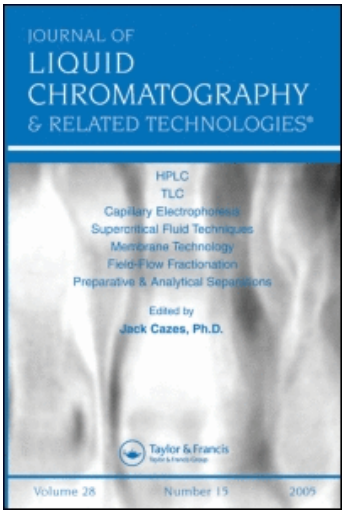

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Hai-Rui Liang[a]; Heikki Vuorela[a]; Pia Vuorela[a]; Raimo Hiltunen[a]; Marja-Liisa Riekkola[b]
[a] Pharmacognosy Division Department of Pharmacy, P.O. Box 56 FIN-00014 University of Helsinki, Finland [b] Laboratory of Analytical Chemistry Department of Chemistry, P.O. Box 55 FIN-00014 University of Helsinki, Finland

To cite this Article Liang, Hai-Rui , Vuorela, Heikki , Vuorela, Pia , Hiltunen, Raimo and Riekkola, Marja-Liisa(1998) 'The Statistical Evaluation of Migration Parameters of Flavonoids in Capillary Electrophoresis with Reference to Structural Descriptors', Journal of Liquid Chromatography & Related Technologies, 21: 5, 625 — 643
To link to this Article: DOI: 10.1080/10826079808005846
URL: http://dx.doi.org/10.1080/10826079808005846

J. LIQ. CHROM. & REL. TECHNOL., 21(5), 625–643 (1998)

THE STATISTICAL EVALUATION OF MIGRATION PARAMETERS OF FLAVONOIDS IN CAPILLARY ELECTROPHORESIS WITH REFERENCE TO STRUCTURAL DESCRIPTORS

Hai-Rui Liang,*[1] Heikki Vuorela,[1] Pia Vuorela,[1]
Raimo Hiltunen,[1] Marja-Liisa Riekkola[2]

[1] Pharmacognosy Division
Department of Pharmacy
P.O. Box 56
FIN-00014 University of Helsinki, Finland

[2] Laboratory of Analytical Chemistry
Department of Chemistry
P.O. Box 55
FIN-00014 University of Helsinki, Finland

ABSTRACT

Under each of 38 buffer conditions, six migration parameters of 14 flavonoids were obtained in capillary zone electrophoresis (CZE) and nine parameters in micellar electrokinetic capillary chromatography (MEKC). A total of 183 structural descriptors for each flavonoid were calculated to represent the molecular structures in a quantitative way. The relationship between the structural descriptors and the migration parameters of flavonoids in CZE or MEKC were evaluated by cluster and factor analyses.

Under all CZE conditions the apparent solute mobility (μ_a) was always the first variable to associate with all molecule-level structural descriptors and the electrotopological state indices (Si) of skeletal carbons, whereas the effective mobility (μe) was closer to Si of oxygens on skeletons. On the other hand, in MEKC retention factor or its logarithm did not depended more on the structural descriptors than other migration parameters. This indicated that the parameter did not represent the properties of the flavonoids better than other parameters. Thus, the results confirmed that hydrophobic interaction was not the only underlying force that influenced the migration behaviour of the flavonoids in MEKC.

INTRODUCTION

The Kovats retention index, logarithm of retention factor (k) and R_M, defined by Bate-Smith and Westall, have been used to describe the chromatographic behaviour of analytes in GC,[1] HPLC,[2] and TLC,[3] respectively. It is relatively well known that the retention is affected by the structural properties of solutes in chromatography.

CE has developed into a powerful complementary technique to HPLC in recent years.[4,5] In capillary zone electrophoresis (CZE), the electrophoretic mobilities (μ_e) of a solute can simply be related to charge to mass ratio of the solute and to the viscosity of the separation medium. Therefore, μ_e seems to express the migration behaviour of solutes better than other migration parameters. On the other hand, retention factor k_m or $\log k_m$ has been accepted to describe the migration behaviour of solutes in MEKC.[6]

However, in fact, various analytical migration parameters have widely been used to describe the migration behaviour of analytes in CE: migration times (t_m), apparent electrophoretic mobilities (μ_a), μ_e,[7,8] and k, logk, k_m, or $\log k_m$.[6,9,10] Besides these, corrected migration times (Δt_m),[11] relative migration parameters (such as relative migration time, t_m'),[12-16] and migration indices[17,18] have also been used with a view to improving the repeatability of analysis. It is important to know which of the parameters can best represent the properties of analytes. In order to find the ideal parameter, it is significant to establish the relationship of the migration parameters with the structural descriptors of analytes because the descriptors represent the structure of a particular compound in a quantitative way. These structural descriptors have largely been applied to the characterisation of chemical structures, as well as to

structure-property and structure-activity correlations.[19,20] The information is topological, geometric, electric and physical. The Wiener number, based on the distance matrix of the molecule, was the first descriptor to have been applied.[21] The molecular connectivity indices, later introduced by Randic[22] and developed by Kier and Hall[23], have commonly been used to encode these structural features such as size, branching, unsaturation, heteroatom content, and cyclicity. The electrotopological state indices (S_i) are atom-level indices, which encodes a combination of electronic, topological, and valence state information.[24-26] There are also other molecular descriptors such as kappa shape indices,[27] topological state indices, and information indices.

