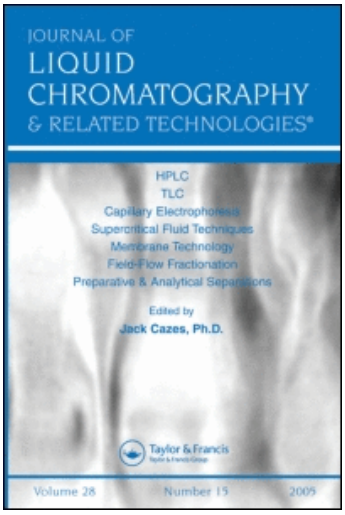

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FACTORS INFLUENCING THE SEPARATION OF IONIC AND NON-IONIC CHEMICAL NATURAL COMPOUNDS IN PLANT EXTRACTS BY CAPILLARY ELECTROPHORESIS

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Abstract

Capillary electrophoresis represents an alternative to the common liquid chromatographic technique in natural product research because of the high-resolution separations obtained by several migration modes. Natural molecules such as flavonoids, flavonoid-O-glycosides, coumarins, phenolic acids, glucosinolates or desulphoglucosinolates, have been resolved by several electrophoretic techniques (free solution capillary electrophoresis with a no-complexing or complexing electrophoretic buffer and by micellar electrokinetic capillary chromatography with a cationic or anionic surfactant).

In free solution capillary electrophoresis (CE), the selectivity of the flavonoid-O-glycosides, using a borate buffer, may be explained by in-situ borate complexation of both the sugar moiety and the cis-1,2-hydroxyl groups on the flavonoid skeleton, and also by the ionization of hydroxyl groups on the flavonoid skeleton in such alkaline pH conditions. In particular, the magnitude of the borate complexation depends on the number of boration sites on the sugar moiety and, consequently, on the sugar configuration.

Micellar electrokinetic capillary chromatography (MECC) based on sodium dodecylsulfate (SDS) and cetyltrimethylammonium chloride (CTACl) micelles was developped for the separation of uncharged natural compounds (coumarin derivatives or desulphoglucosinolates) or charged solutes (phenolic carboxylic acids or glucosinolates). The high efficient separations allowed the resolution of closely related structures.

Table I: Some separations of natural compounds by capillary electrophoresis.

Chemical compounds	Electrophoretic buffer	pH	Voltage (kV)	UV detection (nm)	Ref.
Flavonoids drugs	20 mM borate-50 mM SDS	8.3	+ 20	260	1
Flavonoid-7-O-glycosides	20 mM Tris/HCl-50 mM SDS	7.1	+ 25	280 nm	2
Flavonoid-3-O-glycosides	200 mM boric acid	10.5	+ 20	270 nm	3
Flavonoids	50 mM tetraborate-50 mM phosphate - 42 mM SDS	7.5	+ 20	210	4
Flavonoids of sugarcane	25 mM borate with 20 % CH_3OH	9.5	+ 30	395	5
Iso-α-acids	65 mM phosphate-40 mM SDS	7.6	+ 23	254	6
Hop bitter acids	25 mM Tris-HAc 25 mM SDS	9.0	+ 10	220	7
Glucosinolates and desulpho-glucosinolates	30 mM phosphate 18 mM borate-50 mM CTAB	7.0	- 19	270	8

Table I (continued)

Glucosinolates	30 mM phosphate 18 mM borate- 50 mM SDS	7.0	- 20	235	9
Glucosinolates and desulpho-glucosinolates	idem	idem	- 16	230	
Phenolic acids	30 mM phosphate 18 mM borate- 18 mM borate-	7.0	+ 20	280	10
Phenolic acids	6 mM borate- 10 mM phosphate - 100 mM SDS	8.5	255 195	+ 18	11
Quaternary alkaloids	100 mM AcONa -CH_3OH (85/15)	-	+ 25	254	12

Finally, the on-column UV spectrum of each solute resolved in capillary electrophoresis has been recorded in the 190-360 nm UV range by using a fast-scanning multiple-wavelength UV detector.

INTRODUCTION

Capillary electrophoresis has recently proved to be applicable to a wide range of analytical areas (inorganic and organic anions, inorganic cations, pharmaceutical and biological molecules).

Existing methods used for the analysis of natural compounds involve, generally, high-performance liquid chromatography, gas chromatography, thin-layer chromatography, and supercritical fluid chromatography. Natural molecules may be resolved by both free solution capillary electrophoresis or micellar electrokinetic chromatography. Capillary electrophoresis is attractive in natural product research because of the

high-resolution separations achievable with on-column UV detection. Several papers (1-12) have been published dealing with the separation of natural compounds by CE (Table I). On the other hand, SEITZ et al. (13) used capillary isotachophoresis for the rapid analysis of flavonoids and phenolcarboxylic acids in the phytopharmaceutical industry.

In this report, several mixtures of different natural compound families have been resolved with capillary electrophoresis using different migration techniques.

