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Aggregation and other intermolecular interactions of biological buffers observed by capillary electrophoresis and UV photometry

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Abstract

Electrophoretic and photometric experiments strongly indicate that monovalent anions, which arise by deprotonation of the nitrogen atom in zwitterionic Good's buffers 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO) and 3-morpholinopropanesulfonic acid (MOPS), spontaneously aggregate. Cationic migration of sanguinarine (SA) and chelerythrine (CHE) in highly alkaline 1,3-bis[tris(hydroxymethyl)methylamino]propane (Bis–Tris–propane), in which the concentration of cations of both alkaloids is negligible, may be explained by the existence of an aggregate, which contains uncharged sanguinarine or chelerythrine and one monovalent cation of Bis–Tris–propane at least. Tendency of tris(hydroxymethyl)aminomethane (Tris), bis (2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bis–Tris) and Bis–Tris–propane cations to ion pairing with synthetic cluster borane anions and with fused silica markedly rises up with the size and charge of these cations. The drop in mobility of cluster borane compounds sometimes exceeds 50% of their mobility found at identical pH and ionic strength in buffers with sodium cation. The electroosmosis drop approached 70% if background electrolyte contained Bis–Tris–propane cations instead of sodium cations. Nitrate, taken as a model inorganic ion, and four randomly chosen organic anions interacted markedly less with Tris, Bis–Tris and Bis–Tris–propane cations than cluster borane anions. 2-(*N*-morpholino)ethanesulfonic (MES) acid anions present in background electrolyte affect the ion pairing of Tris, Bis–Tris and Bis–Tris–propane cations with anionic analytes and, in this way influence also mobilites of these anionic analytes. Limited hydrophilicity at least one of interacting species appears to be the most probable cause of observed intermolecular interactions of biological buffers.
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Keywords: Aggregate formation; Buffer composition; Sanguinarine; Chelerythrine; Alkaloids; Borane cluster compounds

1. Introduction

Electrophoretic experiments always take place in solutions with controlled pH and ionic strength. Ideally, constituents of electrolytes used for these controls are indifferent to analyzed or investigated sample constituents as implicitly suppose basic theories of electrophoretic techniques, see, e.g. [1–3]. However, it is easy to understand that an analytically defined role of a background electrolyte constituent is no guaranty for the real absence of its interaction with any analyte.

The a priori identification or even estimation of a contingent non-bonding intermolecular interaction between a background electrolyte constituent and a particular analyte is hard task especially for large analytes of complicated structure. The estimation is impossible as a rule for analytes whose properties are known only partly. It is therefore expedient to utilize buffers and another background electrolyte constituents whose side interactions with analytes either are scarce or entirely absent. The principal reason is that the indifference of background electrolyte constituents to analytes helps in the understanding of experimental findings and simplifies their interpretation. This confirms also our experience from recent electrophoretic interaction studies of naturally occurring benzo[c]phenanthridine alkaloids sanguinarine (SA) and chelerythrine (CHE) with mercapto compounds [4,5] and from exploration on the possibility to reach chiral separations of anionic boron cluster compounds if capillary electrophoresis is used as an experimental

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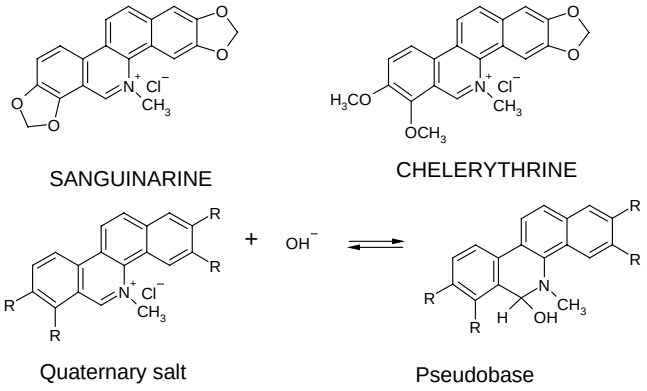


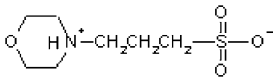
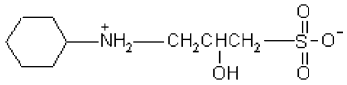
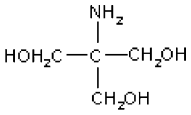
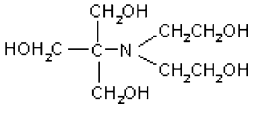
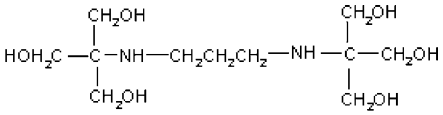
Fig. 1. Structural formulas of quaternary sanguinarine and chelerythrine completed with the scheme for equilibrium between their quaternary and pseudobase forms.

technique [6]. The indifference of background electrolyte constituents is mandatory in the measurement of physicochemical data, see, e.g. [7], and must be therefore carefully verified in such studies.

Alkaloids sanguinarine and chelerythrine (Fig. 1) are heterocyclic nitrogen bases [8] characteristic by acidobasic equilibrium between their quaternary and pseudobase forms in weakly alkaline solutions [9–12]. They have pronounced and very heterogeneous biological and biochemical effects, both beneficial and adverse, see, e.g. [8,13–16], whose list permanently enlarges within last years. Cluster boranes and their derivatives are purely synthetic compounds [17] of practical attractiveness and wide promises [18–20]. Borane cluster anions behave like strong hydrophobic acids having pK_a lower than 3 when they dissolve either in water or in aqueous polar organic solvents made of alcohols or acetonitrile. Number of accessible information on electrophoretic properties and behavior of sanguinarine and chelerythrine in almost neutral and in weakly alkaline solutions is very limited. Such an information on anionic cluster boranes and their derivatives are entirely lacking.

