrode, +0.9 V	[37]
Apigenin, catechin, epicatechin, luteolin, quercetin, rutin/*Ginkgo biloba*	50 mM borate (pH 9.0)	70 cm × 25 μm/16 kV	ED-carbon disc electrode, +1.00 V	[38]
Catechin, epicatechin, kaempferol, quercetin, rutin/*Hippophae rhamnoides*	50 mM borate (pH 9.0)	75 cm × 25 μm/14 kV	ED-carbon disc electrode, +950 mV	[39]
Chlorogenic acid, baicalcin, baicalin	15 mM borate (pH 9.2)	60 cm/20 kV	ED-carbon disc electrode, +0.9 V	[40]
Acacetin, caffeic acid, chlorogenic acid, protocatechuic acid, quercetin, rutin/*Herba cepbalanoplosis segeti, Herba cirsii japonici*	50 mM borate (pH 8.4)	75 cm × 25 μm/15 kV	ED-carbon disc electrode, +0.95 V	[41]
Farrerol, hyperoside, kaempferol, quercetin, scopoletin, umbeliferone/*Rhododendron dauricum*	70 mM borate (pH 9.2)	75 cm × 25 μm/16 kV	ED-carbon disc electrode	[42]
Genistein, genistin, kaempferol, quercetin, rutin/*Flos sophorae*	50 mM borate (pH 9.0)	75 cm × 25 μm/16 kV	ED-carbon disc electrode	[43]
Biochanin A, hesperetin, 5-methoxyflavone, naringenin/-	40 mM ammonium acetate, 15 % ACN (pH 9.5)	NA/NA	Quadrupole ESI-MS	[44]
Chlorogenic acid, rutin/cigarettes	20 mM borate (pH 10)	50 cm × 75 μm/13 kV	Indirect chemiluminiscence	[45]
Rutin/*Aponycum venetum, Jinkgo biloba, Morus alba, Rhododendron dauricum*	20 mM phosphate (pH 7.4)	45 cm × 25 μm/20 kV	ED-carbon disc electrode	[46]
Caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, gallic acid, catechin, kaempferol, morin, myricetin, quercetin, *trans*-resveratrol, rutin/*Hippophae rhamnoides*	20 mM $Na_2B_4O_7$ (pH 9.3)	60 cm (41 cm) × 50 μm/20 kV	UV 210 nm	[47]
Biochanin A, daidzein, daidzin, genistein, puerarin/*Puerarie radix*	20 mM borax-NaOH (pH 10.1)	47 cm (40 cm) × 50 μm/21 kV	UV 200 nm	[48]
Caffeine, catechin, epicatechin, epicatechingallate, epigallocatechin, epigallocatechingallate/tea (black, green)	4 mM $Na_2B_4O_7$, 12 mM K_2HPO_4, 40 mM SDS (pH 7.0)	85 cm (70 cm) × 50 μm/ 30 kV	UV 200 nm, 266 nm	[49]
Calycosin, licochalconel A, licoisoflavone A, liquiritin/*glycyrhizae radix*	100 mM borate (pH 10.5)	58.5 cm (50 cm) × 50 μm/30 kV	UV 210 nm	[50]
Caffeic, cinnamic, dihydrocaffeic, ferulic, gallic, gentisic, o-coumaric, *p*-coumaric, protocatechuic, siringic and vanillic acid, hydroxytyrosol, quercetin, luteolin, oleuropein glycoside, taxifolin, tyrosol/olive oil	45 mM borate (pH 9.6)	47 cm (40 cm) × 50 μm/27 kV	UV 200 nm	[51]
Ascorbic acid, didymin, ferulic acid, hesperidin, narirutin, phenylalanin, phlorin, synephrine, tyrosine/orange juice	35 mM borate, 5% ACN (pH 9.3)	70 cm × 50 μm/21 kV	200–360 nm	[52]
Apigenin, apigenin-7-*O*-glucoside, chlorogenic acid, isoorientin, isoschaftoside, luteolin-4′-*O*-glucoside, luteolin-7-*O*-glucoside, orientin, rutin, schaftoside, vicenin-2, vitexin/*Achillea setacea*	25 mM $Na_2B_4O_7$, 20% MeOH (pH 9.3)	65.5 cm (58 cm) × 50 μm/30 kV	UV 275 nm	[53]
Apigenin, diplacone, mimulone/*Paulownia tomentosa*	20 mM borate, 10 mM SDS, 5% MeOH (pH 10.0)	35 cm (30 cm) × 50 μm/15 kV	UV 280 nm	[54]