EXPERIMENTAL

Materials

All open-tube electrokinetic capillary chromatographic separations were performed with a Spectra-Physics (San Jose, CA, USA) Spectraphoresis 1000 instrument using a 70 cm x 375 µm O.D x 50 µm I.D silica capillary column. Electrokinetic separations were performed at 40°C at a voltage of 15-20 kV. The capillaries were conditioned daily by washing first with 1 M sodium hydroxide (10 min) at 60°C, then with 0.1M sodium hydroxide (10 min) at 40°C, with water at 60°C (10 min) and finally with the electrophoretic buffer (15 min) at 40°C. Between consecutive analyses, the capillary tube was flushed with water (2 min), 0.1M sodium hydroxide (2 min), water (3 min) and finally with the electrophoretic buffer (5 min) in order to improve the migration time and the peak shape reproducibility. For this instrument, analytes for each run were injected in the hydrodynamic mode for 1s.

Data were processed with an IBM PS/2 Model 70 386 computer. Software, operating under IBM OS/2, was supplied by Spectra Physics. This instrument contains a programmable, high-speed scanning, multiple-wavelength UV detector. Working in the fast scanning mode, we were able to record on-column spectra of these natural compounds. The scanning mode was from 195 nm to 360 nm with a 5-nm wavelength increment.

Reagents

All chemicals were of analytical-reagent grade. Commercially available boric acid (Fluka, Buchs, Switzerland), sodium dihydrogen phosphate (Fluka), 0.1 M sodium hydroxide (Fluka) and the surfactant sodium dodecylsulfate were used as received. Water used for dilutions and in buffer solutions was HPLC-grade (Carlo Erba, Milan, Italy).

For free solution capillary electrophoresis under alkaline conditions, the electrophoretic medium composition was 0.2 M boric acid buffer (adjusted at pH 10.5 with 0.1 M NaOH).

For MECC separations under neutral conditions, the electrophoretic medium consisted of sodium dodecyl sulfate (SDS) micelles in Tris-HCl buffer (tris(hydroxymethyl)aminomethane, Sigma, St Louis, MO, USA). Stock solutions to prepare pH buffer were 0.1 M Tris and 0.1 M HCl. The

buffer (pH = 7.1) was prepared by mixing these two stock solutions in the proportion 50:45.7 (v/v) diluted to 100 mL and adjusted to pH = 7.1 by addition of hydrochloric acid. The aqueous buffer composition was 0.05 M Tris-0.046 M HCl with the 0.05 M surfactant concentration. Methanol was used to determine the retention time of a neutral unretained solute (t_o) and anthracene the migration time of the micelles (t_{mc}).

Authentic samples of quercitrin (quercetin-3-O-rhamnoside), peltatoside (quercetin-3-O-arabinoglucoside), isoquercitrin (quercetin-3-O-glucoside), hyperoside (quercetin-3-O-galactoside) and avicularin (quercetin-3-O-arabinoside) were obtained from Extrasynthese (Genay, France). A standard solution of ca. 100 ppm of each flavonoid or flavonoid 7-O-glycoside was prepared in dimethyl sulfoxyde-methanol (80:20, v/v); their different chemical names and structures are detailed in Table II.

Several coumarin derivatives (coumarone-2, coumarin, acetyl-3 coumarin, hydroxy-7 methyl-7 coumarin, methoxy-7 coumarin, methyl-7 coumarin, dimethoxy-5,7 coumarin) were purchased from Aldrich (Milwaukee, WI, USA); their chemical structure were given in Table III.

The glucosinolate standards and their desulfoderivatives were isolated in our laboratory by semi-preparative liquid chromatography after extraction from rapeseed meal or prepared by chemical synthesis.

Finally, each analyte and each solution (water, sodium hydroxide and electrophoretic buffer) was filtered prior to injection through a polypropylene filter (0.2 μm pore size, 25 mm diameter) from Whatman (Maidstone, England).

RESULTS AND DISCUSSION

Free solution capillary electrophoresis with in-situ complexation

Flavonoids constitute a large group of naturally occurring phenols and are widespread components in all parts of plants. These compounds have structures based on the presence of a 2-phenylbenzopyrone group and differ in the pattern of hydroxylation, methylation, and glycosylation, in the degree of unsaturation and in the type and position of sugar links (1). The selectivity of an electrophoretic separation can usually be optimized by the pH and concentration of the electrophoretic buffer.

The separation of several flavonoid-3-O-glycosides in free solution capillary electrophoresis (CE) may be achieved using a complexing borate buffer, which gives a different selectivity compared to a no-complexing phosphate electrophoretic buffer (2-3).

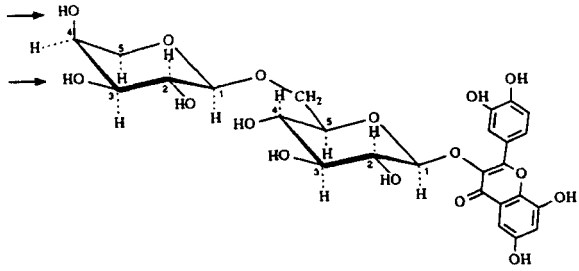
For the solutes listed in Table II, the same flavonoid aglycone (quercetin) is found in association with either a monosaccharide (β-D-glucose, β-D-galactose, α-L-rhamnose, α-L-arabinose) or either a disaccharide (α-L-arabino-β-D-glucoside). A five quercetin-3-O-glycoside mixture was resolved by free solution capillary electrophoresis using two

Table II: Structure of several quercetin-3-O-glycosides resolved by free solution capillary electrophoresis (3).

