

# Preconditions for reasonable detection sensitivity and for zone symmetry in electrophoretic separations of cluster borane anions

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## Abstract

Single-cage boron cluster anions with at least 11 cluster atoms, free of bonded functional groups that strongly absorb UV light, and their cobalt complexes have been the investigated compounds. Their UV-absorption spectra have absolute maxima between 200 and 215 nm. Corresponding molar extinction coefficients that are of the order of  $10^3$ – $10^4$  L mol<sup>-1</sup> cm<sup>-1</sup> indicate medium detection sensitivity. Its reaching requires elimination of background electrolytes that weaken the UV-light beam in any way. Frequently used carboxylic acids and zwitterionic Good's buffers cannot be used as buffering compounds from this reason. Freshly prepared 1 mM solutions of boron cluster compounds in aqueous sodium chloride, chosen as indifferent electrolyte, which contain 20–30% (v/v) of methanol or acetonitrile, give zones free of tailing. After storing in the fridge, zones of the compounds became pronouncedly tailed even if their solutions remain clear and free of precipitation, turbidity or opalescence. The tailing usually disappeared if the acetonitrile or the methanol concentration in samples was 40–60% (v/v) depending on the dissolved compound hydrophobicity. Solutions of extremely hydrophobic compounds, stored in the fridge, require mild heating to 30–40 °C for half an hour for the avoiding of the tailing. Permanent slow decrease in effective mobilities of boron cluster anions was found if background electrolytes contained acetonitrile and  $\beta$ -cyclodextrin. Analogous decrease was not observed with organic anions. Constant mobilities of boron cluster anions have been reached if acetonitrile was replaced with methanol. Analyte zones were more symmetrical in background electrolytes buffered with sodium borate of pH 9 than in background electrolytes buffered with sodium phosphate of pH 7. © 2004 Elsevier B.V. All rights reserved.

**Keywords:** Cluster boranes; Chiral separations; Capillary electrophoresis;  $\beta$ -Cyclodextrin

## 1. Introduction

Boron atoms can bind one another like carbon atoms. However, they have only three valence electrons for four valence orbitals in difference to “electronically exact” carbon. This so-called electron deficiency of boron [1] has pronounced consequences. The principal one is the formation of two-electron three-center bond between boron atoms in addition to standard two-electron two-center bonds, e.g., between boron atoms or between boron and hydrogen atoms [2]. Two-electron covalent bond of three atoms requires trianguloid arrangement of the atoms. Adjoining triangles have common sides. Thus, compounds with higher number of boron atoms

and with adjoining triangles cannot be plain. Bulkier boranes and their derivatives prepared by substitution of boron atoms with carbon and with another main group atoms or with metal atoms, form therefore three-dimensional cages. These cages are symmetrical polyhedrons as a rule. Their shape, symmetry and the skeleton type depend on the number of cage electrons, too [1]. Delocalized cage electrons are denoted as “sterically aromatic” or as “super-aromatic” [2]. Some *closo*-skeletons, which are highly symmetrical, are also extremely stable. Cluster boranes are highly hydrophobic and well soluble in polar organic solvents like alcohols, acetonitrile, ethers, etc. [1,3]. The same holds for their derivatives as a rule. They cannot be usually classified soluble in water even if the highest possible sensitivity of their UV detection is the criterion. Hydrogen atoms bound to skeleton atoms may be substituted with other elements and with inorganic or organic groups.

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Some hydrogen atoms are so acidic that they split off in dry polar organic solvents and their salts with alkali metal cations are strong electrolytes even in these solvents [2]. Carboranes, obtained by the substitution of one or more carbon atoms for skeletal boron atoms, exist only in anionic form in solutions from this reason unless a stable and positively charged group is the hydrogen substituent. Compounds with the negatively charged cage, bearing a cationic substituent, are zwitterionic [4]. Boron cluster anions exhibit pronounced tendency to ion pairing with organic cations [3].

Almost 50,000 of boron cluster compounds have been synthesized since the half of the past century. Some of them are utilized, e.g., in the treatment of radioactive wastewater from nuclear power plants [5,6], in neutron capture therapy of tumors [7,8] or in production of polymers and of another materials having properties that are not accessible with organic carbon compounds [3]. The struggle for practical utilization of synthesized boron cluster compounds markedly strengthens in last years. Majority of synthesized boron cluster compounds is chiral. Investigated possibilities of their use therefore include also alternatives conditioned by or resulting from their chirality. Drugs of new generations [9] and homogeneous stereoselective catalysts [10] are the most promising intentions of the type. Chiral boron cluster compounds are featured by the absence of discrete chiral centers [11,12], which dominate in organic compounds. Their chirality results from overall sterical structure. The term atropisomers introduced for chiral compounds of this type is therefore preferred for sterically different fractions of boron cluster compounds in this article.