So-called biological buffers are effective in the pH range of 5.5–11.5 with respect to their pK_a values [21]. Zwitterionic Good's buffers and glycine-based buffers have anionic group at one end of their molecular chain. pK_a value of the protonated amine group, which is at the opposite end or close to it, determines the applicability range of buffers like 2-(*N*-morpholino)ethanesulfonic acid (MES), 3-morpholinopropanesulfonic acid (MOPS), *N*-Tris(hydroxymethyl) methyl-3-aminopropanesulfonic acid (TAPS) or 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO). Frequently used basic buffers represent tris(hydroxymethyl) aminomethane (Tris), bis (2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bis–Tris) and 1,3-bis[tris(hydroxymethyl)methylamino]propane (Bis–Tris–propane) (Table 1). No electrophoretically important side-interactions of biological buffers with analytes have been reported yet to our knowledge. We used therefore these buffers for the pH control in an electrophoretic interaction study with sanguinarine and chelerythrine [4,5] and in the test on the applicability of capillary electrophoresis for achiral and chiral separations of anionic borane cluster compounds [6]. However, some results from these and linking present studies could be neither understood nor explained unless various side-interactions of biological buffers with investigated compounds of both mentioned types were admitted. The aims of this contribution are the description of effects, which indicate intermolecular interactions of biological buffers, and the description of experiments, which support the existence of these interactions.

Table 1
Biological buffers investigated for aggregation and ion pairing effects

Acronym	Structural formula	pK_a*	Useful pH-range*
MOPS		7.2	6.5–7.9
CAPSO		9.6	8.9–10.3
Tris		8.1	7.0–9.1
Bis–Tris		6.5	5.8–7.2
Bis–Tris–propane		6.8; 9.0	6.3–9.5

* pK_a data and useful pH ranges taken from Ref. [21].

2. Experimental

2.1. Instrumentation

Beckman P/ACE System 5010 (Beckman Instruments, Fullerton, CA, USA) equipped with the filter UV detector, which allowed selection of several detection wavelengths, was used in experiments with alkaloids. The wavelength of 280 nm was chosen as the best accessible alternative for their detection. The constant power input of 0.4 W was applied with the instrument on the uncoated fused silica capillary (Polymicro Technologies, Phoenix, Arizona, USA) of 75 μm i.d. × 375 μm o.d., 50 cm separation length and 57 cm total length. Voltage adjusted on the separation capillary to this power input varied around +15 kV depending on electric conductivity of used buffers. The temperature of the cooling liquid was 25 °C. Laboratory set-up described in details in [6] was used for experiments with cluster boranes and with other anions. Both uncoated and polyacrylamide coated [22] fused silica capillaries of separation length from 38 to 50 cm and total length higher by 10.1 cm, thermostated by the cooling liquid to 25 °C, were used. +10 kV voltage was used in experiments with uncoated capillaries, −10 kV voltage was used in experiments with coated capillaries. These voltages guaranteed the Joule heat input below the 0.4 W limit in all experiments with anions.

The double-beam UV–vis spectrophotometer UNICAM UV 530 (Thermo Spectronic, Cambridge, UK) equipped with quartz cell of 1 cm path length was used for photometric experiments (measurement of spectra of boron cluster compounds and of the apparent UV-light absorption by solutions of buffers). Spectra, measured in the 200–480 nm region, are automatically corrected for the solvent background by the included software.

2.2. Chemicals

Biological buffers MOPS, CAPSO, Tris, Bis–Tris and Bis–Tris–propane given in Table 1 as well as MES and TAPS were from Sigma (Prague, Czech Republic). Sanguinarine and chelerythrine, the gift from the Institute of Medical Chemistry and Biochemistry, Palacky University, Olomouc, Czech Republic, were isolated from the extract of Macleya cordata using the published chromatographic method [23]. Cluster borane species were a gift from the Institute of Inorganic Chemistry, Czech Academy of Sciences, Prague, in which the compounds have been synthesized. For information on the synthesis methods, see [6]. Other chemicals, obtained from Aldrich (Sigma–Aldrich, Prague, Czech Republic) Fluka (Sigma–Aldrich) or Merck (Merck, Prague, Czech Republic), were of analytical grade purity.

2.3. Methods

Fresh uncoated fused silica capillary was activated by its treating with 1 M HNO3 for 30 min, with water for 5 min and with 0.1 M NaOH for 30 min. Activated capillary was washed briefly with background electrolyte using the flushing pressure of 20 psi. Electroosmosis stability was checked prior to experiments by injection of mesityloxide as the electroosmosis marker. Fluctuation of the electroosmotic coefficient around a value in repeated runs was taken as the evidence for the electroosmosis stability. Between analyses, capillary was flushed with background electrolyte for 1 min. Before any change in the buffer composition, capillary was treated with 0.1 M NaOH for 15 min and electroosmosis was stabilized as given above. Capillary was stored overnight in background electrolyte; distilled water was used for longer storing periods. Polyacrylamide coated capillaries, used in some experiments with anions, were cleaned between experiments only by the flushing with the background electrolyte and stored in distilled water.

Except of a few introductory experiments, sanguinarine and chelerythrine were dissolved in approximately 1 mM aqueous solution of hydrochloric acid to a stock solution. Cluster borane compounds have been dissolved in a few drops of acetonitrile and than were diluted to a known proper concentration with water–acetonitrile (4:1 (v/v)) mixture, which contained 9 mM sodium chloride. Sodium nitrate, anionic organic compounds and mesityloxide used for the electroosmosis stability monitoring have been dissolved directly in this water-acetonitrile mixture. Freshly boiled water was used for the preparation of stock solutions of buffers and of mobility standards. All stock solutions have been stored at 5 °C in the fridge. Injected 5×10^{-5} M samples of alkaloids have been prepared daily before use by the pipetting of their stock solutions into the 1:1 mixture of water and the buffer. Samples have been injected for 5 s using the sampling pressure 5 psi (1 psi = 6894.76 Pa). Samples with anions have been injected as described in [6]. Mobilites and electroosmotic coefficients are given as signed values in 10^{-9} m^2 V^{-1} s^{-1} units.

Buffers have been prepared from freshly boiled distilled water. Calculated amount of a weak electrolyte, that acted as the buffering constituent in the final buffer, was potentiometrically titrated to the desired pH with a strong electrolyte, which introduced ions of opposite polarity to ions of the buffering constituent into the buffer. Either approximately 1 M sodium hydroxide or approximately 1 M hydrochloride acids were the strong electrolytes. Stock buffers have been stored in the fridge.

Other experimental details are given in the description of experiments if necessary.