Solutes:1. quercetin-3-O-arabinoglucoside; 2. quercetin-3-O-glucoside; 3. quercetin-3-O-galactoside; 4. quercetin-3-O-rhamnoside; 5. quercetin-3-O-arabinoside.

(Arrows indicate hydroxyl group occurring as boration sites on the sugar molecule, but the same boration sites in the 3',4' positions on the flavonoid skeleton are not shown).

1.



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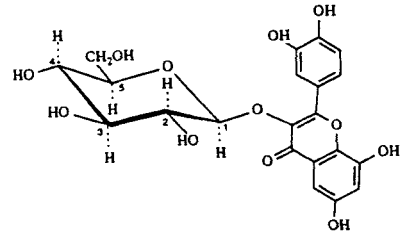

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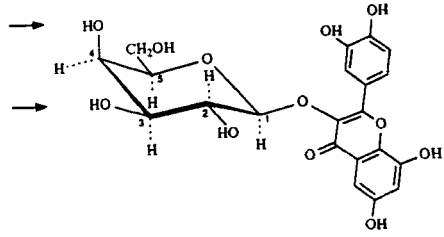

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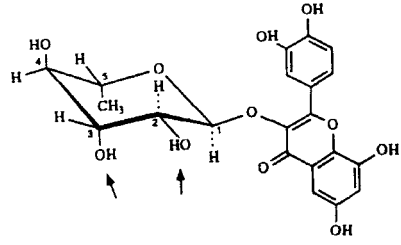
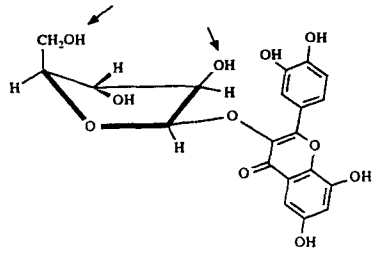
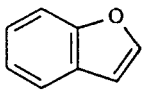
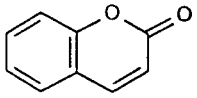
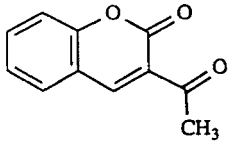
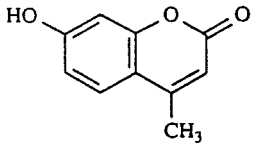
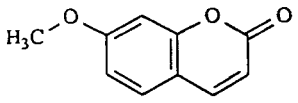
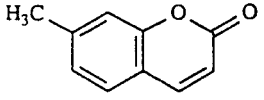
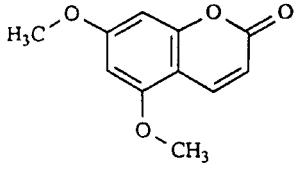


Table II (continued)

5. 

electrolytes having a pH value of 10.5 but differing in their chemical composition (phosphate-borate buffer or boric acid-NaOH buffers). Using a phosphate-borate buffer, the ionization of the flavonoid skeleton is not great enough to allow differentiation among the electrophoretic mobilities of the five solutes. Another analytical approach involves a complex formation equilibrium by adding to the electrophoretic buffer a complexing agent (borate anion) of the saccharide or of the flavonoid skeleton; the retention time of each solute is thus increased as it reacts with boric acid to form a borate anionic complex. Under alkaline conditions, the borate anions react with cis-1,2 hydroxy groups to produce negatively charged borate complexes, which migrate in an electric field. The complex formation reaction is a strongly pH-dependent equilibrium; the increase in the complex ratio becomes greater at higher pH values, inducing a greater negative electrophoretic mobility and, so, resulting in retardation of its migration velocity. Furthermore, the migration time of each flavonoid-3-O- glycoside increases with boric acid concentration (3), due to the decreasing electrosmotic flow with increasing buffer concentration and to the formation of a charged complex borate-flavonoid. In this borate complexation mode, flavonoid-3-O-glycosides migrate in the order of quercetin-O-disaccharide (α-L-arabinoside-β-D-glucoside) and then quercetin-O-monosaccharides (β-D-glucoside, β-D-galactoside, β-L- rhamnoside and α-L-arabinoside). The migration order of these quercetin-3-O-glycosides depends on the structural preference for the formation of the borate complex (Table II). As indicated in Figure 1, quercetin-3-O-rhamnoside migrates more slowly than quercetin-3-O-galactoside; this is probably due to the formation of a more stable complex at the 2- and 3- hydroxyl groups in quercetin-3-O-rhamnoside rather than at the 3- and 4-hydroxyl groups in quercetin-3-O-galactoside or a weaker charge density for the second one (3). Quercetin-3-O-galactoside migrates more slowly than quercetin-3-O-glucoside because of the favourable structure of the galactopyrannosyl unit for formation of a borate complex (presence of the 3,4-cis-diol system). The quercetin-3-O-disaccharide has a lower

Table III: Structures of some coumarin derivatives.