The relationship can be established by statistical methods such as cluster or factor analysis. Cluster analysis is a multivariate procedure for natural detecting groupings in data, based on the measurement of similarity of variables. Factor analysis is a multivariate procedures for decomposing a matrix. These analyses have been used to categorise 17 octadecylsilyl phases[28] and evaluate the relationship between retention and structural descriptors of retinoids.[29]

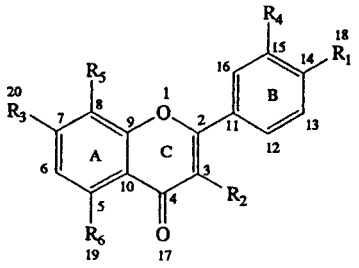
In recent studies, we have investigated the effect of electrolyte solutions and instrumental parameters on the migration behaviour of flavonoids in CE.[12,15] The aim of the present study was to evaluate the relationship between the structural descriptors and the migration parameters of 14 flavonoids in CZE and MEKC by statistical analyses.

EXPERIMENTAL

Chemicals, Apparatus and Procedures of CE Experimental

Chemicals and most of the investigated flavonoids are as described earlier.[12,15] In addition, flavone, avicularin, and vitexin were from Sigma (St. Louis, MO, USA). Structures of the flavonoids are shown in Figure 1.

The separation was performed on a Hewlett-Packard Chemstation 3D CE equipped with an HP diode-array detector (DAD) and an air cooling system for the capillary cassette (Hewlett-Packard, Avondale, PA, USA). The running voltage was kept at 20 kV, detection was at 254 nm, and all experiments were carried out at 25°C.



No	Name	R_1	R_2	R_3	R_4	R_5	R_6
1	flavone	H	H	H	H	H	H
2	epimedin B	OCH_3	O-rha-xyl	O-glc	H	prenyl	OH
3	epimedin C	OCH_3	O-rha-rha	O-glc	H	prenyl	OH
4	icariin	OCH_3	O-rha	O-glc	H	prenyl	OH
5	icariin II	OCH_3	O-rha	OH	H	prenyl	OH
6	luteolin-3′7-O-diglucoside	OH	H	O-glc	O-glc	H	OH
7	luteolin-7-O-glucoside	OH	H	O-glc	OH	H	OH
8	8-OCH_3-isoquercitrin	OH	O-glc	OH	OH	OCH_3	OH
9	vitexin	OH	H	OH	H	C-glc	OH
10	tricin	OH	H	OH	OCH_3	H(5′-OCH_3)	OH
11	quercitrin	OH	O-rha	OH	OH	H	OH
12	avicularin	OH	O-ara	OH	OH	H	OH
13	luteolin	OH	H	OH	OH	H	OH
14	icariin I	OCH_3	OH	O-glc	H	prenyl	OH

glc: glucose; rha: rhamnose; xyl: xylose, ara: arabinose.

Figure 1. Structures of the investigated flavonoids.

The buffer solutions containing 20, 25, 30, 40, or 50 mM borate and 0 or 48 mM SDS were prepared and adjusted to pH 10.5 each, at 25°C for CZE and MEKC. The pH values of the buffers containing 20 mM borate and 0 or 48 mM SDS were adjusted to 10.5, 10.0, 9.5, 9.0, and 8.0 at 25°C to obtain solutions of different pH for CZE and MEKC. Similarly, β-cyclodextrin (CD) was added to buffer solutions of 20 mM borate with 0 or 48 mM SDS to give solutions containing 2.5, 5, 7.5, 10, or 15 mM for CZE-CD and MEKC-CD. Again, the pH was adjusted to 10.5 at 25°C. Finally, 1,3-diaminopropane was added to solutions of 20 mM borate containing 5 mM β-CD to give solutions containing 1, 2, 3, 4, 5, or 6 mM of 1,3-diaminopropane (Org) for CZE-CD-Org. The pH was then adjusted to 10.5 at 25°C. A total of 38 buffer conditions were obtained in different CE systems.

The migration time of a neutral marker t_0 was experimentally measured by injecting methanol, which is assumed not to interact with the micelles. The migration time of micellar marker t_{mc} was measured using anthracene as the marker.[8] The other procedures of CE experimental are as described earlier.[12,15]

Calculation of Migration Parameters in CZE and MEKC

The migration times (t_m) in this study were obtained from a single source under similar experimental conditions. The relative standard errors of t_m from 4 replicates were less than 1%.

In CZE, the effective mobility of a solute is calculated from the experimental results as follows:

$$\mu_e = -[(1/t_0)-(1/t_m)]\,(L_{det}L_{tot})/V \quad (1)$$

where μ_e is the effective mobility, L_{det} is effective capillary length (to the detector) (cm), L_{tot} is total capillary length, and V is applied voltage. Similarly, the electroosmotic flow and apparent mobilities, μ_{EOF} and μ_a, are calculated using:

$$\mu_{EOF} = (L_{det}L_{tot})/(t_0V) \quad (2)$$

$$\mu_a = (L_{det}L_{tot})/(t_mV) \quad (3)$$

In addition, the corrected migration time (Δt_m) was calculated as t_m-t_0, the relative migration time (t'_m) as t_m/t_0, and the retention factor (k) as $(t_m-t_0)/t_0$.

In MEKC, neutral molecules partition between two moving phases. Terabe et al.[6] have derived the following equation for the retention factor of an electrically neutral solute:

$$k_m = (t_m-t_0)/\{t_0[1-(t_m/t_{mc})]\} \quad (4)$$

As t_{mc} becomes infinite (the micellar phase becomes stationary), the equation reduces to the analogous for conventional chromatography.

The calculation of μ_a, μ_e, Δt_m, t_m' and k values in MEKC is assumed to be the same as that in CZE.