3. Results and discussion

3.1. Aggregation of deprotonated zwitterionic buffers

The equilibrium between charged, quaternary, and uncharged, pseudobase forms of alkaloids sanguinarine and chelerythrine in aqueous solutions (Fig. 1) may be formulated

as a reversible acidobasic interaction [9]:

$$Q^+ + H_2O \leftrightarrow QOH + H^+ \quad (1)$$

pK_{R+} values [9] reported for this equilibrium in primary communications [10–12] suggest their dependence on conditions of measurement of raw data. Therefore, we calculated the pK_{R+} values of sanguinarine and chelerythrine using the method published in [24] for the needs of studies [4,5] from pH-dependent effective mobilities of sanguinarine and chelerythrine. These dependencies have been measured in the pH range 6.0–10. Zwitterionic Good's buffers MES, MOPS, TAPS and CAPSO served for the pH control. The decrease of effective mobilities of both alkaloids to zero with increasing pH follows from equilibrium (1). This decrease was obtained with 5×10^{-5} M sanguinarine if pH was controlled with buffers of ionic strength $I = 30$ mM including CAPSO (Fig. 2A). Identical result was obtained if the ionic strength of these background electrolytes was increased to 120 mM with sodium chloride (Fig. 2B) or sodium acetate. However, anionic migration of sanguinarine was found in CAPSO pH = 9.5 and $I = 120$ mM (Fig. 2C). Apparent anionic mobility of sanguinarine increased if pH of the CAPSO of $I = 120$ mM rose up to 10.0 (Fig. 2D).

These findings evidence that the concentration of CAPSO and its pH are substantial for the anionic migration of sanguinarine. Background electrolyte containing CAPSO adjusted to the working pH with sodium hydroxide as single constituent is free of any other anions except of hydroxyls, which, however, convert sanguinarine cations to the uncharged sanguinarine pseudobase (Fig. 1, Eq. 1). Anionic migration of sanguinarine in more concentrated CAPSO of pH 9.5 or 10 (Figs. 2C and D), must be therefore the consequence of including of sanguinarine in a supramolecular formation whose negative charge is given by CAPSO anions. Zwitterionic CAPSO (Table 1) changes to the CAPSO anion by the deprotonation of the nitrogen atom. The anion has monovalent sulfate group at one side and hydrophobic group at the opposite side. Cyclohexyl is its main part. The concentration of anions increases with the increasing concentration of CAPSO and with its pH.

Indication that aggregation capability is not specific for CAPSO was got during the measurement of UV-spectra of anionic cluster boranes and their derivatives. These compounds have been dissolved in MOPS buffer, which was adjusted to pH 7 with sodium hydroxide and then mixed with acetonitrile as the solubilization agent for the compounds in 4:1 (v/v) ratio. The exploration for the cause

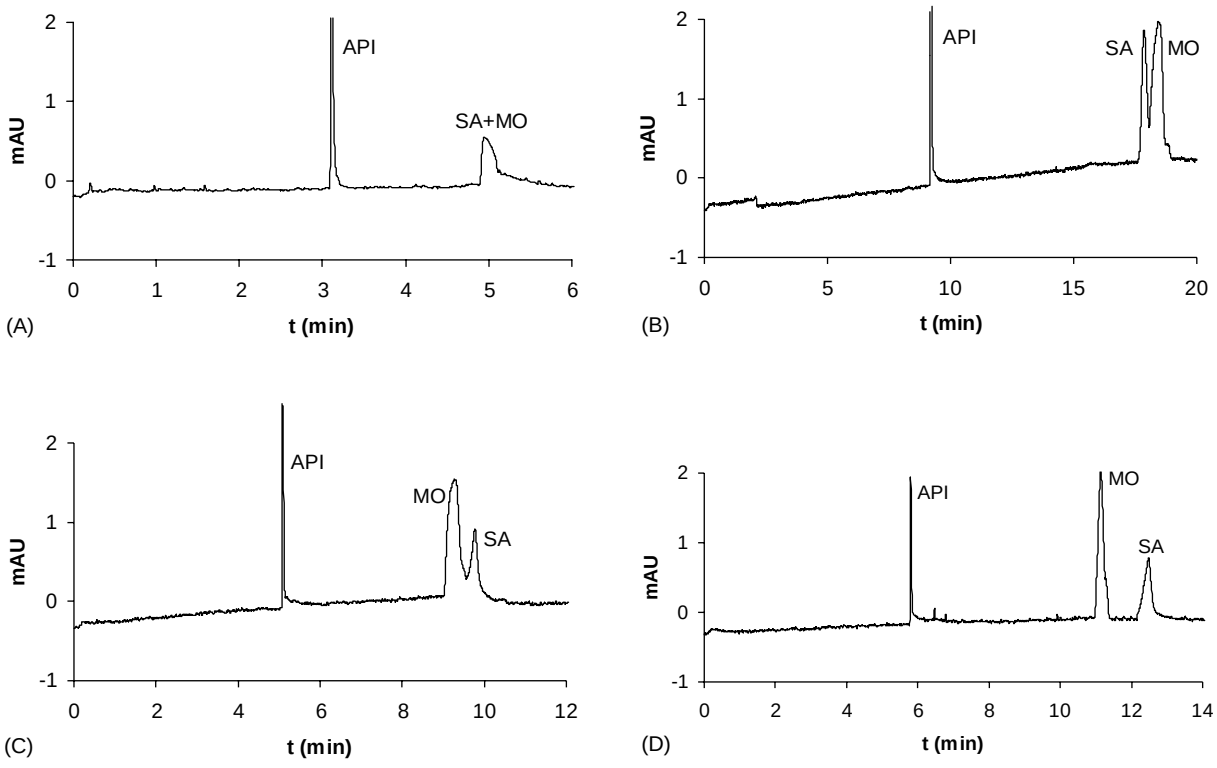


Fig. 2. The influence of the CAPSO pH and ionic strength, I, on apparent mobility of sanguinarine at constant Joule heat input 0.4 W into the uncoated separation capillary. Background electrolytes: (A) CAPSO of pH 9 and $I = 30$ mM; (B) CAPSO of pH 9.5 and $I = 30$ mM containing the addition of 90 mM NaCl, which increases the total ionic strength of the background electrolyte to 120 mM; (C) CAPSO of $I = 120$ mM, pH 9.5; (D) CAPSO of $I = 120$ mM, pH 10.0. pH of the background electrolyte was adjusted with NaOH. Voltage applied on the separation capillary migrated around +15 kV depending on electric conductivity of used buffers. Peak identity: API: 1-aminopyridinium iodide (cationic mobility standard); SA: sanguinarine injected for 5 s in concentration of 50 μM; MO: mesityloxide used as an uncharged electroosmosis marker. Instrument: Beckman P/ACE 5010. For other details, see Section 2.