1	Coumarone	
2	Coumarin	
3	3-Acetylcoumarin	
4	7-Hydroxy-4-methylcoumarin	
5	7-Methoxycoumarin	
6	7-Methylcoumarin	
7	5,7-Dimethoxycoumarin	

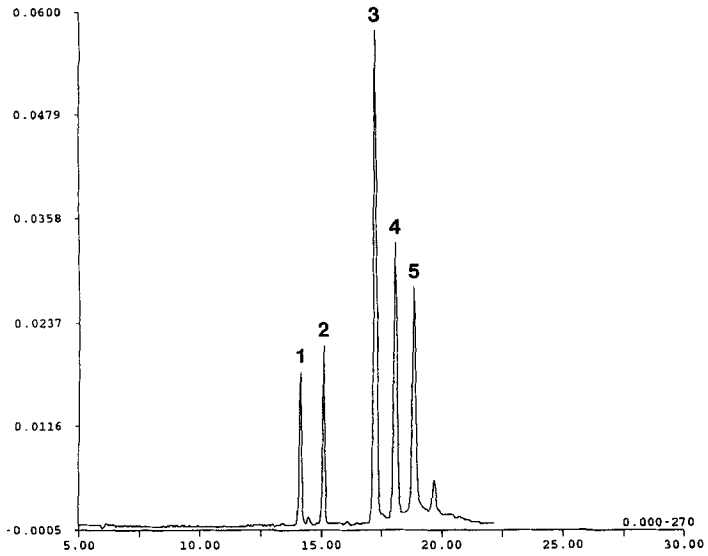


Figure 1: Separation of several flavonoid-3-O-glycosides by free solution capillary electrophoresis with an in-situ complexation (3).

Applied voltage: + 21.5 kV; capillary: 70 cm x 50 μm I.D; running buffer: 0.2 M H_3BO_3 (pH 10.5); detection wavelength: 270 nm; temperature: 40 °C; electrosmotic velocity: 5.62 cm/s. Solutes:1. quercetin-3-O-arabinoglucoside; 2. quercetin-3-O-glucoside; 3. quercetin-3-O-galactoside; 4. quercetin-3-O-rhamnoside; 5. quercetin-3-O-arabinoside.

electrophoretic mobility than the quercetin-3-O-monosaccharides because of its lower charge density. However, this disaccharide has cis-3,4 hydroxyl groups which can form diol-complexes with borate. Finally, the α-L-arabino-pyranoside unit has a pair of cis-hydroxyl complex at opposite ends of the α-L- arabino-furanoside unit, which has obviously no cis-1,2-diol system but can probably form a borate complex between the 2- and 5-hydroxyl groups. The secondary complexation equilibrium provides a further source of selectivity, but contributes to smaller peak efficiencies (for example,147 000 theoretical plates for quercetin-3-O-glucoside).

So, the formation of sugar-borate complexes is of particular value in the CE separation of flavonoid-3-O-glycosides having the same flavonoid aglycone but differing in their sugar moieties. The extent of borate complexation of flavonoid-3-O-glycoside is greater with an alkaline aqueous buffer and with a high boric acid concentration. The magnitude of the borate complexation depends on the number of boration sites on sugar moiety and of their charge density; flavonoid-3-O-glycosides migrated in the increasing order of quercetin-3-O-disaccharide (α-L-arabinoside-β-D-glucoside) and then of quercetin-3-O-monosaccharides(β-D-glucoside, β-D-galactoside, β-L-rhamnoside and α-L-arabinoside).

Micellar electrokinetic capillary chromatography

Micellar electrokinetic capillary chromatography (MECC), first reported in 1984 by TERABE et al. (14-15) provides interesting selectivity for both neutral as well as ionic compounds. In MECC, neutral solutes partition between the aqueous phase and micellar pseudo-stationary phase and migrate with a velocity depending on their hydrophobicity. Solutes of intermediate micelle solubility will elute within the range defined by t_o, the migration time of an unretained solute, and t_{mc} the migration time of a fully dissolved solute in the micelle (anthracene). Usually, anionic surfactants such as sodium dodecylsulfate (SDS) or cationic surfactants such as cetyltrimethylammonium chloride (CTACl) will be added to the electrolyte buffer at a concentration in solution greater than the critical micelle concentration (cmc). Micelles formed by SDS, as the concentration in solution exceeds the critical micelle concentration (cmc) move toward the anode, at an opposite direction of the electrosmotic flow. On the other hand, micelles formed by CTACl, as the concentration in solution exceeds the critical micelle concentration (cmc) move toward the cathode, at the opposite direction of the electrosmotic flow. The selectivity may be different between the micelle CTACl and the SDS micelle, because they differ in their size, association number and the nature of the polar head.

1) Mechanism based on hydrophobic interactions

a) Coumarins:

Coumarins represent an important class of compounds having biologically active properties. These chemical compounds occur in numerous plant families, particularly Gutiferae, Rutaceae and Umbelliferae (16). Current methods for the analysis of coumarins involve HPLC techniques (16-18). Seven coumarin derivatives (with hydroxy- or methoxy-substituents) were separated by MECC using phosphate-borate