Present research in boron cluster chemistry focuses on synthesis of new boron cluster compounds and on ways of their use. Synthesized compounds are identified and characterized by mass and IR spectrometry, advanced NMR techniques, X-ray diffraction analysis and by alike techniques. Necessary methods are developed as an unsplitable part of the synthetic research. Other analytical techniques including chromatography and electrophoresis, which dominate in research linked in any way with the utilization of organic and common inorganic compounds, have been seldom applied to boron cluster compounds. It evokes a supposition that analytical methods, developed for organic compounds, may be used for boron cluster compounds either directly or after a simple modification because boron cluster compounds are a special class of organic compounds or their mere analogy.

However, reality is different. Boranes and their derivatives are based on chemical bonds not occurring in organic compounds. Their chemical reactivity and sterical structure depend, except others, on overall number of atoms forming the clusters and on their chemical identity, on the number of electrons delocalized in a given cluster and on exo-skeletally bonded atoms or groups, which substitute hydrogen atoms bound to cluster atoms. Many properties of boron cluster compounds are therefore markedly different from properties of organic compounds as briefly mentioned above. It is well

founded to expect that unique structure and many unusual properties of boron cluster compounds reflect in some way also in their intermolecular non-bonding interactions. These interactions determine the course and the result of both electrophoretic and chromatographic separations.

Liquid chromatography (LC) proved effective in achiral analyses of boron cluster compounds with more than approximately nine atoms in a cluster [13] and in chiral analyses of zwitterionic compounds of this type see, e.g. [4,14,15]. But, the technique failed in chiral splitting of anionic boron cluster compounds with the exception of two compounds [14]. Since this first communication, non-additional chromatographic chiral splitting of an anionic boron cluster compound was reported despite much higher attractivity of anionic boron cluster compounds for syntheses compared to zwitterionic compounds [16]. Cause for the minimal success of liquid chromatography in chiral splitting of boron cluster anions is unclear considering full replaceability of LC and capillary electrophoresis (CE) in chiral separations of organic compounds and theoretical conceptions based on it [17,18]. Published articles do not supply explanation for this surprising fact. It is evident that the explanation has to consider different properties of boron cluster compounds and organic compounds, differences in behavior of boron cluster compounds and of organic carbon compounds in electrophoretic and chromatographic separation systems and, perhaps, differences in interactions of boron cluster compounds and of organic compounds with particular chiral selectors. Necessary input knowledge are extensively accessible for organic compounds. But, they are almost completely missing for boron cluster compounds. The main reason is that knowledge on non-bonding intermolecular interactions of boron cluster compounds are unimportant as a rule for syntheses of new boron cluster compounds. Thus, they are out of interest of the synthesists engaged in boron chemistry.

The number of reports on both achiral and chiral separations of boron cluster compounds by LC is negligible compared to the number of alike reports dealing with organic compounds. Single communication was published yet on CE separations of anionic boron cluster compounds [19]. Fortunately, the article demonstrates wide capability of CE to split anionic boron cluster compounds chirally supposing proper chiral selector is used. This article also points out to unexpected results found in the study and to differences in the behavior of organic ions and anionic boron cluster compounds at conditions of electrophoretic separations. Additional differences are given in the article [20], accepted for publication in this issue. The capability of CE to separate boron cluster anions both achirally and chirally offers a way to the accumulation of knowledge on properties of boron cluster compounds important for their electrophoretic separations. Such studies are also a contribution to recognizing of behavior of boron cluster compounds in solutions. It is reasonable to expect that electrophoretic studies will be helpful in the disclosing of causes of the apparent incapability of LC to split anionic boron cluster compounds chirally.

Electrophoretic chiral separations of boron cluster anions reported in [19] do not meet standards common in electrophoretic separations of organic ions. Their drawbacks are low sensitivity of UV-photometric detection and broad, pronouncedly tailing peaks, which cause low efficiency of both achiral and chiral separations of boron cluster compounds. It is unreasonable to investigate capability of various chiral selectors for chiral splitting of boron cluster anions if the shape of their zones is distorted by enormous tailing to which probably contributes low detection sensitivity. We undertook, therefore, this study that aimed at the search for causes of both drawbacks and for methods of their elimination. Detection sensitivity and separation efficiency are important both in CE and LC. This study, is therefore, also a step to chiral chromatographic separations and preparations of anionogenic boron cluster compounds.