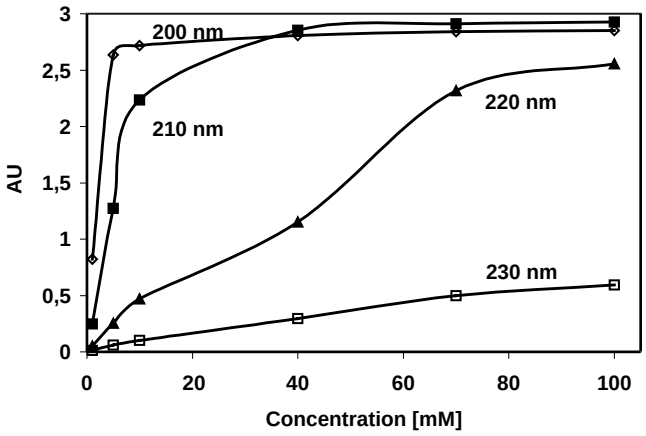


Fig. 3. The dependence of the weakening of the UV-light beam, expressed in absorbance units, on the concentration of MOPS buffers, ranging from 0.5 to 10 mM, adjusted to pH 7.0 with sodium hydroxide. Wellenlength of the light beam in nanometers is given as parameter. Instrument: UV–vis spectrophotometer UNICAM UV 530 equipped with quartz cell of 1 cm optical length.

of low reproducibility of measured spectra in the range 200–230 nm revealed pronounced decrease in the intensity of the UV-light beam passing through the aqueous MOPS solutions adjusted to pH 7 with sodium hydroxide. The weakening of the light beam, expressed in absorbance units, did not change linearly with the MOPS concentration (Fig. 3). This finding excludes the absorption of the light by the MOPS solution as the cause of its weakening. The addition of acetonitrile up to 20% (v/v) did not affect meaningfully the weakening of the UV-light beam (Fig. 3).

The aggregation capability of aqueous solutions of CAPSO and MOPS, free of another anions except of hydroxyls, was explored as the function of the difference (pH–pK_a). 50 mM solutions of CAPSO and MOPS were acidified with hydrochloric acid three units below their reported pK_a [21]. Another 50 mM samples of these compounds were adjusted with sodium hydroxide to pH = pK_a and to pH = pK_a + 3. The acidification to pH = pK_a − 3 transfers the buffers to their zwitterionic forms. Zwitterionic and anionic forms are present in 1:1 ratio at pH = pK_a and zwitterionic form is absent at pH = pK_a + 3.

Table 2
The decrease of intensity of the UV-light beam, formally expressed in absorbance units, in 50 mM MOPS and CAPSO adjusted either with hydrochloric acid or with sodium hydroxide to various (pH–pK_a) values

Zwitterionic compound	pH–pK_a	Absorbance units at wavelength (nm)			
		200	210	220	230
MOPS	−3	0.159	0.051	0.018	0.013
	0	3.172	3.147	1.764	0.319
	3	3.297	3.285	3.026	0.663
CAPSO	−3	0.053	0.023	0.012	0.11
	0	3.05	2.897	0.854	0.167
	3	3.262	3.249	2.740	0.513

Instrument: UV–vis spectrophotometer UNICAM UV 530. For other details, see text.

Table 3 documents practical absence of weakening of the UV-light beam, which passes through 1 cm layer of 50 mM zwitterionic CAPSO or MOPS. In contrary, strong weakening of the UV-light beam was found at pH = pK_a and at pH = pK_a + 3 in both CAPSO and MOPS.

Optical transparency of solutions of zwitterionic CAPSO and MOPS (Table 2) evidences that these buffers do not contain chromophores, which absorb the UV-light in the range of 200–230 nm. The deprotonation of the nitrogen atom is single change in CAPSO and MOPS if they change from zwitterions to anions. It is evident from their structural formulas (Table 1) that deprotonation is not linked with the formation of a chromophore. The creation of a supramolecular formation which disperses the passing light [25–27] is the most probable explanation for the experimentally found weakening of the UV-light beam at pH = pK_a and higher. The described experiment also indicates that the aggregation is a spontaneous process, which needs neither initiation centers nor an outside stimulus.

The probability of dissolution of more than one sanguinarine ion or molecule in one aggregate is very low with respect to submillimolar sanguinarine concentrations in electrophoretic experiments. Let us therefore suppose the simplest possible result of interaction between sanguinarine and the anionic CAPSO aggregate: single sanguinarine particle, Q, dissolved in one CAPSO aggregate $(CAPSO^-)_m$,

Table 3
Limiting mobilites of the charged forms of sanguinarine and chelerythrine, μ_Q and of their uncharged forms, μ_{QOH}, in various buffers obtained at the calculation of pK_{R+} values of these alkaloids from their pH-dependent effective mobilities

Background electrolyte	I_s (mM)	Sanguinarine		Chelerythrine	
		μ_Q	μ_{QOH}	μ_Q	μ_{QOH}
ZWB[a]–Na^+	30	17.4 ± 0.6	0.3 ± 0.7	15.3 ± 0.5	1.2 ± 1.3
ZWB[b]–Na^+ + NaCl	120	17.5 ± 0.6	−0.2 ± 1.2	16.2 ± 0.4	−1.9 ± 2.5
ZWB[a]–Na^+	120	13.6 ± 0.8	−2.7 ± 0.6	12.9 ± 0.5	−0.5 ± 1.7
BB[c]–Cl^-	30	20.7 ± 0.9	1.9 ± 0.7	18.8 ± 0.5	3.1 ± 2.1

Mobilites are given as signed values in 10^{-9} m^2 V^{-1} s^{-1} units.

[a] Zwitterionic Good's buffers MES, MOPS, TAPS and CAPSO adjusted with 1 M NaOH to pH 6.0, 6.5–8.0, 8.8 and 9.0–10.0, respectively.

[b] Buffer contribution to ionic strength of the background electrolyte, I_s = 120 mM, is 30 mM, NaCl contribution is 90 mM.