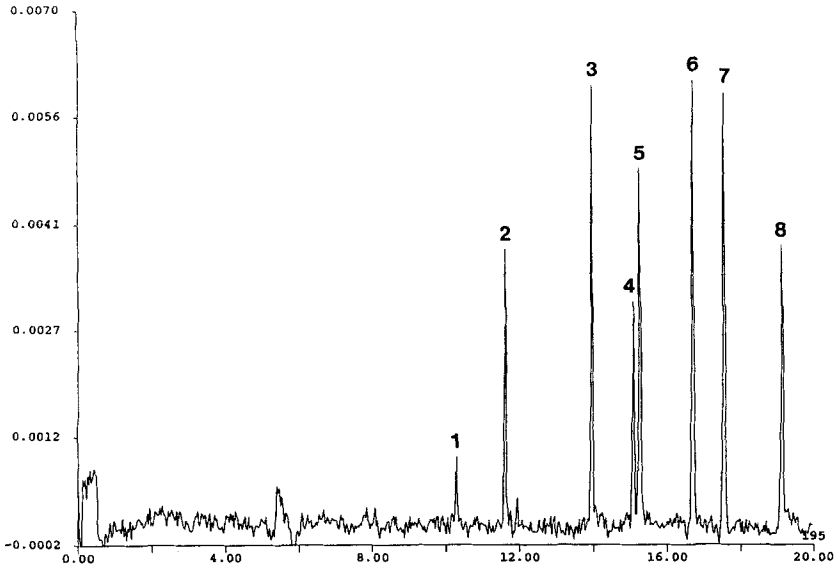


Figure 2: MECC-analysis of a mixture of coumarin derivatives. Applied voltage:18 kV; capillary: 70 cm x 50 μm i.d; phosphate-borate buffer/0.1 M SDS pH=7.0; detection wavelength195 nm. Solutes:1. coumarone-2; 2. coumarin; 3. acetyl-3 coumarin; 4. hydroxy-7 methyl-7 coumarin; 5. methoxy-7 coumarin; 6. methyl-7 coumarin; 7. dimethoxy-5,7 coumarin. 8. neutral marker (sudan III).

buffer (pH 7) and SDS as anionic surfactant; their chemical structures are shown in Table III. The retention mechanism with this neutral running buffer is mainly based on hydrophobic interactions between coumarins and the hydrophobic core of micelles; high efficiency separation of coumarins can be obtained as shown in Figure 2; the number of theoretical plates was fairly high (440 000 for acetyl-3 coumarin and 450 000 for dimethoxy-5,7 coumarin), more than one order of magnitude than the values usually encountered in HPLC. Scanning all wavelengths for the duration of one chromatogram and saving each data point provided by a post-run analyse, three-dimensional plot showing absorbance vs. wavelength vs. elution

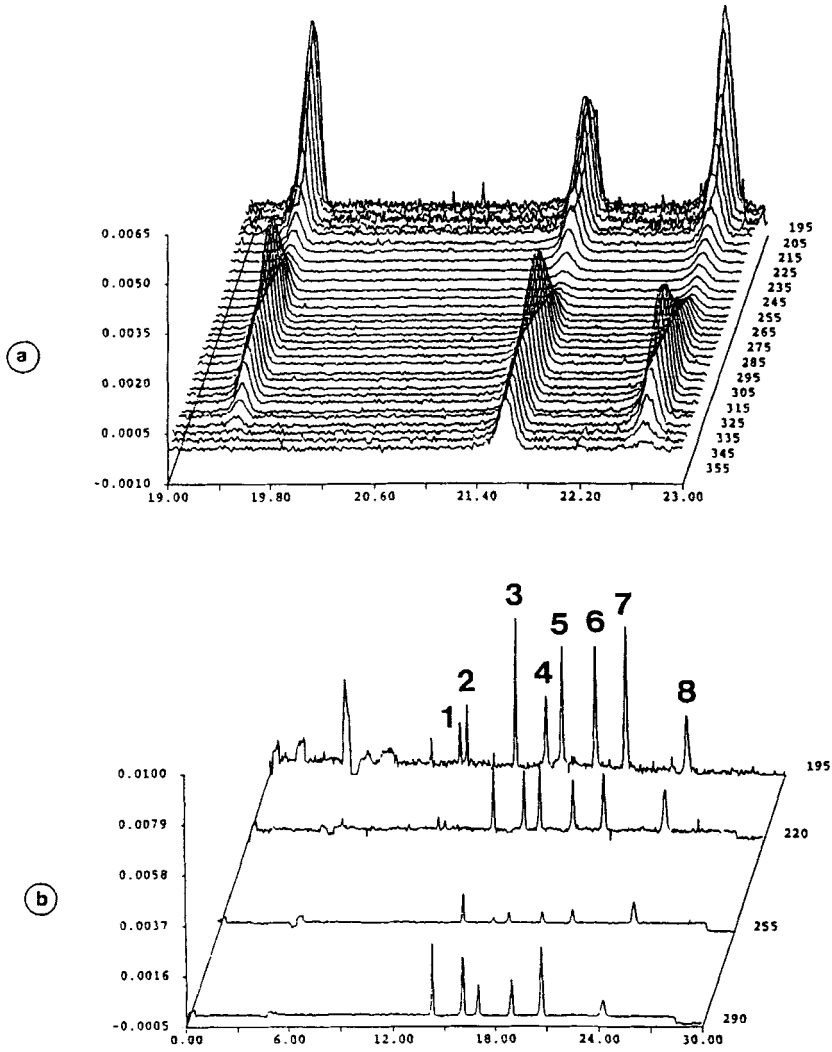


Figure 3: Separation of a mixture of coumarin derivatives by MECC. Conditions as in Figure 2 except applied voltage: + 14 kV.
a) Three-dimensional plot showing absorbance (AU) vs. wavelength (nm) vs. elution time (min).
b) Influence of the UV detector wavelength (195, 220, 255 and 290 nm) upon sensitivity.