Table 1 (*Continued*)

Analytes/matrix	BGE	Capillary/voltage	Detection	Reference
Acteoside, 3,7-dihydroxyquercetin, 20-hydroxyecdysone, rutin/*Lamium maculatum*	30 mM borate (pH 9.47)	51 cm (43.4 cm) × 75 μm/20 kV	UV 254 nm	[55]
Catechin, gallic acid, kaempferol, quercetin, quercitrin/*Morus alba*	150 mM boric acid (pH 10.0)	51 cm (42.5 cm) × 50 μm/18 kV	UV 270 nm	[56]
Apigenin, caffeic acid, ferulic acid, luteolin, quercetin, rutin/propolis	50 mM borate (pH 9.2)	60 cm (50 cm) × 75 μm/23 kV	UV 262 nm	[57]
Baicalein, baicalin, oroxylin A, oroxylin A-7-*O*-glucuronide, wogonin, wogonin-7-*O*-glucuronide/*Scutellarie radix*	20 mM NaH_2PO_4, 25 mM $Na_2B_4O_7$ (pH 7.24)	NA/NA	UV 254 nm	[58]
Quercetin, naringenin	200 mM $Na_2B_4O_7$, 50 mM SDS (pH 8.5)	50 cm × 50 μm/18 kV	UV 200-360 nm	[59]
Benzoic, cinnamic, 3,4-dimethoxycinnamic and p-coumaric acids, chrysin, galangin, methyl and propyl 4-hydroxybenzoates, pinocembrin and its 12 derivatives/propolis	25 mM borate, 50 mM SDS, 10% ACN (pH 9.3)	64.5 cm (56 cm) × 50 μm/30 kV	UV 200 nm	[60]
Caffeic, chlorogenic, ferulic and protocatechuic acid, flavone, quercitrin, rutin/-	30 mM NaH_2PO_4, 30 mM Na_2HPO_4 (pH 7.0)	87 cm (80 cm) × 50 μm/30 kV	UV 220 nm	[61]
D-catechin, epicatechin, myricetin, quercetin, rutin/-	30 mM Na_2HPO_4 (pH 8.85)	67 cm (60 cm) × 50 μm/20 kV	UV 220, 380 nm	[61]

Abbreviations: ACN: acetonitrile, AOT: bis(2-ethylhexyl)sodium sulfosuccinate, cAMP: cyclic adenosine 3′,5′-monophosphate, CD: cyclodextrin, ED-electrochemical detection, FEP: fluorinated ethylene-propylene copolymer, HEC: 2-hydroxyethylcellulose, HP-CD: hydroxypropyl-cyclodextrine, MeOH: methanol, MOPSO: β-hydroxy-4-morpholinopropanesulfonic acid, NA: not available, poly-(GMA-co-NVP): poly(glycidylmethacrylate-co-*N*-vinylpyrrolidone), SC: sodium cholate, TAPS-*N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid, TBAOH: tetrabutylammonium hydroxide, TRIS: tris(hydroxymethylamino)methane.