## 2. Experimental

### 2.1. Instrumentation

The double-beam UV–vis spectrophotometer UNICAM UV 530 (Thermo Spectronic, Cambridge, UK) was equipped with the fused silica cell of 1 cm optical length and with Vision 3.5 software (Unicam, Cambridge, UK) for the measurement of spectra of boron cluster compounds. The electrophoretic laboratory set-up based on a Spellman CZE 1000R high-voltage power supply (Plainview, NY, USA) and a Jasco 875 UV–vis spectrophotometer (Tokyo, Japan) is described in details in [19]. The separation lengths of both uncoated and polyacrylamide-coated [21] fused silica capillaries of  $75 \mu\text{m} \times \text{i.d. } 360 \mu\text{m}$  o.d. ranged from 38 to 53 cm. Their total length was higher by 10.1 cm. Uncoated capillaries were rinsed with 0.5 M sodium hydroxide for approximately 5 min at the beginning of the working day. During the working day, they have been rinsed with 0.5 M sodium hydroxide before a change in the background electrolyte composition except of a change in the chiral selector concentration. Subsequent rinsing with background electrolyte (BGE) for approximately 2 min was repeated till constant migration time of mesityloxy serving as an electroosmosis marker was reached. Conditioned capillaries were rinsed with background electrolyte between runs. Temperature of the thermostating liquid was set at  $25.0^\circ\text{C}$ . Current flowing through the separation capillary was from 3 to  $6 \mu\text{A}$  at the 10 kV driving voltage depending on electric conductivity of the background electrolyte and on the capillary length. Optimal detection wavelength ranged from 200 to 215 nm for investigated boron cluster compounds and from 210 to 220 nm for the sample containing nitrate anion and organic acids. The detector output was monitored, stored and processed by the intelligent chromatographic integrator CSW 1.7 (DataApex, Prague, Czech Republic).

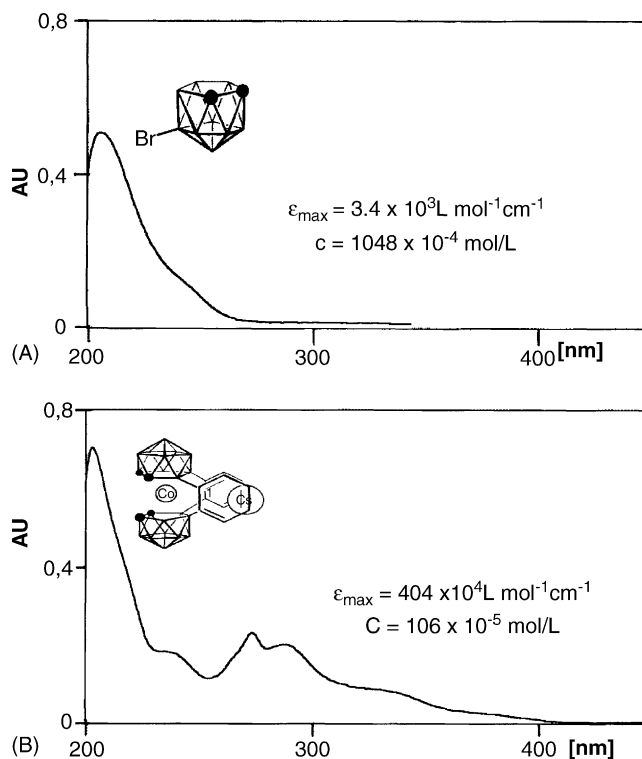


Fig. 1. Optical spectrum of (A) the simple dicarbollide anion [*nido*-5-Br-7,8-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>]<sup>-</sup> and (B) the cesium salt of the bridged anionic cobalt bis(dicarbollide) complex [*closo*-4,8',8,4'-(Ph)<sub>2</sub>-(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>10</sub>)<sub>2</sub>-3-Co]<sup>-</sup>.