[c] Basic buffers Bis–Tris and Bis–Tris–propane adjusted with 1 M HCl to pH 6.0–6.5 and 7.0–9.5, respectively.

to a mixed aggregate $Q(CAPSO^-)_m$:

$$Q + (CAPSO^-)_m \leftrightarrow Q(CAPSO^-)_m \quad (2)$$

In this case, sanguinarine is in solution in three coexisting chemical forms: charged, Q^+, uncharged, QOH, and aggregated $Q(CAPSO^-)_m$. The forms, bound by fast and reversible equilibria (1) and (2), have electrophoretic mobilities μ_Q, μ_{QOH} and $\mu_{Q,m}$, respectively. Consequently, they cannot be separated from each other and migrate in a common mixed zone [28,29]. Mobility of the uncharged pseudobase form, μ_{QOH}, is zero. Effective mobility of the zone, μ_{eff}, which contains the forms Q^+, QOH and $Q(CAPSO^-)_m$ in equilibrium concentrations $[Q^+]$, [QOH] and $[Q(CAPSO^-)_m]$, respectively, therefore is [28,29]:

$$\mu_{eff} = \frac{1}{c_T}([Q^+]_{\mu_Q} + [Q(CAPSO^-)_m]\mu_{Q,m}) \quad (3)$$

Total analytical concentration of sanguinarine, c_T, is $c_T = [Q^+] + [QOH] + [Q(CAPSO^-)_m]$. For experimentally observable anionic migration, domination of the contribution of anionicly migrating fraction of sanguinarine over the contribution of its cationicly migrating fraction is necessary:

$$[Q(CAPSO^-)_m]|\mu_{Q,m}| > [Q^+]\mu_Q \quad (4)$$

Identical conclusion is reached if interaction of more than one sanguinarine ion or molecule with anionic aggregates is considered.

It follows from Eqs. (1) and (4) that higher pH supports the domination of the contribution of the aggregated alkaloid in μ_{eff}. Faster anionic migration at higher pH, which must be therefore expected from Eqs. (1) and (4) was found for sanguinarine in CAPSO of ionic strength of 120 mM (Figs. 2C and D). Chelerythrine pK_{R+} is higher approximately by one unit than that of sanguinarine [4,5,10–12]. Its experimentally observable anionic migration should be therefore expected at pH, which cannot be reached with CAPSO.

The structural formulas of CAPSO, MOPS and of other zwitterionic Good's buffers bearing sulfate group are alike. It implies that the tendency of their anions to the formation of aggregates may be common for Good's buffers of the type. Alike tendency may be expected for zwitterionic buffers based on glycine.

3.2. Interactions of biological buffers with sanguinarine and chelerythrine

Mobilities of the charged (acid) form of sanguinarine and chelerythrine, u_Q, may be measured experimentally. They are accessible also as "side-products" in the calculation of equilibrium constants from pH-dependent effective mobilities together with mobilities of the conjugated uncharged (basic) forms, u_{QOH}, [5,24] (Table 3). Different effective mobilites u_Q obtained for sanguinarine and chelerythrine in various buffers in the pK_a calculation (Table 2), which hold for pH 6.0 and lower, as well as different effective mobilities of these alkaloids measured in various background electrolytes at pH 6.0 (Table 4) may be ascribed to the ion pairing of cations of these alkaloids with MES anions present in different concentrations.

Table 4
Experimentally measured ionic mobilities of sanguinarine and chelerythrine, given as signed values in 10^{-9} m^2 V^{-1} s^{-1} units, in various background electrolytes

Background electrolyte	pH	I_s (mM)	μ_Q	
			Sanguinarine	Chelerythrine
MES–Na^+	6.0	30	17.76 ± 0.02	16.14 ± 0.02
MES–Na^+ + NaCl	6.0	120[a]	17.98 ± 0.10	16.07 ± 0.07
MES–Na^+	6.0	120	14.12 ± 0.03	12.93 ± 0.02
Bis–Tris–Cl^-	6.0	30	20.64 ± 0.01	19.10 ± 0.04
Acetate–Na^+	4.2	30	22.35 ± 0.06	20.05 ± 0.05
Phosphate–Na^+	2.7	30	23.57 ± 0.04	22.00 ± 0.06

[a] Buffer contribution to ionic strength, I_s, is 30 mM, NaCl contribution is 90 mM.

Limiting mobilities calculated for uncharged pseudobase forms of sanguinarine and chelerythrine, μ_{QOH}, must be zero. Values, which differ meaningfully from zero, evidence therefore measurable participation of a side-interaction of the uncharged form on its transport. The reason for negative μ_{QOH} of uncharged sanguinarine and chelerythrine in zwitterionic buffers is discussed in the previous chapter. The experimental pH dependence of sanguinarine and chelerythrine mobilities in Tris and Bis–Tris–propane buffers of constant ionic strength $I = 30$ mM, which were adjusted with hydrochloric acid, is in Fig. 4. The dependence, measured also at the constant power input 0.4 W, supports the credibility of positive μ_{QOH} values for sanguinarine and chelerythrine obtained in the pK_{R+} calculation (Table 2). Higher uncertainty of μ_{QOH} for chelerythrine indicates that used raw mobilities have not been measured up to sufficiently high pH.

Positive limiting mobilities for uncharged sanguinarine and chelerythrine, μ_{QOH}, in Bis–Tris–propane may be explained by the existence of a supramolecular aggregate or

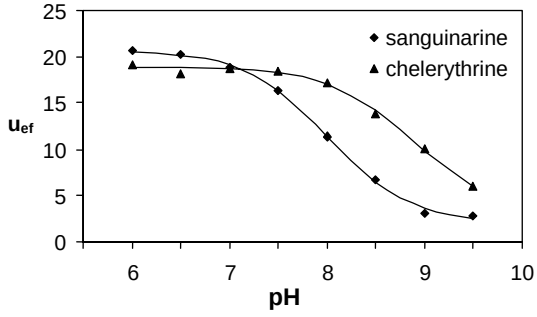


Fig. 4. The pH-dependence of effective mobilities of sanguinarine and chelerythrine, μ_{ef}. Working pH of Bis–Tris (pH 6.0) and Bis–Tris–propane buffers (pH 6.5–9.5) having constant ionic strength $I = 30$ mM was adjusted with hydrochloric acid. Mobilities have been measured at constant Joule heat input 0.4 W into the separation capillary. Applied voltage ranged around +15 kV depending on electric conductivity of used buffers. Fifty micromoler samples of SA and CHE have been injected for 5 s. Instrument: Beckman P/ACE 5010. For other details, see Section 2.

a complex, which contains at least one Bis–Tris–propane cation. Bis–Tris–propane solution of $I = 30$ mM, which was adjusted to pH 7 with hydrochloric acid, was transparent for the UV-light 200–230 nm. It suggests that the hypothetical aggregate is small. Polar organic solvents including aliphatic alcohols are better solvents for sanguinarine and chelerythrine than water. This indicates that either their hydroxyls or their hydrocarbonaceous parts interact in some way with uncharged pseudobase of sanguinarine or chelerythrine. Bis–Tris–propane contains six hydroxyls occurring in alcohols as well as one fragment of propane molecule. It is possible to imagine that Bis–Tris–propane interacts with uncharged sanguinarine or chelerythrine in a comparable, or, perhaps, even in the identical way, as alcohols. In this case, the considered interaction might be classified as an interaction closely related to solvation or, maybe, as a kind of solvation.