The results of other studies [17,23,24] indicate that the CE compares well with HPLC in terms of precision, linearity and limit of detection. For example Wang et al. [24] developed both CZE and HPLC methods for the assay of nine flavonoids in wine grapes. In this case the CZE seems to be slightly more sensitive than HPLC (the LODs fell within 0.08–0.21 and 0.030–0.210 μg/ml for HPLC and CZE, respectively). Reproducibility of either method was very similar (e.g. R.S.D. of run-to-run reproducibility of retention times ranged from 0.34 to 0.53% for HPLC and from 0.24 to 0.39% for CZE; R.S.D. of run-to-run integrated peak area varied from 0.70 to 3.82% for HPLC and 0.79 to 3.91% for CZE). Also the recovery experiment gave comparable results for both methods (93.3–107% for HPLC and 90.1–99.8% for CZE). Linear range is similar for both methods (0.1–80 and 0.100–150 μg/ml for HPLC and CZE, respectively; $r \geq 0.9990$).

4. Electrophoretic determination of various kinds of isomers including chiral separations

Allen et al. [25] applied CZE for fast screening of the enantiomeric purity of flavonoids biosynthesized by transgenic leguminous plants. Simultaneous chiral separation of mixtures containing four enantiomers, namely *R*-vestitone, *S*-vestitone and their metabolites (+)-medicarpin and (−)-medicarpin was achieved by adding two different cyclodextrins into the BGE (HP-β-CD and HP-γ-CD facilitated the separation of the vestitones and the medicarpins, respectively). The addition of methanol into the BGE with the two CDs improved also the separation.

Various types of cyclodextrins were tested to resolve enantiomers of catechin and epicatechin. Only 6-*O*-α-D-glucosyl-β-cyclodextrin (6G-β-CD) and HP-γ-CD were effective in their separation, but only 6G-β-CD allowed to separate them from other catechins. The method was applied to the analysis of real samples of teas and tea beverages [26].

Dantuluri et al. [27] were the first in finding separation conditions for the resolution of highly sulfated flavanoids and flavonoids. The possibility of chiral separation of (±)-catechin sulfate (CS) enantiomers and (−)-epicatechin sulfate (ECS) and (+)-CS diastereomers was examined by applying various chiral selectors. Because of high charge density of these compounds it was possible to separate them with BGEs containing β-CD either in pressurized capillary under the positive voltage or under the reversed polarity conditions; the latter technique gave better results.

Two papers dealing with the separation of *cis*- and *trans*-resveratrol as positional isomers were published [28,29]. Nevado et al. [28] used simple borate buffer to separate these isomers whereas Gu et al. [29] devised a MEKC method for the same purpose. In either case the limits of detection and migration times of both isomers were similar but in [29] the linear calibration range for *trans*-resveratrol was wider (45.6 μg to 22.8 mg/l in [29] and 500 μg to 20 mg/l in [28]). Both methods were applied for the determination of *cis*- and *trans*-resveratrol in wines but SPE with C-18 cartridge for the sample pretreatment was required.

Table 2
List of analytical matrices

Plant/drug	References
Achillea setacea	[53]
Aponycum venetum	[46]
Cepbalanoplosis segeti herba	[41]
Chocolate	[19]
Cigarettes	[45]
Cinnamomum camphora	[37]
Cirsii japonici herba	[41]
Cocoa	[19]
Crataegus	[30]
Fagopyrum esculentum	[23,34]
Ginkgo biloba	[38,46]
Glycyrrhizae radix	[50]
Hippophae rhamnoides	[16,39,47]
Hypericum perforatum	[31]
Jam	[18]
Lamium maculatum	[55]
Ligustrum lucidum	[37]
Morus alba	[46,56]
Olive (oil, leaves—*Eucomnia ulmoides*)	[20,51]
Orange juice	[52]
Paulownia tomentosa	[54]
Propolis	[57,60]
Puerariae radix	[48]
Rhododendron dauricum	[42,46]
Sambucus flos	[30]
Scutellariae radix	[36,58]
Sesame oil	[17]
Sophorae flos	[37,43]
Tea beverage	[26]
Tea (black, green)	[49]
Wine	[14,15,17,28,29,32,33]
Wine (grapes)	[24]