### 2.2. Chemicals and methods

Cluster boron compounds used in this study have been synthesized in the Institute of Inorganic Chemistry, Czech Academy of Sciences, Řež near Prague, Czech Republic, using published methods specified in [19]. Their systematic and schematic formulas are given in Figs. 1–6 except of the fused borane *i*-B<sub>18</sub>H<sub>22</sub> and of [*nido*-7-MeS-7,8-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>]<sup>-</sup>.  $\beta$ -Cyclodextrin and mesityloxy were from Sigma (Sigma–Aldrich, Prague, Czech Republic). Acetonitrile, 99.93 + percentage of HPLC grade, lot no. U04517, was from Aldrich, methanol of HPLC grade was from Fluka (Buchs, Switzerland). Other chemicals including those used for preparation of buffers and for the capillary coating [21], obtained from different suppliers, were of analytical grade purity. Distilled and freshly boiled water was used for the preparation of stock solutions of buffers and of other solutions.

Stock solutions of 5 mM sodium phosphate buffer of pH 7 and of 30 mM sodium borate buffer of pH 9 have identical ionic strength,  $I = 11.1 \text{ mM}$ . The buffers were prepared either from equimolar mixture of sodium phosphate with disodium phosphate or from boric acid, respectively. Their working pH was adjusted with 4 M NaOH potentiometrically. Background electrolytes were prepared daily by mixing of a stock solution of a buffer, stored in a fridge, with acetonitrile or methanol. The mixing ratio is given in the description of individual experiments.  $\beta$ -Cyclodextrin was dissolved as a chiral selector

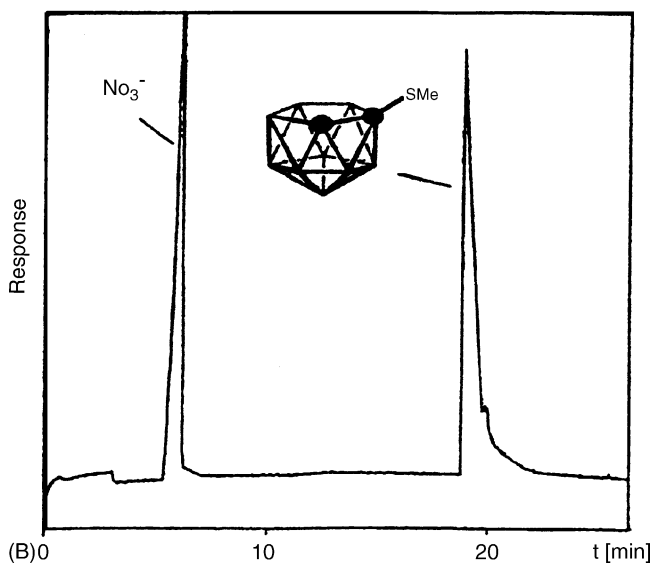
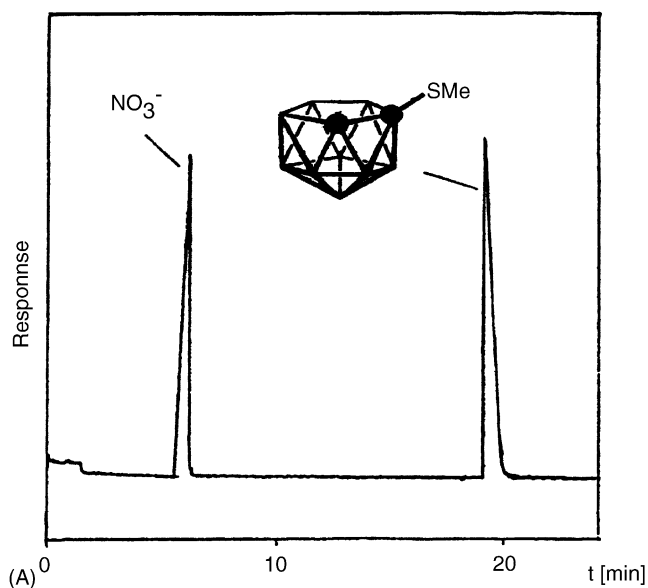


Fig. 2. Deterioration of shapes of  $[nido-7-MeS-7,8-C_2B_9H_{11}]^-$  and nitrate anion at repeated analyses in a polyacrylamide coated fused silica capillary if the anions were dissolved in 6.6 mM sodium chloride–(4:1, v/v) acetonitrile and stored for one week in a frig. (A) First injection, (B) seventh injection. Experimental details: capillary dimensions, 63.1 cm (53 cm separation length)  $\times$  75  $\mu$ m i.d.  $\times$  360  $\mu$ m o.d.; background electrolyte, 5 mM sodium phosphate buffer of pH 7 and  $I = 11.1$  mM acetonitrile; (7:3, v/v) voltage,  $-10$  kV.

in this liquid if necessary. Background electrolyte was filtered through 0.45  $\mu$ m nylon filter and degassed by sonication before use.