Table 1

The Definition of Structural Descriptors

Molecule-Level Structural Descriptors

Molecular Connectivity Indices ${}^{m}\chi_t^{v}$ [23]

${}^{0\text{-}10}\chi$: connectivity simple path indices X0 to X10

${}^{0\text{-}10}\chi^{v}$: connectivity valence path indices XV0 to XVP10

${}^{3\text{-}10}\chi_{ch}$: connectivity simple chain indices XCH3 to XCH10

${}^{3\text{-}10}\chi^{v}_{ch}$: connectivity valence chain indices XVCH3 to XVCH10

${}^{3,4}\chi_{c/pc}$, ${}^{3,4}\chi^{v}_{c/pc}$: connectivity simple or valence cluster/path indices

Shape Indices[27]

${}^{1\text{-}3}\kappa$: Kappa simple indices K1 to K3; ${}^{0\text{-}3}\kappa_{\alpha}$: Kappa alpha indices K0 to K3

FW: molecular weights

NASC: the number of atoms of element carbon

ND1-3: number of vertices for which delta δ =1-3

W: Wiener number,[21] WT: total Wiener number, WP: Wiener's P number

PLATTF: Platt's F number

Information Indices

SHIND: the Shannon index; IDG, IDWG: Bonchev-Trinajstic information indices, NORMIDG, NORMIDWG: normalised Bonchev-Trinajstic information indices

Graph Path Counts

NTPATH: total number of paths; NXP2-NXP10: counts of path subgraphs; NXCH3-NXCH10: counts of chain subgraphs

Total Topological Indices

TESTS2: total electrotopological state index based on electrotopological state indices; TOTOP: the total topological indices; SUMI: sum of the intrinsic state value I; SUMDELI: sum of delta-I values

Atom-Level Structural Descriptors[24]

S_i: the electrotopological state index for atom i

$S_{C\ 2\text{-}16}$, $S_{O\ 1,\ 17\text{-}20}$: the electrotopological state indices for atom C 2-16, O 1, 17-20

Table 2

Representatives of Migration Parameters of 14 Flavonoids Separated in CZE

	Migration Parameters CZE[b]					
No[a]	t_m(Min)	$\mu_a(10^{-5}cm^2)$ Sec.*V	$-\mu_e(10^{-5}cm^2)$ Sec.*V	k	Δt_m(Min)	t_m'
1	4.78	50.99	0.00	0.00	0.00	1.00
2	5.51	44.27	6.72	0.15	0.73	1.15
3	5.72	42.59	8.40	0.20	0.94	1.20
4	6.46	37.75	13.24	0.35	1.68	1.35
5	6.97	35.00	16.00	0.46	2.19	1.46
6	6.92	35.20	15.79	0.45	2.14	1.45
7	6.97	35.00	16.00	0.46	2.19	1.46
8	9.19	26.52	24.47	0.92	4.41	1.92
9	9.74	25.02	25.97	1.04	4.96	2.04
10	8.07	30.22	20.77	0.69	3.29	1.69
11	9.94	24.52	26.47	1.08	5.16	2.08
12	10.21	23.89	27.11	1.13	5.43	2.14
13	11.61	21.00	29.99	1.43	6.83	2.43
14	6.57	37.08	13.91	0.38	1.79	1.37

[a] The explanation of No. 1-14 sees Figure 1.
[b] Buffer conditions: borate 20mM at pH 10.5 (S0B20).

Structural Descriptor Generation

The molecular structures of the flavonoids were sketched as hydrogen-suppressed diagrams and stored as connection tables. A total of 183 descriptors for each flavonoid were calculated by the Molconn-X 1.0 software (Lowell H. Hall, Hall Associates Consulting, Eastern Nazarene College, Quincy, MA, USA) (Table 1).

Statistical Analysis

Excel v.5.1 and Systat 6.0 for Windows (Systat, Chicago, IL, USA) were used as statistics programs. The satistical anlysis included cluster and factor

Table 3

Representatives of Migration Parameters of 14 Flavonoids Separated in CZE-CD and CZE-CD-Org

No[a]	Migration Parameters					
	t_m(Min)	$\mu_a(10^{-5}cm^2)$ Sec.*V	$-\mu_e(10^{-5}cm^2)$ Sec.*V	k	Δt_m(Min)	t_m'
	CZE[b]-CD (β-Cyclodextrin)[b]					
1	5.29	46.04	0.00	0.00	0.00	1.00
2	6.06	40.21	5.83	0.15	0.77	1.15
3	6.61	36.90	9.14	0.25	1.32	1.25
4	6.81	35.80	10.24	0.29	1.52	1.29
5	6.94	35.11	10.93	0.31	1.65	1.31
6	7.30	33.38	12.66	0.38	2.01	1.38
7	7.24	33.68	12.36	0.37	1.95	1.37
8	9.42	25.87	20.17	0.78	4.13	1.78
9	8.70	28.02	18.02	0.64	3.40	1.64
10	11.56	21.09	24.95	1.18	6.27	2.19
11	8.84	27.56	18.48	0.67	3.55	1.67
12	11.65	20.92	25.11	1.20	6.36	2.20
13	8.73	27.93	18.11	0.65	3.44	1.65
14	6.94	35.11	10.93	0.31	1.65	1.31
	CZE-CD-Org (1,3-Diaminopropane)[c]					
1	6.13	39.75	0.00	0.00	0.00	1.00
2	6.92	35.24	4.51	0.13	0.79	1.13
3	7.26	33.59	6.16	0.18	1.13	1.18
4	8.11	30.04	9.71	0.32	1.98	1.32
5	8.11	30.04	9.71	0.32	1.98	1.32
6	8.77	27.79	11.96	0.43	2.64	1.43
7	8.77	27.79	11.96	0.43	2.64	1.43
8	14.21	17.15	22.60	1.32	8.08	2.32
9	10.83	22.50	17.25	0.77	4.70	1.77
10	15.70	15.53	24.23	1.56	9.57	2.56
11	10.98	22.19	17.56	0.79	4.85	1.79
12	16.81	14.50	25.25	1.74	10.68	2.74
13	10.61	22.98	16.77	0.73	4.48	1.73
14	8.11	30.04	9.71	0.32	1.98	1.32

[a] The explanation of No. 1-14 sees Figure 1. [b] Buffer conditions: S0B20, CD 15mM. [c] Buffer conditions: S0B20, CD 5mM, Org 6mM.