5. On-line methods utilized for the sample pretreatment

On-line coupling of ITP and CZE (ITP-CZE) has been used recently for the determination of antioxidants in various matrices; here the background electrolyte used in the CZE step may be generally different from the LE and TE used in the preceding ITP phase but the simplest way is to utilize the TE or LE from the ITP step as the BGE in the CZE phase. The ITP-CZE technique allows improving the limits of detection substantially and at the same time it involves a pre-separation step enabling the removal of unwanted matrix from the minor analytes of interest when analyzing complex samples such as plant materials or wines [30–32].

The ITP pre-separation and pre-concentration step was carried out in fluorinated ethylene-propylene copolymer capillary (9.0 cm × 0.8 mm i.d.) linked to a CZE column operated with UV detection. Picric acid and 1-nitroso-2-naphthol were used as coloured markers to ensure proper timing of the introduction of the stacked flavonoid ITP zones into the CZE capillary.

Another example of possible on-line pre-concentration of analytes leading to the improvement of limits of quantification is integration of a flow-injection (FI) system with a CE analyzer [33]. The FI system conducted automated solid-phase extraction (SPE) of analytes before the CE analysis of wine samples for flavonoids. The analytes were initially retained on a C-18

Table 3
List of analytes

Substance	References
Acacetin	[41]
Apigenin	[24,32,38,53,54,57]
Apigenin-7-*O*-glucoside	[53]
Apigenin sulfate	[27]
Baicalcin	[40]
Baicalein	[24,36,58]
Baicalin	[36,40,58]
Biochanin A	[44,48]
Caffeic acid	[24,30,32,33,41,47,51,57,61]
Calycosin	[50]
Catechin	[14,19,21,22,26,32,33,38,39,47,49,56,61]
Catechin-3-gallate	[22,26]
Catechin sulfate	[27]
Chlorogenic acid	[15,23,30,31,40,41,45,47,53,61]
Chrysin	[60]
Cinnamic acid	[30,51,60]
Daidzein	[37,48]
Daidzin	[48]
Didymin	[52]
Dihydrocaffeic acid	[51]
3,7-Dihydroxyquercetin	[55]
3,4-Dimethoxycinnamic acid	[60]
Diplacone	[54]
Dodecylgallate	[17,18,20]
Epicatechin	[14,19,21,26,32-34,38,39,49,61]
Epicatechingallate	[19,22,26,49]
Epicatechin sulfate	[27]
Epigallocatechin	[19,22,26,49]
Epigallocatechin-3-gallate	[19,22,26,49]
Farrerol	[42]
Ferulic acid	[15,30,32,47,51,52,57,61]
Flavone	[61]
6,2′,3′-Flavonoid sulfate	[27]
Galangin	[24,60]
Gallic acid	[22,32,33,47,51,56]
Gallocatechin	[19,22]
Gallocatechingallate	[22]
Genistein	[43,48]
Genistin	[43]
Gentisic acid	[15,33,51]
Hesperetin	[24,44]
Hesperidin	[21,52]
Hyperoside	[23,30,31,34,42]
Icariin	[21]
Ikarisoside A	[21]
Isoferulic acid	[30]
Isoorientin	[53]
Isoquercitrin	[30,31]
Isorhamnetin	[16]
Isoschaftoside	[53]
Kaempferol	[16,21,24,32,39,42,43,47,56]
Kaempferol-3-*O*-rhamnoside	[21]
Licochalconel A	[50]
Licoisoflavone A	[50]
Liquiritin	[50]
Luteolin	[24,38,51,57]
Luteolin-4′-*O*-glucoside	[53]
Luteolin-7-*O*-glucoside	[53]
Medicarpin	[25]
5-Methoxyflavone	[44]
Mimulone	[54]
Morin	[47]
Myricetin	[14,24,33,47,61]