Weighed amounts of cluster boranes have been dissolved in a few drops of acetonitrile and then diluted with 6.6 mM aqueous solution of sodium chloride mixed in 4:1 (v/v) ratio with acetonitrile to 1 mM solutions for the measurement of their spectra. These solutions have been used also in electrophoretic experiments as a rule. Sodium nitrate and organic acids (tropic acid, 3,5-dinitrobenzoic acid and folic

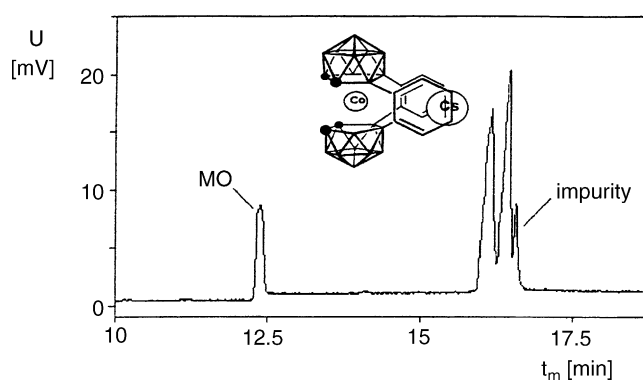


Fig. 3. Chiral separation of  $[closo-4,8',8,4'-(Ph)_2-(1,2-C_2B_9H_{10})_2-3-Co]^-$  with mesityloxide (MO) as an electroosmosis marker in background electrolyte consisting of 5 mM sodium phosphate pH 7–acetonitrile mixed with it in (7:3, v/v) ratio and of 3 mM  $\beta$ -cyclodextrin added as a chiral selector. Experimental details: uncoated fused silica capillary of 63.1 cm (53 cm separation length)  $\times$  75  $\mu$ m i.d.  $\times$  360  $\mu$ m o.d. Voltage 10 kV. Separation efficiency 59,000 and 45,000 theoretical plates for the less and the more retained peaks; mobility difference  $\Delta\mu = 0.6$ .

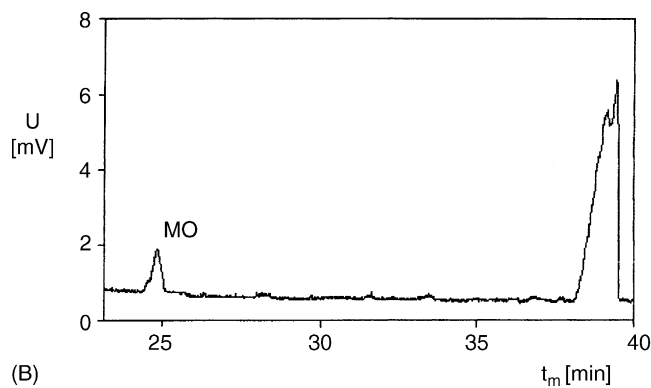
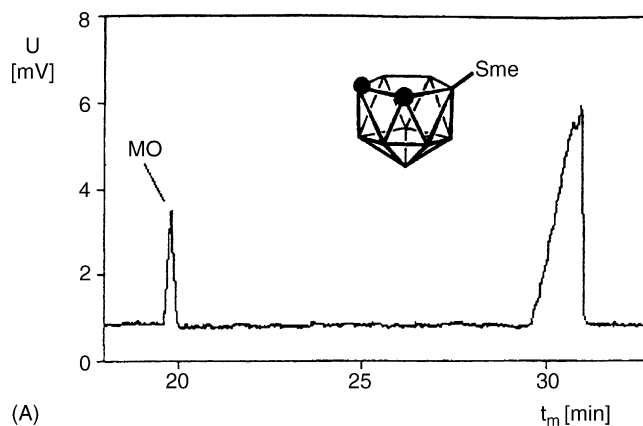


Fig. 4. Chiral separation of  $[nido-9-MeS-7,8-C_2B_9H_{11}]^-$  with mesityloxide (MO) as an electroosmosis marker in background electrolyte based either on (A) 5 mM sodium phosphate of pH 7 and  $I = 11.1$  mM or on (B) 30 mM sodium borate of pH 9 and  $I = 11.1$ , mixed in 1:1 (v/v) ratio with methanol. 2.7 mM  $\beta$ -cyclodextrin is a chiral selector. Absolute value of the mobility difference of splitted peaks: 0.14. Other experimental details as in Fig. 3.