Table 4

Representatives of Migration Parameters of 14 Flavonoids Separated in Different MEKC Systems

	Migration Parameters								
				MEKC[b]					
No[a]	t_m(Min)	μ_a[c]	$-\mu_e$[c]	k	logk	Δt(Min)	t'	k_m	$logk_m$
1	20.72	11.76	38.23	3.25	0.51	15.84	4.25	8.80	0.94
2	19.60	12.44	37.55	3.02	0.48	14.72	4.02	7.49	0.87
3	19.60	12.44	37.55	3.02	0.48	14.72	4.02	7.49	0.87
4	19.60	12.44	37.55	3.02	0.48	14.72	4.02	7.49	0.87
5	17.84	13.66	36.33	2.66	0.43	12.96	3.66	5.82	0.77
6	7.39	32.98	17.01	0.52	-0.28	2.51	1.52	0.67	-0.17
7	7.45	32.71	17.28	0.53	-0.28	2.57	1.53	0.68	-0.17
8	9.10	26.80	23.19	0.87	-0.06	4.22	1.87	1.20	0.08
9	9.99	24.40	25.59	1.05	0.02	5.11	2.05	1.51	0.18
10	11.69	20.85	29.14	1.40	0.15	6.81	2.40	2.17	0.34
11	10.16	23.98	26.01	1.08	0.03	5.28	2.04	1.57	0.20
12	10.80	22.57	27.42	1.22	0.09	5.92	2.22	1.81	0.26
13	11.90	20.49	29.50	1.44	0.16	7.02	2.44	2.26	0.35
14	17.84	13.66	36.33	2.66	0.42	13.00	3.66	5.82	0.77
				MEKC-CD[d]					
1	35.00	6.96	37.56	5.39	0.73	29.53	6.39		
2	30.36	8.03	36.49	4.55	0.66	24.89	5.55		
3	30.36	8.03	36.49	4.55	0.66	24.89	5.55		
4	31.11	7.84	36.68	4.68	0.67	25.63	5.68		
5	28.40	8.58	35.94	4.19	0.62	22.93	5.19		
6	8.43	28.90	15.62	0.54	-0.27	2.96	1.54		
7	8.43	28.90	15.62	0.54	-0.27	2.96	1.54		
8	12.51	19.48	25.04	1.29	0.11	7.04	2.29		
9	13.06	18.66	25.86	1.39	0.14	7.59	2.39		
10	12.80	19.04	25.48	1.34	0.13	7.33	2.34		
11	12.00	20.31	24.21	1.19	0.08	6.52	2.19		
12	13.98	17.44	27.08	1.55	0.19	8.50	2.55		
13	12.13	20.10	24.42	1.21	0.08	6.65	2.21		
14	28.40	8.58	35.94	4.19	0.62	22.93	5.19		

[a] The explanation of No. 1-14 sees Figure 1. [b] Buffer condition: SDS 48mM and borate 20mM at pH10.5. [c] Units are the same as in Table 2. [d] Buffer condition: SDS 48mM, borate 20mM and β-CD 15mM at pH 10.5.

analyses. Cluster analysis was performed using single, complete, or ward linkage with Pearson correlation or Euclidean distance. In the factor analysis, the common factor model was selected and the matrix types of correlation with Varimax rotation were used.

RESULTS

Under each of 38 buffer conditions, six migration parameters (t_m, μ_a, μ_e, Δt_m, t_m', k) in CZE or nine (t_m, μ_a, μ_e, Δt_m, t_m', k, logk, k_m, $logk_m$) in MEKC were obtained. The CZE systems included the buffers with the different pH and the different concentrations of borate (CZE), β-cyclodextrin (CZE-CD) and 1,3-diaminopropane (CZE-CD-Org). Similarly, the MEKC systems included the buffers with the different pH and the different concentrations of borate (MEKC) and β-cyclodextrin (MEKC-CD). The representatives of migration parameters from CZE, CZE-CD, CZE-CD-Org, MEKC, and MEKC-CD are listed in Tables 2-4.

Most of the structural descriptors are molecule-level and the electrotopological state indices (S_i) are atom-level. The representatives from different kinds of structural descriptors are compiled in Table 5.

The relationships between the structural descriptors and the migration parameters in CZE or in MEKC were evaluated by cluster analysis. We tested single, complete, and ward linkages with the Pearson and Euclidean distances. We selected Pearson correlations as the basis for similarity or dissimilarity among migration parameters and structural descriptors because the structure of the cluster was influenced by the scales of the variables when using Euclidean distances; but Pearson correlation standardise each variable and thus force the weights to be equal. Results were similar in single and complete linkages with the Pearson correlation.

Firstly, the relationships between the migration parameters in CZE and the structural descriptors were evaluated by cluster analysis. The data set of migration parameters in CZE and molecule-level descriptors was mainly clustered into: 1 molecule-level descriptors, 2 the migration parameters μ_a and 3 the other migration data (t_m, μ_e, Δt_m, t_m', k).

Most of the molecule-level descriptors are bulky topological indices, which were correlated with each other. Figure 2 shows that under all CZE conditions, μ_a is always the first migration parameter to associate with all these

Table 5

Representatives of Structural Descriptors of 14 Flavonoids

No[a]	FW	SHIND	ND2	$^{0}\chi$	$^{5}\chi$	$^{5}\chi_{p}$	$^{6}\chi_{ch}$	^{2}K	$^{1}K_{\alpha}$	NASC
1	222.243	1.160	11	11.665	4.478	1.260	0.241	12.055	9.996	15
2	808.785	1.724	17	41.455	14.514	4.675	0.320	46.502	43.449	38
3	822.812	1.743	16	42.325	14.768	4.818	0.310	47.478	44.423	39
4	676.669	1.644	14	35.024	12.131	3.828	0.265	39.213	36.314	33
5	514.528	1.519	11	27.016	9.227	2.868	0.219	29.970	27.270	27
6	610.524	1.633	13	31.162	10.844	3.196	0.270	34.338	31.668	27
7	448.382	1.505	10	23.154	7.979	2.271	0.225	25.104	22.637	21
8	494.408	1.544	9	25.602	9.007	2.495	0.204	28.019	25.462	22
9	446.410	1.468	10	23.154	8.284	2.719	0.230	25.104	22.676	22
10	330.293	1.280	8	17.430	5.907	1.595	0.167	18.781	16.467	17
11	448.382	1.505	8	23.317	8.145	2.315	0.214	25.104	22.637	21
12	434.355	1.491	9	22.447	7.921	2.112	0.169	24.135	21.673	20
13	286.240	1.322	7	15.146	5.166	1.359	0.179	15.879	13.625	15
14	530.527	1.532	12	27.723	9.529	2.923	0.219	30.947	28.204	27