3.3. Interactions of basic biological buffers with anions

Unexpected deviations in mobilities of ions are frequently explained by their ion pairing with ions of opposite charge present in background electrolyte. Strong tendency to ion pairing features anionic cluster borane compounds [18]. Mobilites of cluster borane anions in buffers with sodium cation, which is considered free of the ion pairing capability, were therefore compared with mobilites of the compounds in buffers containing much bulkier cations of basic biological buffers. Uncoated fused silica capillary was chosen for these experiments. Mobilities of boron cluster anions in MOPS or in phosphate buffer adjusted with sodium hydroxide to pH 7.0 were identical within the limit of experimental error. The same was found when pH of the buffers was adjusted with Tris. However, mobilities measured in buffers adjusted with Tris were lower than in buffers adjusted with sodium hydroxide [6] (Table 5). The influence of Bis–Tris and Bis–Tris–propane on mobilities of cluster boron anions was therefore explored only with MOPS.

Table 5
The electroosmotic coefficient, μ_{eo}, given as signed value in $10^{-9}\,m^2\,V^{-1}\,s^{-1}$ units, in MOPS of different analytical concentrations as the principal buffering constituent, which was adjusted to the working pH and ionic strength, I_s, with NaOH, Tris, Bis–Tris and Bis–Tris–propane

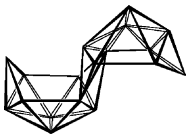
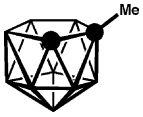
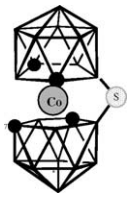
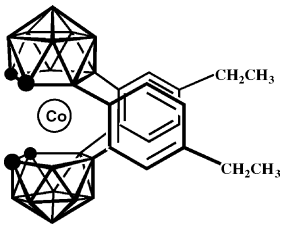
Base	Buffer pH = 7.0, I_s = 11.6 mM		Buffer pH 7.2, I_s = 30 mM	
	μ_{eo}	$\Delta\mu_{eo}$ (%)	μ_{eo}	$\Delta\mu_{eo}$ (%)
NaOH	68	–	49	–
Tris	60	12	44	10
Bis–Tris	43	37	36	28
Bis–Tris–propane	21	69	16	67

The decrease of the electroosmotic coefficient, $\Delta\mu_{eo}$, given in %, relates to the buffer with sodium cation. Electroosmotic coefficients are the means of at least three consecutive measurement with mesityolxide as the electroosmosis marker, made at +10 kV driving voltage. Uncoated 75 μm i.d. × 360 μm o.d. fused silica capillary (50 cm total length, 38.9 cm detection length) was thermostated by liquid to 25 °C. For details on the used laboratory set-up, see Ref. [6].

Electroosmotic flow strongly decreased with the size of the base utilized for the adjustment of the MOPS pH (Table 6). The decrease reached 70% if pH of MOPS was adjusted with Bis–Tris–propane instead of sodium hydroxide. Consequently, the polyacrylamide-coated capillary must be used for the mobility measurements in MOPS adjusted with Bis–Tris–propane. Mobilities of boron cluster anions decreased with the increasing size of the buffer cation. The decrease depended on the structural type of the particular boron cluster anion, which was linked with its size and hydrophobicity (Table 6). The highest decreases exceeded 50% of mobility, which was found for the respective boron cluster anion in MOPS adjusted with sodium hydroxide. Experiments with a set of anions consisting of four randomly chosen organic anions and of nitrate, whose ion pairing capability was expected to be lower than that of organic cations, supplied unexpected results (Table 7). Ionic mobility of nitrate in MOPS adjusted with sodium hydroxide, -57.7 ± 0.3, was markedly below the tabulated range -75.4 to -68.3 [30]. Identical nitrate mobility, -57.8 ± 0.3, was found when Tris was used for the pH adjusting instead of sodium hydroxide. However, higher nitrate mobilities, 59.9 ± 0.4 and 59.1 ± 0.5 have been found in MOPS buffers whose pH was adjusted with Bis–Tris and Bis–Tris–propane. Higher mobility found for the nitrate anion in MOPS buffers adjusted with these bases is credible with respect to high precision of experiments as well as with respect to higher mobilites found in these buffers with some organic anions, too (Table 7). Reasons for very low nitrate mobility in MOPS–sodium and MOPS–Tris buffers as well as for the increase of nitrate mobility in MOPS buffer adjusted to pH 7.0 with Bis–Tris and Bis–Tris–propane cations are unclear. Mobilites found for phthalate in 30 mM MOPS adjusted with various bases to pH 7.0 corresponds merely to mobility -35.3 reported in [30] for monovalent phthalate with pK_a 2.95 than to mobilities ranging from -52.7 to -45.4 reported for divalent phthalate with pK_a 5.408. Surprisingly, higher mobilities of both nitrate and organic anions have been found as a rule if hydrochloric acid was used instead of MOPS for the adjustment of the working pH 7.0 of Tris, Bis–Tris and Bis–Tris–propane buffers (Table 8). The highest mobility increase was found for the nitrate anion in chloride–Tris buffer. Its mobility reached the lower limit of reported nitrate mobilities [30].

Tabels 7 and 8 evidence that mobilities of anions may be affected by the buffer anion(s). It is well-founded to expect that various side-interactions of anionic buffer constituents with another constituents of the buffer or background electrolyte as well as products of these interactions enable this seemingly improbable effect. In the case of data summarized in Tabels 7 and 8, some of such effects may be the aggregation of MOPS anions and the ion pairing of MOPS anions with TRI, Bis–Tris and Bis–Tris–propane cations.