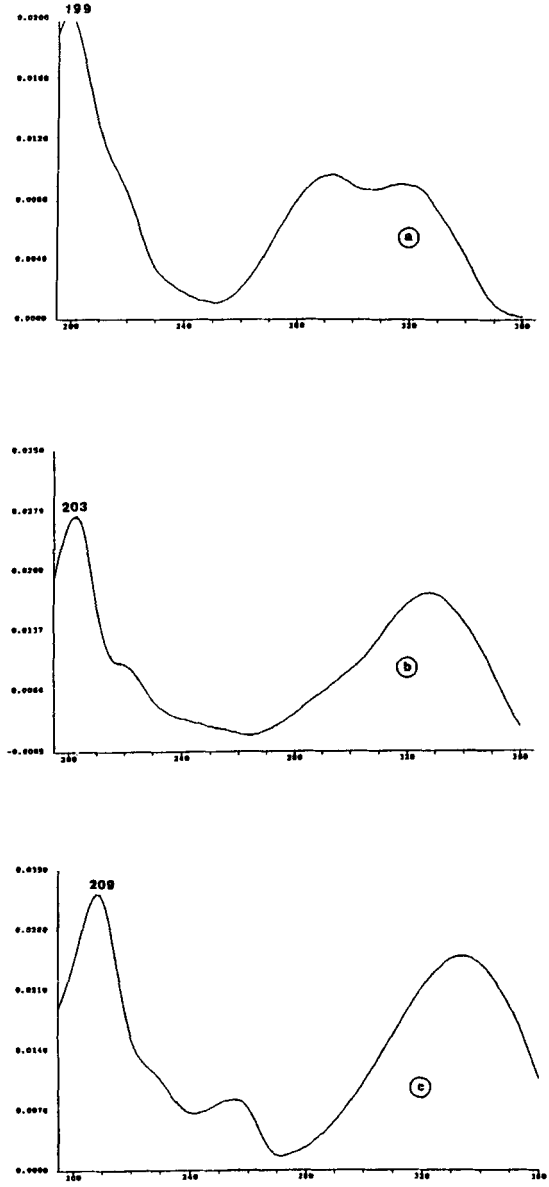


Figure 4: On-line spectra of selected coumarins in the 195-360 nm range.
a) dimethoxy-5,7 coumarin; b) methyl-7 coumarin;
c) 7-methoxycoumarin.

Table IV: a) Absorption wavelength maxima of coumarins.

* First value is the largest maxima with proceeding values in decreasing order of intensity.

Solutes	λ_{max} (nm)		
coumarone-2	195	274	
coumarin	195	282	315
acetyl-3 coumarin	212	305	
hydroxy-7 methyl-4 coumarin	203	327	220
methoxy-7 coumarin	203	328	221
methyl-7 coumarin	199	292	317
dimethoxy-5,7 coumarin	209	333	257

time (Figure 3a). The selection of the absorption maxima wavelengths for flavones increases the sensitivity and the specificity of the flavonoid detection (Figure 3b). So, the detection sensitivity increases at 195 nm compared to 255 nm or even 220 nm-wavelength. The UV spectra of dimethoxy-5,7, methyl-7 coumarin and methoxy-7 coumarin are shown in Figure 4; their main spectral features are reported in Table IV. The separation of a synthetic mixture of several coumarins has been achieved by MECC using a 0.1 M SDS surfactant with a phosphate-borate buffer (pH 7). The capacity factor of each solute has been determined using sudan III as micelle migration marker (Table IV); closely related structure compounds such as methoxy-7 and methyl-7 coumarins have different k'-values due to a slight hydrophobic structure difference. Neutral species are separated based upon their differential partitioning between the aqueous mobile phase and the hydrophobic interior of the SDS micelles. The more hydrophilic solutes with hydroxy or methoxy side chain elute faster than the more hydrophobic solutes with methyl side chain, such as

Table IV: b) Capacity factors of coumarins resolved by MECC. Electrolyte: phosphate-buffer buffer, 0.1 M SDS, pH 7. Capillary: 70 cm x 50 μm; applied voltage: + 18 kV. Methanol and soudan III were markers for electroosmotic and micelle migration velocities solutes.

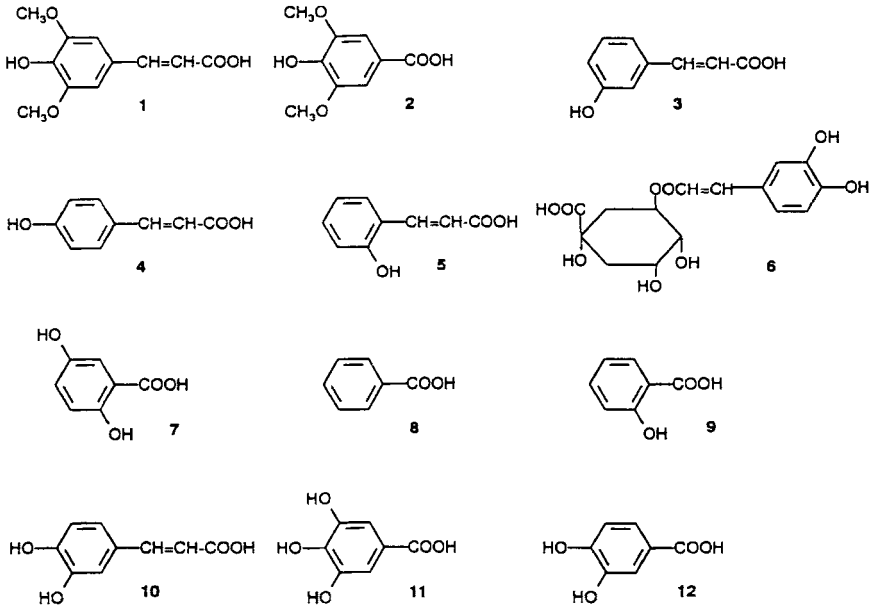
Solutes	t_m (min)	k'
coumarone-2	10.31	1.77
coumarin	11.6	2.65
acetyl-3 coumarin	14.0	5.46
hydroxy-7 methyl-4 coumarin	15.07	7.77
methoxy-7 coumarin	15.24	8.27
methyl-7 coumarin	16.71	15.28
dimethoxy-5,7 coumarin	17.64	26.76
soudan III	19.15	$+\infty$