No[a]	NORMIDG	TESTS2	W	S_{C3}	S_{C4}	S_{C6}	S_{C}^{7}	S_{C11}	S_{C12}	S_{C15}
1	6.870	14.242	500	1.539	-0.009	1.835	1.861	0.912	1.924	1.921
2	10.429	129.370	13024	-0.561	-0.958	1.054	-0.160	0.239	1.537	1.566
3	10.479	136.628	13628	-0.563	-0.959	1.055	-0.160	0.238	1.539	1.567
4	9.932	79.683	8181	-0.487	-0.887	1.083	-0.115	0.302	1.569	1.595
5	9.178	40.769	4030	-0.397	-0.785	1.054	-0.253	0.393	1.619	1.640
6	9.557	71.410	6968	1.046	-0.683	1.035	-0.174	1.163	1.217	1.176
7	8.709	33.991	2997	1.156	-0.596	0.957	-0.446	0.448	1.513	1.397
8	9.023	50.654	3364	-0.552	-0.907	0.920	-0.386	0.066	1.078	1.132
9	8.736	27.401	2794	1.158	-0.489	1.047	-0.242	0.065	1.238	1.259
10	7.893	11.163	1282	-0.700	-0.868	0.280	1.113	0.371	1.590	1.624
11	8.663	40.708	2677	-0.552	-0.907	0.920	-0.386	0.064	1.078	1.132
12	7.493	37.577	2474	-0.548	-0.904	0.918	-0.385	0.068	1.076	1.130
13	9.243	9.759	896	1.158	-0.489	1.047	-0.242	0.060	1.238	-0.353
14	7.399	43.141	4454	-0.700	-0.868	0.280	1.113	0.371	1.590	1.624

[a] The explanation of No. 1-14 sees Figure 1.
[b] The explanation of the structural descriptors sees Table 1.

descriptors. In the set of the migration data and the atom-level descriptors, Figure 3 shows μ_a was closer to the electrotopological state indices S_i of carbons 2-16 ($S_{C\,2\text{-}16}$) (Figure 1), indicating that the μ_a depended more strongly upon the $S_{C\,2\text{-}16}$ than any other migration parameters.

Then, the relationships between the structural descriptors and the migration parameters in MEKC were evaluated. In the cluster analysis, k, logk, k_m or $logk_m$ did not stand out from the other parameters (t_m, μ_e, Δt_m, t_m') in

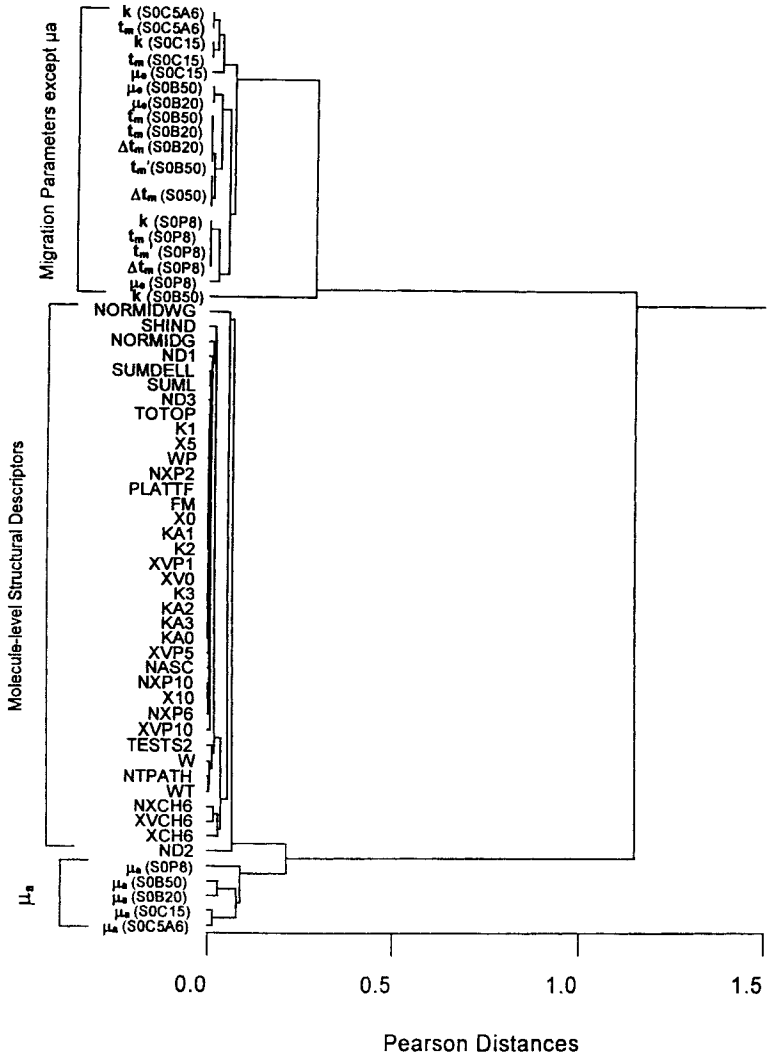


Figure 2. Cluster tree of molecule-level structural descriptors and migration parameters in CZE. The explanation of the molecule-level structural descriptors sees Table 1. Migration parameters obtained under different buffer conditions in CZE: S0B20, S0B50 — SDS 0, borate 20 or 50 mM at pH 10.5; S0P8 — borate 20 mM at pH 8; S0C15—borate 20 mM, β-CD 15 mM at pH 10.5; S0C5A6 — borate 20 mM, β-CD 5 mM, 1,3-diaminopropane 6 mM at pH 10.5.