Table 3 (*Continued*)

Substance	References
Naringenin	[24,44,59]
Naringin	[21]
Narirutin	[52]
o-Coumaric acid	[51]
Octylgallate	[17,18,20]
Orientin	[53]
Oroxylin A	[58]
Oroxylin A-7-*O*-glucuronide	[58]
p-Coumaric acid	[30,32,33,47,51,60]
Pinocembrin	[60]
Propylgallate	[17,18,20]
Protocatechuic acid	[32,41,51,61]
Puerarin	[48]
Quercetin	[14,16,21,24,30-32,34,36-39,41-43,47,51,56,57,59,61]
Quercetin sulfate	[27]
Quercitrin	[31,32,56,61]
Resveratrol	[14,28,29,33,47]
2″-*O*-Rhamnosylicariside	[21]
Rutin	[23,30-32,34,37-39,41,43,45-47,53,55,57,61]
Schaftoside	[53]
Scopoletin	[42]
Syringic acid	[32,51]
Taxifolin	[51]
Tiliroside	[21]
Umbeliferone	[42]
Vanillic acid	[15,32,51]
Vestitone	[25]
Vicenin-2	[53]
Vitexin	[30,32,53]
Vitexin-2-*O*″-rhamnoside	[30]
Wogonin	[21,58]
Wogonin-7-*O*-glucuronide	[58]

SPE minicolumn and thereafter they were eluted by methanol directly into the CE autosampler through a programmable arm. Compared to ITP-CZE the FI-CE method was approximately 10-times less sensitive (the LODs of most analytes were 0.03 mg/l in the case of ITP-CZE [32] whereas for FI-CE [33] the LODs ranged from 0.14 to 0.36 mg/l except of *trans*-resveratrol whose LOD was 0.05 mg/l). On the other hand the calibration range for FI-CE was much wider (0.05–100 mg/l) compared to ITP-CZE (0.125–5 μg/ml [32]). The repeatability of migration times ($n = 6$) was favourable (the R.S.D. values did not exceed 1.9% but they were below 1% for most analytes). The repeatability of peak areas ($n = 6$) was worse: for nine substances the R.S.D. ranged from 0.01 to 1.59% but for ferulic acid and quercetin the R.S.D. were 8.02 and 10.98%, respectively [32]. In the case of FI-CE only the repeatability data of peak areas ($n = 11$) were available (the R.S.D. ranged from 3.2 to 7.1%). The recovery experiments were accomplished only in the FI-CE paper [33] and the recoveries varied between 92 and 110% (at different concentration levels).

6. Detection techniques

Besides the widely used UV spectrophotometric detection in CZE of polyphenols (35 papers) electrochemical detection (ED) was a frequent alternative (11 papers) thanks to the fact that most of the polyphenols are easily oxidized. Although the UV detection is the most common detection technique in CZE, its main disadvantage is usually lower sensitivity compared to UV detection in HPLC. This can be overcome either by the extension of light pathway (wider bore capillaries or the so called "bubble cells" [23]) or by applying the technique of sample stacking (see Section 5). Generally ED afforded higher sensitivity than UV detection (LOD values ranged from 10^{-8} to 10^{-7} g/ml of an analyte for ED and from 10^{-5} to 10^{-7} g/ml for UV detection) and good selectivity [10]; consequently the ED is favorable in the CZE analysis of plant materials for polyphenols because compounds such as proteins, carbohydrates and lipids normally do not interfere. The "bubble cell" extended capillary [23] and ED [34] were employed in the analysis of phenolic compounds in buckwheat (*Fagopyrum esculentum*); the latter detection techniques was slightly more sensitive (the LOD ranged from 0.5 to 2.5 μg/ml of an analyte for UV detection and from 0.2 to 0.5 μg/ml for ED).