methyl-7 coumarin. The selectivity obtained by MECC allows a slight hydrophobic structural difference to satisfactorily resolve these solutes.

b) Phenolic carboxylic acids

The migration behaviour of several phenolic carboxylic acids (Table V) has been studied with MECC using SDS as surfactant. Neutral phenolic acids migrate faster than anionic acids in the experimental conditions (pH = 8.5), indicating that the separation of the anionic solutes involves not only hydrophobic interactions but also electrophoretic mobilities (Figure 5). The ability of MECC to separate structural isomers was demonstrated by the separation of three coumaric acid isomers. Since each coumaric acid has a characteristic UV absorption profile, analysis at several wavelengths can provide additional identification of the components.

Table V: Structures of several phenolic carboxylic acids.
Solutes:1. sinapic acid; 2. syringic acid; 3. 3-coumaric acid; 4. 4-coumaric; 5. 2-coumaric; 6. chlorogenic acid; 7. gentisic acid; 8. benzoic acid; 9. salicylic acid;10. caffeic acid; 11. gallic acid; 12. protocatechic acid.



2) Mechanism involving ion-pair formations:

Glucosinolates (GSL) are a special class of thioglucosides, the structures of which vary mainly with the nature of the substituent R, which may be aliphatic or aromatic (Table VI). Glucosinolates occur in every species of the plant family Cruiferae, specially in the Brassicaca genus involved in animal and human feeding. Glucosinolates are easily broken down by endogenous myrosinase or in vivo by the bacterial flora of the intestine. Toxic hydrolytic products such as oxazolidine-2-thione, isothiocyanates, thiocyanates and nitriles may be released. The main *chromatographic* interest lies in the isolation and separation of these compounds and their degradation products.

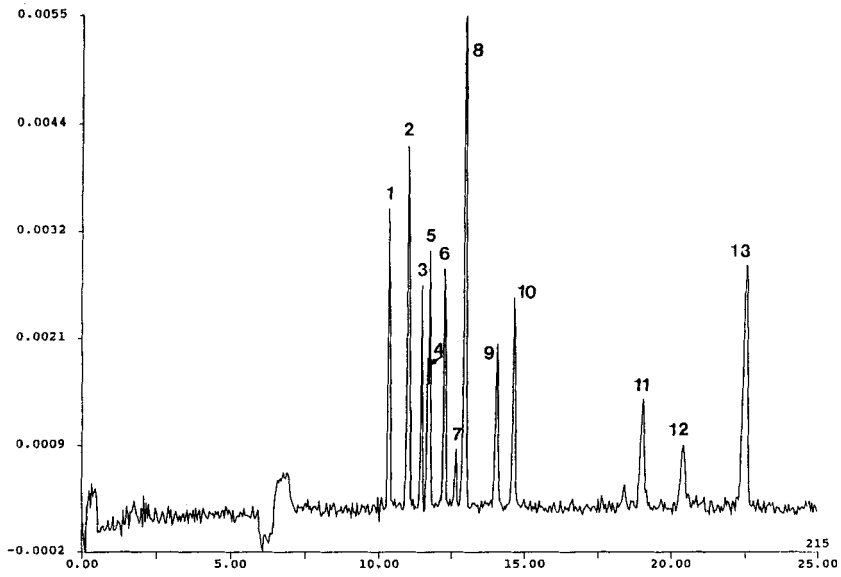
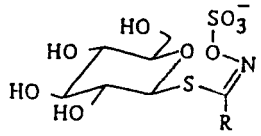


Figure 5: Separation of a natural mixture of phenolic carboxylic acids by micellar electrokinetic capillary chromatography. Applied voltage: +18 kV; capillary: 70 cm x 50 μm I.D; running buffer 6 mM borate -10 mM phosphate /100 mM SDS (pH=8.5); wavelength detection: 215 nm; temperature: 40 °C. Solutes:1. sinapic acid; 2. syringic acid; 3. 3-coumaric acid; 4. 4-coumaric; 5. 2-coumaric; 6. unidentified; 7. chlorogenic acid; 8. gentisic acid; 9. benzoic acid; 10. salicylic acid; 11. caffeic acid; 12. gallic acid; 13. protocatechic acid.