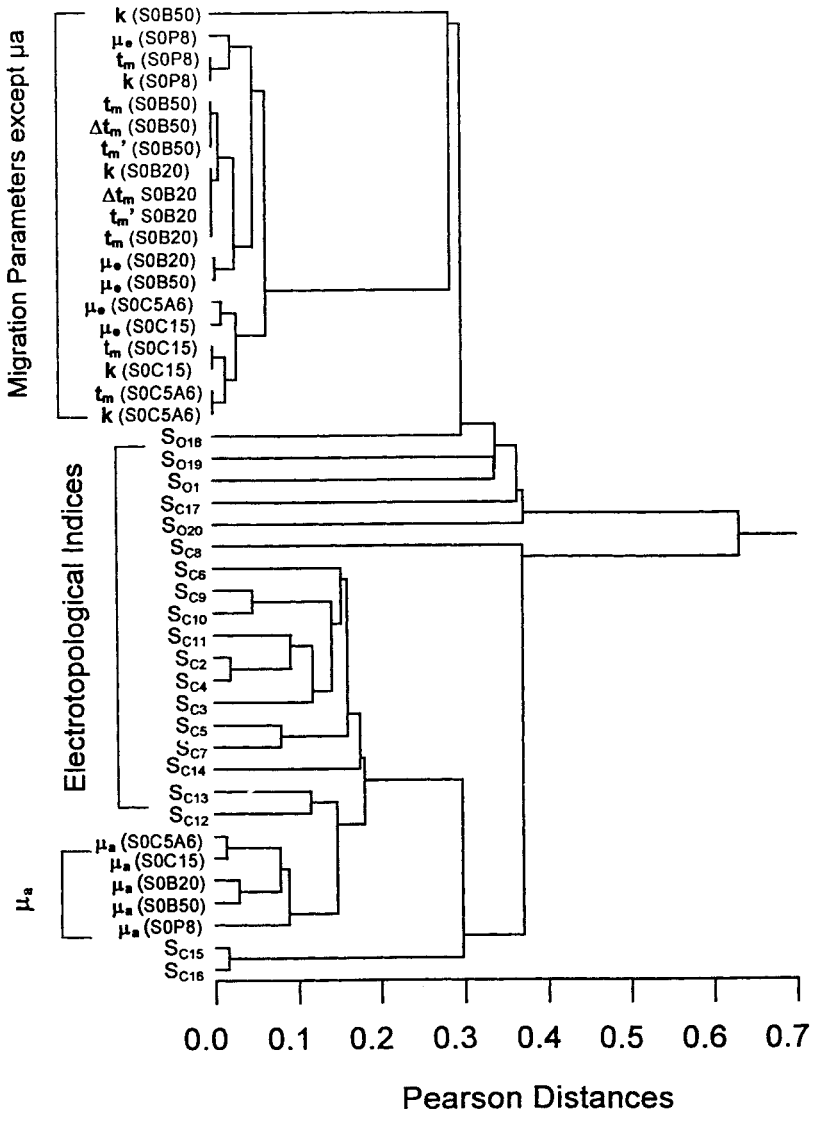


Figure 3. Cluster tree of electrotopological state indices $S_{C\ 1\text{-}16,\ O1,\ 17\text{-}20}$ and migration parameters in CZE. The explanation of the atom-level structural descriptors sees Table 1. The explanation of buffer conditions sees Figure 2.