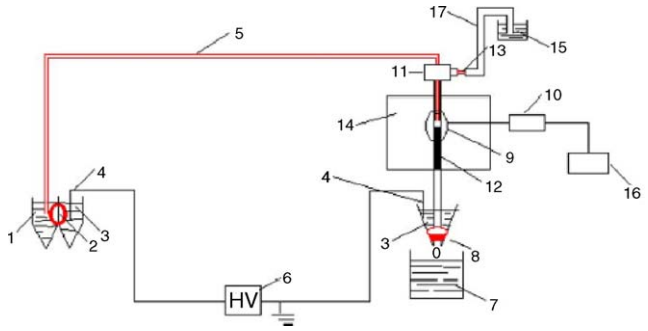

Fig. 2. Schematic diagram of CE chemiluminescence system: (1) running buffer cell; (2) frit, (3) electrode buffer cells; (4) Pt electrodes; (5) electrophoretic capillary; (6) high-voltage power supply; (7) waste reservoir; (8) silicone seal; (9) PMT; (10) amplifier; (11) T-connector; (12) reaction tube; (13) capillary restrictor; (14) dark box; (15) $K_3Fe(CN)_6$ bottle; (16) computer with A/D card; (17) reagent tube. Reprinted from [45] with permission from Elsevier.

Chinese authors used an ED (amperometric) system based on the end-column wall-jet configuration in which the working electrode is placed at the outlet of the separation capillary and detection is performed in the same solution reservoir that contains the grounding electrode for the CE instrument [35]. In all papers cited a three-electrode cell consisting of a carbon disc working electrode, a platinum auxiliary electrode and saturated calomel electrode as the reference electrode was utilized; working potentials were optimized by hydrodynamic voltammetry [34,36–43,46].

Surprisingly mass-spectrometric detection appeared only once [44] in the papers reviewed and its sensitivity was the same (0.5 mg/l for hesperetin and naringenin) or 10-times worse (2 mg/l for biochanin A) compared to UV detection.

Pre-separation step in the ITP-CZE [30–32] was monitored by auxiliary conductivity detection.

On-column chemiluminescence quenching was another option for detecting separated analytes (see Fig. 2) [45]. Luminol was added to the BGE and introduced at the head of separation capillary during electrophoresis. An alkaline potassium hexacyanoferrate solution merged with the BGE at the outlet of the

separation capillary. The limits of detection were by two orders lower (10^{-5} mol/l) compared to the UV detection (10^{-3} mol/l) when the analysis was performed under the same separation conditions and with the same capillary. On the other the presence of relatively wide reaction capillary (0.8 mm i.d.) connected to the end of the separation capillary influenced negatively the separation efficiency and resolution. Such indirect chemiluminescence detection was applied for the determination of rutin and chlorogenic acid in cigarettes. Since the sample matrix inhibited the chemiluminescence reaction too, correction by means of the standard addition method was necessary.

7. Evaluation of antioxidation activity of polyphenols by electromigration methods

CZE was utilized for evaluating antioxidation activity of polyphenols and plant extracts. Cao et al. [46] developed an indirect CZE method capable of determining hydroxyl radical generated by Fenton reaction. Salicylic acid is allowed to react with hydroxyl radical to originate 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA) that are separated and determined by CZE with ED. If rutin or plant extracts containing rutin are introduced into the reaction mixture, the generation of hydroxyl radical and accordingly the formation of 2,3-DHBA and 2,5-DHBA is suppressed. Rapid and simple determination of hydroxyl radical scavenging activity of plant extracts can be carried out in this way.

Recently paper dealing with the on-column monitoring of reaction kinetics for the determination of antioxidation potential of various polyphenols was published [47]. Both antioxidants and hydrogen peroxide were introduced into the CZE capillary by means of autosampler. After fixed incubation period the voltage was switched on and the reaction products were separated. Rutin, chlorogenic acid, *p*-coumaric acid, quercetin, caffeic acid and gallic acid were tested either as individual compounds or in various combinations. The rate constant of their oxidation was calculated and compared with that of ascorbic acid as the reference substance. This method was also applied to the determination of antioxidant potential of *Hippophae rhamnoides* extract. Unavailability of commercial pneumatic autosampler utilized in this study is certain disadvantage of this method.