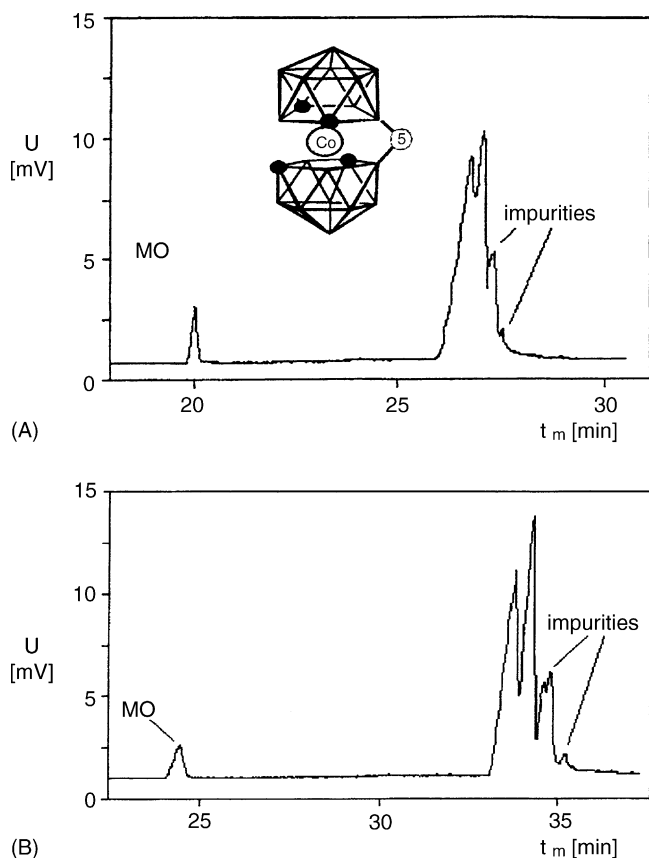


Fig. 5. Chiral separation of  $[closo-6,6'-\mu-S<(1,7-C_2B_9H_{10})_2-2-Co]^-$  with mesityloxide (MO) as an electroosmosis marker in background electrolyte based either on (A) 5 mM sodium phosphate of pH 7 and  $I = 11.1$  mM or on (B) 30 mM sodium borate of pH 9 and  $I = 11.1$ , mixed in 1:1 (v/v) ratio with methanol. The 2.7 mM  $\beta$ -cyclodextrin is a chiral selector. Absolute value of the mobility difference of splitted peaks: 0.24. Other experimental details as in Fig. 3.

acid) have been dissolved in 3.3 mM sodium chloride. Their submillimolar concentrations, giving responses comparable in height with those of boron cluster compounds, have been prepared by the try-and-error method. Samples have been injected in the separation capillary by the difference in the hydrostatic pressure between the capillary inlet and outlet. The length of injected zones, controlled by injection time, was around 1 mm.

### 2.3. Calculations

The definition equation

$$\mu_{app} = \frac{Ll}{t_m V} \quad (1)$$

served for calculation of apparent mobility of an ion,  $\mu_{app}$ , from its migration time,  $t_m$ , applied voltage,  $V$ , total length of the capillary,  $L$  and from its separation length,  $l$ . Migration time of the mobility marker,  $t_0$ , was inserted in (1) instead of  $t_m$  in calculation of the electroosmotic coefficient,  $\mu_{eo}$ . Ionic mobility of an analyte at the absence of a chiral selector,  $\mu$ ,