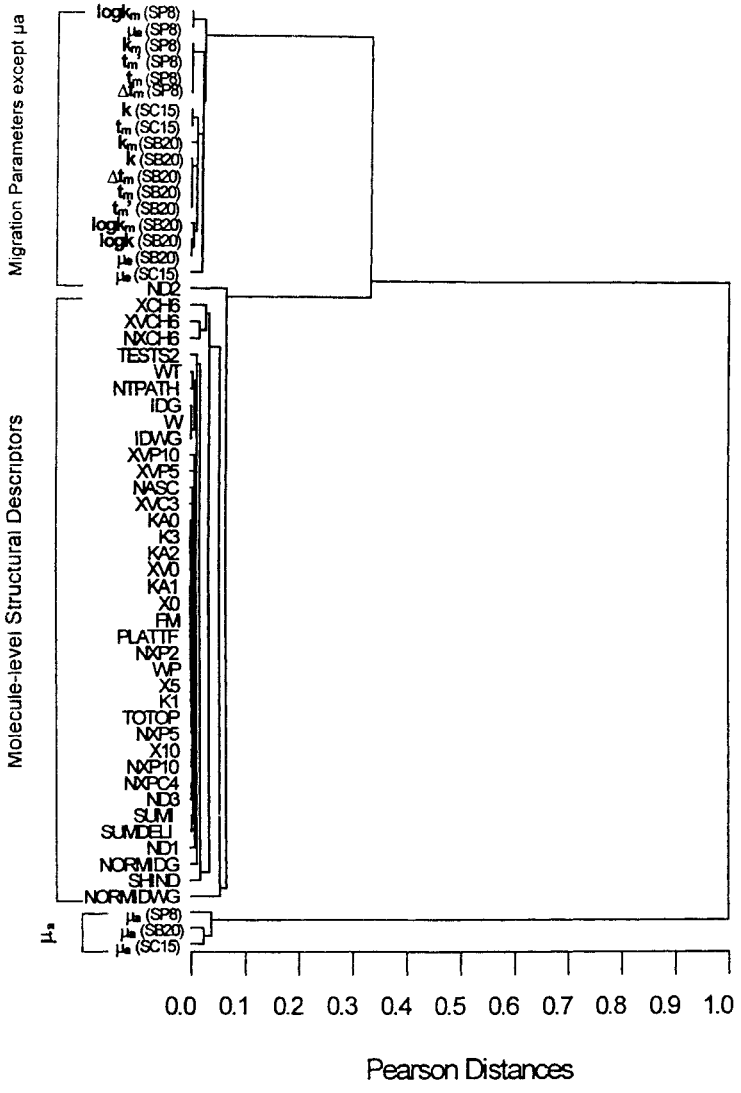


Figure 4. Cluster tree of molecule-level structural descriptors and migration parameters in MEKC. The explanation of the molecule-level structural descriptors sees Table 1. Migration parameters obtained under different buffer conditions in MEKC: SP8 — borate 20 mM, SDS 48 mM at pH 8.0; SC15 — borate 20 mM, SDS 48 mM, β-CD 15 mM at pH 10.5; SB20 — borate 20 mM, SDS 48 mM at pH 10.5.

relating to molecule- or atom-level descriptors (Figure 4). In order to confirm the results we undertook factor analysis. Factor analysis was performed on a correlation matrix including structural descriptors and migration parameters, with individual descriptors and migration parameters in CZE or MEKC expressed as a percentage of all variables.

In general, the results of the factor analysis were consistent with those of the cluster analysis. The first two factors obtained without or with Varimax rotation explained over 85% of the variance. The factor patterns were similar in the two analyses with and without rotation. In the data set of descriptors and migration parameters in CZE, the Factor 1 and 2 loading plot shows that μ_a and the structural descriptors, both with positive loadings, do not form distinct clusters, whereas the descriptors and other parameters (t_m, μ_e, Δt_m, t_m' or k) with negative loadings form two very distinct clusters. (Figure 5). This further indicated that μ_a was closer to the descriptors than t_m, μ_e, Δt_m, t_m' or k. On the other hand, in the data set of descriptors and migration parameters in MEKC, the factor analysis showed no obvious difference between retention factor k_m or its logarithm and t_m, μ_e, Δt_m, t_m', k or logk in relation to structural descriptors.

DISCUSSION

In CZE, although we had expected that μ_e were more related to the structure descriptors than other parameters, the results indicated that μ_a had closer distance to all molecular-level descriptors and all $S_{C\ 2\text{-}16}$ atom-level descriptors than any other parameters. The molecule-level descriptors mainly encode the size and complexity and bonding of the molecules. The electrotopological state indices S_i for an atom encodes information about both the topological environment of that atom and the electronic interactions it has with all other atoms in the molecule. The $S_{C\ 2\text{-}16}$ represented the properties of flavonoid skeletons.

Separation in CZE is achieved via the distinct migration velocities of analytes under the influence of an electric field. The migration velocity (v) of a given analyte is essentially the combined net effect of μ_e and μ_{EOF}, which is given by

$$v = (\mu_e + \mu_{EOF})\ (V/L_{tot}) = \mu_a\ (V/L_{tot}) \qquad (5)$$

In our study, total capillary length and applied voltage are constant. Thus, velocity of an analyte as a function of its structure depends upon its μ_a.

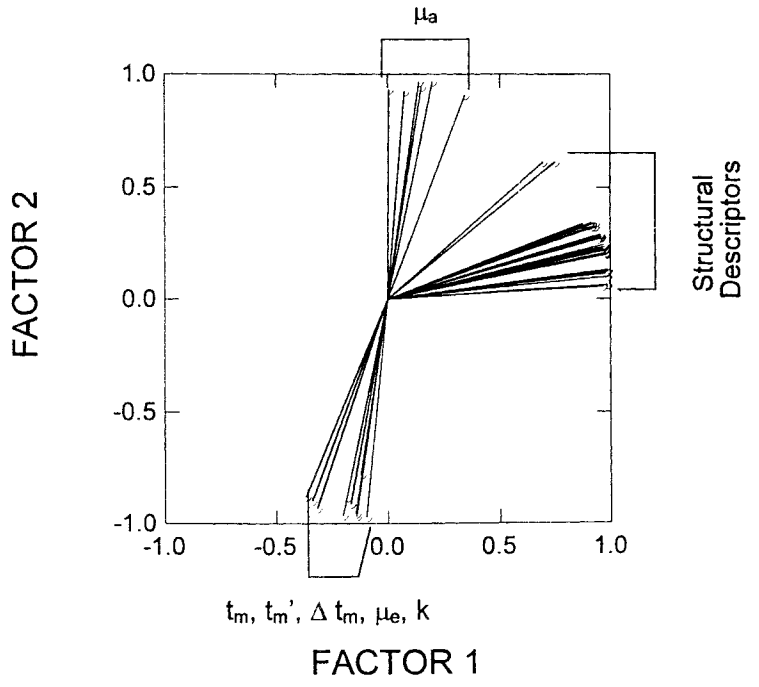


Figure 5. Factor loading plot of molecule-level structural descriptors and migration parameters in CZE. The structural descriptors and migration parameters obtained under different buffer conditions are the same as in Figure 2.