Table 6
Mobilities of anionic boron cluster compounds, listed in the order of their increasing hydrophobicity, in 30 mM MOPS buffers adjusted to pH 7.0 with sodium hydroxide, Tris, Bis–Tris (B–T) and Bis–Tris–propane (B–Tp)

Formula		Buffer cation			
Systematic	Schematic	Na^+	$Tris^+$	$B–T^+$	B–Tp[a]
$[i–B_{18}H_{21}]^-$		−29.0	−28.0	−23.0	−20.5
[nido-7-Me-7,8-$C_2B_9H_{11}$]$^-$		−33.0	−30.5	−29.1	−18.8
[closo-6,6′-μ-S<(1,7-$C_2B_9H_{10}$-2-Co]$^-$		−23.7	−22.5	−20.3	−9.1
[closo-4,8′;8,4′-(EtPh)$_2$-(1,2-$C_2B_9H_{10}$)$_2$-3-Co]$^-$		−16.8	−15.6	−15.5	<−7

Mobilities are given as signed values in 10^{-9} m^2 V^{-1} s^{-1} units. Mobilities are the mean of at least three consecutive measurements made at 10 kV driving voltage in uncoated 75 μm i.d. × 360 μm o.d. fused silica capillary (50 cm total length, 38.9 cm detection length), which was thermostated by liquid to 25 °C. Electroosmosis was measured with mesityloxide. For details on the used laboratory set-up, see Ref. [6]. Ionic strength of background electrolytes adjusted with monovalent bases was 11.6 mM.

[a] Mixture of monovalent and divalent cations of Bis–Tris–propane.

Table 7
Mobilities of nitrate anion and of several randomly chosen organic anions, given as signed values in 10^{-9} m^2 V^{-1} s^{-1} units, in 30 mM MOPS adjusted to pH 7.0 with sodium hydroxide, Tris, Bis–Tris (B–T) and Bis–Tris–propane (B–Tp).

Anion	Buffer cation			
	Na^+	$Tris^+$	$B–T^+$[a]	B–Tp[a]
Nitrate	−57.7 ± 0.3[b]	−57.8 ± 0.3	−59.9 ± 0.4	−59.1 ± 0.5
Phthalate	−35.8 ± 0.3	−34.4 ± 0.2	−37.0 ± 0.3	−30.3 ± 0.4
3,5-Dinitrobenzoate	−18.0 ± 0.1	−18.4 ± 0.1	−20.8 ± 0.2	−21.3 ± 0.2
Tropate	−15.8 ± 0.1	−16.3 ± 0.1	−18.8 ± 0.1	−19.3 ± 0.2
Folate	−19.0 ± 0.2	−19.1 ± 0.3	−20.6 ± 0.3	−18.2 ± 0.3

Mobilities in the table are the mean of at least three measurements made in the polyacrylamide coated [22] 75 μm i.d. × 360 μm o.d. fused silica capillary (50 cm total length, 38.9 cm detection length), which was thermostated by liquid to 25 °C. The highest Joule heat input in the capillary was 0.12 W/m at the 10 kV driving voltage. Ionic strength of the background electrolyte was 11.6 mM if its pH 7.0 was adjusted with a monovalent base.

[a] Mixture of monovalent and divalent cations of Bis–Tris–propane.

[b] Mobilites given in [30] are: nitrate from −75.4 to −68.3; divalent phthalate from −52.7 to −45.4, monovalent phthalate −35.3.

4. Closing discussion

Experimental results obtained by electrophoresis and photometry evidence the aggregation of anions of Good's buffers CAPSO or MOPS. Probability that these aggregates may be classified as micelles is high. Experiments verifying it are in progress. However, there is one unclear incompatibility in relevant results. Fig. 2 implies that the CAPSO micelles create when the concentration of CAPSO anions is higher than 30 mM. However, Fig. 3 quotes strong weakening of the UV-light beam in solution with lower concentration of CAPSO anions. This indicates that further investigation of the aggregation of anions of Good's buffers and its effect on mobilities of analytes is

Table 8
Mobilities of nitrate anion and of several randomly chosen organic anions, given as signed values in 10^{-9} m^2 V^{-1} s^{-1} units, in 11.6 mM hydrochloric acid adjusted to pH 7.0 with Tris, Bis–Tris and Bis–Tris–propane as buffering constituents.

Anion	Buffering constituent		
	Tris	Bis–Tris	Bis–Tris–propane
Nitrate	−68.4 ± 1.0[a]	−63.7 ± 0.2	−64.5 ± 0.4
Phthalate	−40.0 ± 0.8[a]	−40.2 ± 0.4	−35.0 ± 0.5
3,5-Dinitrobenzoate	−24.0 ± 0.3	−21.0 ± 0.4	−22.4 ± 0.4
Tropate	−21.7 ± 0.2	−19.1 ± 0.5	−20.4 ± 0.4
Folate	–	−21.7 ± 0.3	−20.0 ± 0.3

For experimental details, see Table 7.
[a] Mobilites given in [30] are: nitrate from −75.4 to −68.3; divalent phthalate from −52.7 to −45.4, monovalent phthalate −35.3.

necessary. Methods established in investigation of the micellization including those used in [27] have to be used in target experiments. Such a research should be extended to basic buffers, too. This indicates strong weakening of the UV-light beam, which passes radially through 75 μm i.d. fused silica capillary filled with 12 mM aqueous solution of Bis–Tris (Fig. 5). Its pH was adjusted to pH 7.0 with hydrochloric acid. Dispersion of the passing beam by aggregates of Bis–Tris is again a possible explanation of its weakening in the range of short wavelengths with respect to the absence of chromophores in the Bis–Tris molecule (Fig. 1). The highest formal absorbancies found with 12 mM Tris and with 12 mM Bis–Tris–propane were 0.004 and 0.01 AU, respectively, at identical conditions and pH.

Hydrophobic parts of anions of zwitterionic buffers are not purely hydrocarbonaceous as the dodecylsulfate anion. Thus, it is reasonable to expect that separation selectivity of their aggregates for a given pair of analytes might be different from the separation selectivity of the dodecylsulfate micelles. Various chemical compositions of hydrophobic parts of anions of various Good's buffers should reflect in various separation selectivity of their aggregates for a given pair of analytes. Aggregates of Good's buffers are therefore a new tool in the tuning of selectivity of separations.