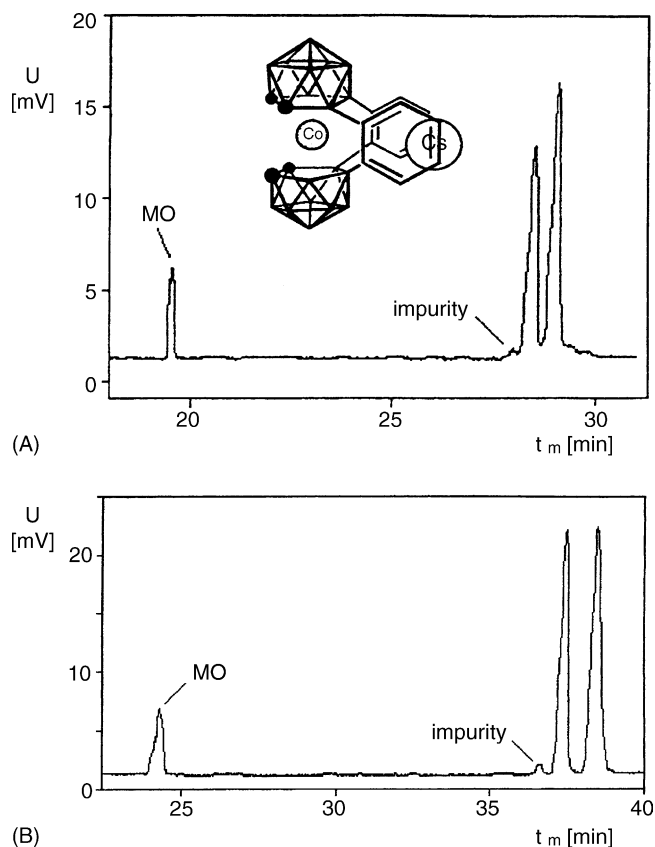


Fig. 6. Chiral separation of  $[closo-4,8',8,4'-(Ph)_2-(1,2-C_2B_9H_{10})_2-3-Co]^-$  with mesityloxide (MO) as an electroosmosis marker in background electrolyte based either on (A) 5 mM sodium phosphate of pH 7 and  $I = 11.1$  mM or on (B) 30 mM sodium borate of pH 9 and  $I = 11.1$ , which is mixed in 1:1 (v/v) ratio with methanol. The 2.7 mM  $\beta$ -cyclodextrin is a chiral selector. Separation characteristics are in Table 2. Other experimental details as in Fig. 3.

and its effective mobility at the presence of a selector,  $\mu_{eff}$ , have been obtained as the difference ( $\mu_{app} - \mu_{eo}$ ). Mobilities and electroosmotic coefficients were given as signed values in  $10^{-9} m^2 V^{-1} s^{-1}$  units. Separation selectivity,  $S$ , of species 1 and 2 was calculated from their apparent mobilities  $\mu_{1,app}$  and  $\mu_{2,app}$ , respectively, using the definition equation [18]:

$$S = \left| \frac{\mu_{1,app} - \mu_{2,app}}{0.5(\mu_{1,app} + \mu_{2,app})} \right| \quad (2)$$

Separation efficiency,  $N$ , peak asymmetry,  $A_s$ , and resolution,  $R$ , have been calculated in standard chromatographic way by calculation routines implemented in the CSW 1.7 software. The direction of the peak migration against the surrounding solvent determined the peak front.

## 3. Results and discussion

Knowledge on behavior of boron cluster compounds at conditions of chromatographic [4,13–15] or electrophoretic [22,23] separations are scarce. Knowledge on their contin-

gent interactions with constituents of electrophoretic separation systems including the capillary wall and its coating are absent. Compositions of electrophoretic separation systems were therefore chosen intuitively based on experience from electrophoretic separations of boron cluster compounds in the exploration study [19] and with respect to results of the study on side-interactions of biological buffers [20].

### 3.1. Detection sensitivity

UV-spectra of boron cluster compounds are not published in communications on their syntheses. However, they are necessary for optimized photometric detection. UV–vis spectra of several representatives of various structural types of boron cluster compounds have been therefore measured as described in Section 2. Main UV-light absorption maximum was found between 200 and 210 nm in spectra of single-cage cluster anions. Extinction coefficients for this maxima range from  $1.4 \times 10^3$  to  $3.4 \times 10^3$  L mol<sup>-1</sup> cm<sup>-1</sup> disregarding the substitution of boron atoms in their cage with carbon atoms and disregarding substituents bonded to the carbaborane cage. These maxima either survive in sandwich cobalt bis(dicarbollide) complexes, both bridged and unbridged, or shift slightly to higher wavelengths. Corresponding extinction coefficients are higher by one order of magnitude. Substituents bound to cage atoms instead of hydrogen atoms shift the main light absorption maximum to higher wavelengths seldom and slightly unless the substituent exhibits pronounced UV-light absorption.