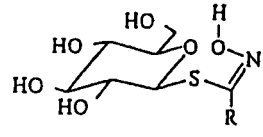
A micelle provides both ionic and hydrophobic sites of interactions , so a mixture of anionic and neutral solutes may be resolved by MECC. The migration behaviour of several glucosinolates and desulphoglucosinolates was studied in MECC using CTACl as cationic surfactant. When CTACl is used, the positively charged micelle is adsorbed on the negatively charged capillary wall, giving a positively charged wall; the electrosmotic flow is faster than the electrophoretic migration of the micelle and in these running conditions, all the solutes are detected at the positive end.

Table VI: Structure of glucosinolates (A) and desulphoglucosinolates (B).

R-group	Systematic name	Trivial name
CH_2=CH-CH_2-	allyl	sinigrin
CH_2=CH-CH(OH)-CH_2-	2-hydroxybut-3-enyl	progoitrin
C_6H_5-CH_2	benzyl	glucotropaeolin
indole-3-CH_2	indol-3-ylmethyl	glucobrassicin



A



B

A mixture of anionic and neutral desulphoglucosinolates is resolved using an electrophoretic buffer containing 0.018 M phosphate, 0.030 M borate (pH 10.5) and 0.050 M CTACl (Figure 6). All neutral desulphoglucosinolates (DSGSL) are separated based upon their differential partitioning between the aqueous mobile phase and the hydrophobic interior of the micelles (14). These neutral solutes elute in order of increasing hydrophobicity; the more hydrophilic solutes with hydroxy alkenyl side chain (desulphoprogoitrin) elute faster than the more hydrophobic solutes with methyl indolyl side chain (desulphoglucobrassicin). So, the selectivity obtained by MECC allows the satisfactory resolution of these compounds (15-16).

Neutral desulphoglucosinolates migrated faster than anionic glucosinolates. If the glucosinolate anions were moving only under the influence of electrophoretic migration and electrosmotic flow, they would elute before the neutral desulphoglucosinolates which interact with the

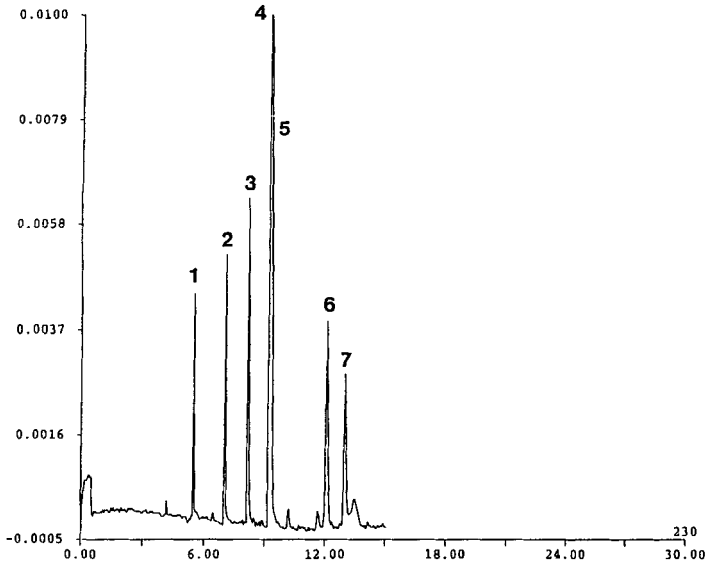


Figure 6: Separation of a mixture of neutral desulphoglucosinolates and anionic glucosinolates by micellar electrokinetic capillary chromatography.

Applied voltage: -20 kV; capillary: 72 cm x 50 μm I.D; running buffer: 0.080 M phosphate/ 0.05M CTACl (pH=7); UV detection wavelength: 230 nm.

Solutes:1. desulphoprogoitrin; 2. desulphoglucotropaeolin; 3. progoitrin; 4. desulphoglucobrassicin; 5. sinigrin; 6. glucotropaeolin; 7. glucobrassicin.

micellar phase. The migration order suggests the formation of an ion-pair between CTACl monomer and glucosinolate anion, and an easier penetration of this ion-pair into the interior of the micelle (8). Consequently, the migration of a desulphoglucosinolate depends not only on its electrophoretic mobility, on its distribution ratio between the micellar phase and the aqueous phase, but also on the secondary chemical equilibrium of ion-pairing formation between a desulphoglucosinolate molecule and a CTACl monomer.

CONCLUSION

Several capillary electrophoresis methods may be used to analyse plant extracts. Free solution capillary electrophoresis of borate complexes was appropriate for the separation of a mixture of flavonoid-O-glycosides having the same flavonoid aglycone but differing by their sugar moiety. The migration order of these flavonoid-O-glycosides in free solution capillary electrophoresis with a borate electrophoretic medium is explained by the in-situ borate complexation of both the sugar moiety and the cis-1,2-hydroxyl groups on flavonoid skeleton. The magnitude of the borate complexation and consequently the migration order depend on the boration site number on sugar moiety and consequently on the sugar configuration.

MECC was useful for the analysis of natural compounds differing only by slight structure difference using either the anionic surfactant SDS (coumarin derivatives) or the cationic surfactant CTACl (neutral desulphoglucosinolates). The separation of anionic glucosinolates and neutral desulphoglucosinolates has been simultaneously achieved by micellar electrokinetic capillary chromatography using a cationic surfactant (CTACl); hydrophobic interactions and also ion-pairing mechanisms between CTACl monomer and glucosinolate anion are involved.

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