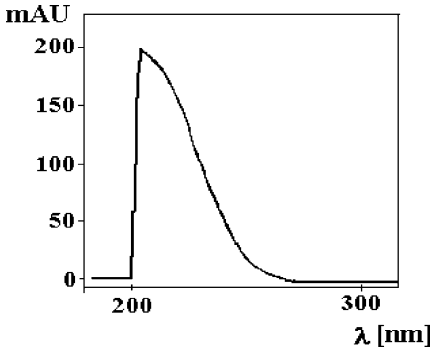


Fig. 5. The weakening of the UV-light beam expressed as absorbance in 12 mM Bis–Tris, which was adjusted to pH 7.0 with hydrochloric acid. The light beam passed radially through the 75 μm i.d. × 360 μm o.d. fused silica capillary coated with polyacrylamide [22]. Instrument: UV–vis spectrophotometer Jasco 875 UV adapted [6] for measurements with fused silica capillaries.

Monotonous decrease in mobilities of anionic analytes with the increasing size of organic cations, which may be ascribed to their ion pairing with these cations, was found only with anionic cluster boranes. Main reasons for it might be the pronounced hydrophobicity of boron cluster compounds even if they are charged [18] and, perhaps, the capability of basic buffers to interact with highly hydrophobic molecules also with another mechanism. The latter indicates their interaction with hydrophobic uncharged sanguinarine and chelerythrine (see Section 3.2) that are highly hydrophobic, too [9].

The reported side-interactions of biological buffers on migration of analytes are heterogeneous. Inspection for properties of species, which participate in them, implies that limited hydrophilicity of at least one of the interacting species is the precondition for any from observed side-interaction. This is single generalizing conclusion, which may be done from our experiments now. Pronounced and sometimes complex influence of side effects of biological buffers on mobilities of analytes supports the idea that electrophoretic mobility is only exceptionally a specific characteristic of an ion in analytical separation systems. It is more realistic to consider the mobility a complex characteristic, which depends on the analyte characteristics as well as on the composition of solution in which the analyte migrates.

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References

[1] P. Boček, M. Deml, P. Gebauer, V. Dolník, Analytical Isotachophoresis, VCH, Weinheim, 1988.
[2] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Applications, Elsevier, Amsterdam, 1992.
[3] P. Camilleri (Ed.), Capillary Electrophoresis: Theory and Practice, second ed., CRC Press, Boca Raton, FL, 1998.
[4] P. Barták, V. Šimánek, M. Vlčková, J. Ulrichová, R. Vespalec, J. Phys. Org. Chem. 16 (2003) 803.
[5] R. Vespalec, P. Barták, V. Šimánek, M. Vlčková, J. Chromatogr. B 797 (2003) 357.
[6] V. Slavíček, B. Grüner, R. Vespalec, J. Chromatogr. A 984 (2003) 121.
[7] F.J.C. Rossotti, H. Rossotti, The Determination of Stability Constants, McGraw-Hill, New York, 1961.

[8] V. Šimánek, in: A. Brosi (Ed.), The Alkaloids, vol. 26, Academic Press, Orlando, FL, 1985, p. 185.
[9] J. Dostál, M. Potáček, Collect. Czech. Chem. Commun. 55 (1990) 2840.
[10] J. Kovář, K. Šimek, E. Kožoušková, H. Klukanová, J. Slavík, Collect Czech. Chem. Commun. 55 (1985) 1312.
[11] D. Walterová, V. Preininger, F. Grambal, V. Šimánek, F. Šantavý, Heterocycles 14 (1980) 597.
[12] M.E. Perelson, I.V. Persiyanova, T.S. Semenova, I.E. Konylova, Khim. Prirod. Soedin. (1984) 337 (in Russian).
[13] D. Walterová, J. Ulrichová, J. Válka, J. Vičar, C. Vavrečková, E. Táborská, R.J. Harkrader, D.L. Meyer, H. Èerná, V. Šimánek, Acta Univ. Palacky Olomouc Fac. Med. (Olomouc) 139 (1995) 7.
[14] M. Wink, Alkaloids. Biochemistry, Ecology and Medical Applications, Plenum Press, London, 1998, p. 265.
[15] M.M. Chaturvedi, A.K. Kumar, B.G. Darnay, G.B.N. Chainy, S. Agarval, B.B. Agarval, J. Biol. Chem. 272 (1997) 30129.
[16] M. Stiborová, V. Šimánek, E. Frei, P. Hobza, J. Ulrichová, Chem. Biol. Interact. 287 (2002) 231.
[17] R.E. Williams, Chem. Rev. 92 (1992) 177.
[18] J. Plešek, Chem. Rev. 92 (1992) 269.
[19] A.H. Soloway, W. Tjarks, B.A. Barnum, F.-G. Rong, R.F. Barth, I.M. Codogni, J.G. Wilson, Chem. Rev. 98 (1998) 1515.
[20] F. Teixidor, M.A. Flores, C. Viñas, Organometalics 18 (1999) 5409.
[21] Biochemicals and Reagents for Research, Life Science, Sigma Catalogue, 1999, p. 1910.
[22] M. Vespalcová, D. Gregorová, J. Sep. Sci. 26 (2003) 727.
[23] J. Dostál, E. Táborská, J. Slavík, Fitoterapia 63 (1992) 67.
[24] P. Barták, P. Bednář, Z. Stránský, P. Boček, R. Vespalec, J. Chromatogr. A 878 (2000) 249.
[25] J. Pazourek, J. Chmelík, J. Chromatogr. A 715 (1995) 259.
[26] J. Janoušková, M. Budinská, J. Plocková, J. Chmelík, J. Chromatogr. A 914 (2001) 183.
[27] E. Tesařová, Z. Tuzar, K. Nesmìrák, Z. Bosáková, B. Gaš, Talanta 54 (2001) 643.
[28] A. Tiselius, Nova Acta Reg. Soc. Sci. Uppsaliensis 7 (1930) 1.
[29] F. Foret, L. Køivánková, P. Boček, Capillary Zone Electrophoresis, VCH, Weinheim, 1993, p. 15.
[30] J. Pospíchal, P. Gebauer, P. Boček, Chem. Rev. 89 (1989) 419.