In MEKC, it is generally accepted that the migration is based on the hydrophobicity of solutes. Hydrophobicity is most often described by the retention factor or its logarithm.[6] Thus k, logk, k_m or $logk_m$ had been expected to represent the property and migration of the flavonoids better than any other parameters. However, in the statistical analyses, k, logk, k_m, or $logk_m$ did not stand out from the other parameters (t_m, μ_e, Δt_m, t_m') in relating to molecule- or atom-level structural indices. This is due probably to the fact that the equation derived k_m (see equation 4) in MEKC is limited to neutral solutes and the calculation is biased by the uncertainties in the true ionic electrophoretic mobility.[30,31] Flavonoids with hydroxyl substitution are weak acids and their apparent charge depends on their pK_a values. The pK_a values were thought to vary between 7.3 and 12.5 due to the presence of phenoxyls, but only several pK_a values out of over 4000 flavonoids from plant resources have been determined.[15] At pH > pK_a + 2, all analytes will be ionised, and the effective

electrophoretic mobility will be equal to the actual ionic mobility. Under our conditions, especially in higher pH buffers, most of the phenoxyl groups of the flavonoids were ionised. Furthermore, as seen in Tables 1 and 2, analytes **6** and **7** had almost no hydrophobic interaction with the micelle and hydrophobic interaction of the analytes **8-13** with the micelle was not predominant either. Thus, the results suggested that hydrophobic interaction was not the only underlying force that influences the migration behaviour of flavonoids in MEKC.[9,10] In addition to hydrophobicity, the migration behaviour of flavonoids also depended upon μ_e and μ_{EOF}.

CONCLUSION

In CZE systems including CZE-CD, CZE-CD-Org, the migration parameter μ_a depended the most strongly on the molecule-level structural descriptors among the six migration parameters, indicating that μ_a best represents the structural properties of the flavonoids in CZE. In MEKC, retention factor or its logarithm did not depend more strongly on the structural descriptors than other migration parameters, indicating it did not better represent the properties of the flavonoids. The migration behaviour of the flavonoids with hydroxyl substitution depended upon μ_e and μ_{EOF} in addition to hydrophobicity. None of the selected nine migration parameters could perfectly represent the migration of the charged and uncharged flavonoids in MEKC.

ACKNOWLEDGMENT

This work was supported by a grant (to H.-R. Liang) from the Finnish Cultural Foundation, which is greatly appreciated.

REFERENCES

1. T. F. Woloszyn, P. C. Jurs, Anal. Chem., **64**, 3059-3063 (1992).

2. R. Kaliszan, A. Kaliszan, T. A. G. Noctor, W. P. Purcell, I. W. Wainer, J. Chromatogr., **609**, 69-81 (1992).

3. F. J. García-March, G. M. Antón-Fos, F. Pérez-Giménez, M. T. Salabert-Salvador, R. A. Cercós-del-Pozo, J. V. de Julián-Ortiz, J. Chromatogr. A, **719**, 45-51 (1996).

4. C. A. Monnig, R. T. Kennedy, Anal. Chem., **66**, 280R-314R (1994).

5. R. L. St. Claire, Anal. Chem., **68**, 569R-586R (1996).

6. S. Terabe, K. Otsuka, T. Ando, Anal. Chem., **57**, 834-841 (1985).

7. S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann, H. M. Widmer, Anal. Chem., **63**, 1541-1547 (1991).

8. C. Sainthorant, Ph. Morin, M. Dreux, A. Baudry, N. Goetz, J. Chromatogr. A, **717**, 167-179 (1995).

9. S. Yang, J. G. Bumgarner, L. F. R. Kruk, M. G. Khaledi, J. Chromatogr. A, **721**, 323-335 (1996).

10. S. Yang, M. G. Khaledi, Anal. Chem., **67**, 499-510 (1995).

11. P. Lukkari, H. Vuorela, M.-L. Riekkola, J. Chromatogr., **652**, 451-457 (1993).

12. H.-R. Liang, H. Sirén, M.-L. Riekkola, P. Vuorela, H. Vuorela, R. Hiltunen, J. Chromatogr. A, **746**, 123-129 (1996).

13. P. G. Pietta, P. L. Mauri, L. Zini, C. Gardana, J. Chromatogr., **680**,175-179 (1994).

14. P. G. Pietta, P. L. Mauri, A. Bruno, C. Gardana, Electrophoresis, **15**, 1326-1331 (1994).

15. H.-R. Liang, H. Sirén, P. Jyske, M.-L. Riekkola, P. Vuorela, H. Vuorela, R. Hiltunen, J. Chromatogr. Sci., **35**, 117-125 (1997).

16. C. P. Palmer, B. G. M. Vandeginste, J. Chromatogr. A, **718**, 153-165 (1995).

17. T. T. Lee, E. S. Yeung, Anal. Chem., **63**, 2842-2848 (1991).

18. H. Sirén, J. H. Jumppanen, K. Manninen, M.-L. Riekkola, Electrophoresis, **15**, 779-784 (1994).

19. R. Kaliszan, **Quantitative Structure-Chromatographic Retention Relationships**, Wiley Interscience, New York, 1987.

20. L. B. Kier, L. H. Hall, **Molecular Connectivity in Chemistry and Drug Research**, Academic press Inc., New York, 1976.

21. H. Wiener, J. Am. Chem. Soc., **69**, 2636-2638 (1947).

22. M. Randic, J. Am. Chem. Soc., **97**, 6609-6615 (1975).

23. L. B. Kier, L. H. Hall, **Molecular Connectivity in Structure-Activity Analysis**, John Wiley & Sons, New York, 1986.

24. L. B. Kier, L. H. Hall, Pharm. Res., **7**, 801-807 (1990).

25. L. B. Kier, L. H. Hall, J. W. Frazer, J. Math. Chem., **7**, 229-241 (1991).

26. L. H. Hall, L. B. Kier, J. Chem. Inf. Comput. Sci., **35**, 1039-1045 (1995).

27. L. B. Kier, Acta Pharm. Jugosl., **36**, 171-188 (1986).

28. B. A. Olsen, G. R. Sullivan, J. Chromatogr. A, **692**, 147-159 (1995).

29. M. Salo, H. Sirén, P. Volin, S. Wiedmer, H. Vuorela, J. Chromatogr. A, **728**, 83-88 (1996).

30. S. Terabe, M. Shibata, Y. Miyashita, J. Chromatogr., **480**, 403-411 (1989).

31. C. Quang, J. K. Strasters, M. G. Khaledi, Anal. Chem., **66**, 1646-1653 (1994).

Received May 27, 1997
Accepted June 24, 1997
Manuscript 4492