8. Miscellaneous

Demianová et al. [14] devised a quick and repeatable NACE method using 5 mM malonate and 9.6 mM tetrabutyammoniumhydroxide in anhydrous methanol as the BGE and coated (poly(glycidylmethacrylate-co-*N*-vinylpyrrolidone)) capillaries for the determination of polyphenols in wines. Application of such capillaries shortened the time of analysis and improved repeatability in comparison with conventional bare capillaries. The electroosmotic flow (EOF) in coated capillaries was significantly reduced (4×10^{-9} m^2 V^{-1} s^{-1}). The lifetime of treated capillaries was 40–45 days.

In spite of the fact that lab-on-chip technology undergoes rapid development in recent decade, only one paper using the microchip-CE technique was dealing with the separation of phenol derivatives of natural origin. Scampicchio et al. [15] employed the microchip-CE method with amperometric detection (see Fig. 3) for the separation of hydroxyphenylcarboxylic acids, namely chlorogenic, ferulic, gentisic and vanilic acid and for their determination in real wine samples. The method was fairly reproducible (R.S.D. corresponding to the repeatability of migration times ranged from 1.4 to 3.0% and that of peak areas from 3.1 to 6.7%) with good linearity (linear range 50–300 μM for chlorogenic acid and gentisic acid with correlations coefficients 0.998 and 0.996, respectively) and high sensitivity (LOD 10 μM of chlorogenic and gentisic acid; S/N = 3); simple sample pre-treatment and insignificant sample and BGE consumption were the major advantages.

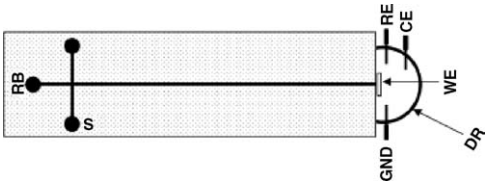

Fig. 3. Schematic diagram of the electrophoretic glass microchip system coupled with a screen-printed working electrode detector. S, sample reservoir; RB, run buffer reservoir; WE, working electrode; RE, reference electrode; CE, counter electrode; GND, ground electrode; DR, detector reservoir. Reprinted from [15] with permission from Elsevier.

9. Concluding remarks

In spite of the fact that the MEKC of polyphenols possesses higher separation efficiency than classical CZE, the CZE has been utilized more frequently than MEKC for the determination of flavonoids (the ratio of the number of CZE/MEKC papers dealing with flavonoids and published in the recent 5 years is 3). Apparently the conventional CZE with alkaline borate-based BGEs exhibits sufficient separation effect for the resolution of polyphenols in complex mixtures probably thanks to the complex-formation ability of borate. On the other hand, it seems that the danger of using alkaline BGEs for the analysis of such strong reductants as polyphenols (that can be easily oxidized by dissolved oxygen) has not yet been much considered by practicing analysts. In some cases, especially for compounds with similar structure but different lipophility, the use of micelles is advantageous since the electrophoretic behaviour of the analytes is affected by more factors (compared to CZE) that can be appropriately manipulated and optimized (surfactant concentration, addition of organic solvent).

In addition to the CE analysis of complex mixtures of polyphenols including chiral separations, the determination of the level of antioxidation activity of individual compounds or plant extracts is feasible. On-line coupling of electromigration methods with pre-separation methods (especially with ITP) facilitates integration of automated sample clean-up and analyte pre-concentration with the analysis proper. Microchip CE provides rapid analysis with small sample and solvent consumption.

When considering these facts it can be noted that CE became a powerful analytical technique suitable for the separation of

polyphenols that often compares well with HPLC in terms of precision, linearity and limit of detection.

Acknowledgements

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