Steep weakening of the light absorption of boron cluster compounds with increasing length of the UV-light features boron cluster compounds (Fig. 1). Medium extinction coefficients allow only medium sensitivity of UV-photometric detection. Its reaching requires detection at the most absorbed wavelength. Further, background electrolytes and their constituents that weaken the passing light beam in the wavelength range 200–215 nm have to be eliminated. Carboxylic aliphatic acids and zwitterionic Good's buffers [20] are frequently used buffers constituents of this kind. The carboxyl group is the chromophore whose UV-absorption pronouncedly rises up if the UV-light wavelength decreases below 230 nm. Zwitterionic Good's buffers are free of chromophores absorbing above 200 nm, however they create aggregates, which strongly disperse the UV light up to 220 nm [20]. Only phosphate and borate remain as buffering anions transparent for UV-light. Cations of basic biological buffers decrease mobility of boron cluster anions by ion pairing [3,20]. Therefore, sodium phosphate of pH 7 was selected as the potentially optimal buffer for this study. Sodium borate of pH 9 was tested as its alternative applicable in uncoated capillaries.

### 3.2. Dissolution power of the sample solvent

Acetonitrile and methanol belong to solvents commonly used in syntheses of boron cluster compounds. Many cesium

and ammonium salts of boron cluster anions dissolve extremely slowly in sodium phosphate pH 7 containing 30–40% (v/v) of these organic solvents, which proved proper for many chiral separations with  $\beta$ -cyclodextrin as a chiral selector [19]. In first experiments, solid samples were dissolved in a few drops of acetonitrile and then were diluted with 6.6 mM sodium chloride containing at least 20% (v/v) of acetonitrile in order to minimize stacking effects. Electric conductivity of 6.6 mM sodium chloride is a bit lower than that of the sodium phosphate buffer of pH 7 and of ionic strength,  $I = 11.1$  mM. Narrow triangular zones have been obtained with freshly prepared samples even if they were injected into uncoated fused silica capillary filled with sodium phosphate free of an organic solvent. However, zones of all boron cluster compounds became tailed within a few days of their storing in a fridge. Only the most hydrophobic samples precipitated or become opalescent during this time. The peak shape deterioration was independent of the organic solvent type present in the background electrolyte and of its content. Acetonitrile was added up to 30% (v/v), methanol up to 60% (v/v). Consecutive injections of samples stored in the fridge into a polyacrylamide coated capillary, which was filled with sodium phosphate buffer of pH 7 containing 30% of acetonitrile, caused step-by-step worsening of the shape of zones of boron cluster anions. The shape distortion was observable also with the peak of nitrate, which was added in the sample as the injection marker and as the mobility marker (Fig. 2).

There are two possible causes of formation of narrow trianguloid peaks that are observed after injection of freshly prepared solutions of boron cluster compounds in an uncoated capillary filled with sodium phosphate buffer free of a polar organic solvent. Such a peak shape may be the consequences of high stability of the first solvating layer, which forms at dissolution of these compounds in acetonitrile or methanol. The stability of trianguloid zones may be supported by slow aggregation of desolvated boron cluster anions due to their coulombic repulsion. Deterioration of the shape of peaks at the absence of observable precipitation of majority of samples may be the ascribed to the formation of non-precipitating colloid aggregates in the sample and to their very slow dissolution in background electrolyte with higher content of an organic solvent. Negative charge of aggregated anions hampers their precipitation. The acetonitrile content in the sample solvent was therefore increased to 40–60% (v/v) depending on hydrophobicity of the dissolved compound in order to retain its sufficient dissolution power at decreased temperature. Higher organic solvent concentrations have not been used in order to avoid a risk of precipitation of sodium chloride, which secures reasonable electric conductivity of injected samples with respect to that of background electrolyte. Vials with extremely hydrophobic samples, which precipitated or become turbid during storing in the fridge, have been gently heated to 30–40 °C in the water bath for half an hour and then shaken. These remedies avoided distortion of peaks of investigated cluster boranes.

### 3.3. Acetonitrile as a competing agent in chiral separations

The interaction of cluster borane anions with the  $\beta$ -cyclodextrin cavity is so strong that their inclusion in the cavity is effectively permanent. The absence of repeated complexation of analyte atropisomers with the chiral selector cavity disables their chiral discrimination. Acetonitrile was preferred as the competing agent, which weakens this excessive inclusion, because of low viscosity of its mixtures with water. It reflects in higher separation speed in background electrolytes with acetonitrile compared to that in background electrolytes with methanol [19]. Sodium phosphate pH 7–acetonitrile (7:3, v/v) was the solvent for  $\beta$ -cyclodextrin as the chiral selector. Narrow triangular peaks of tested compounds have been obtained. The influence of the  $\beta$ -cyclodextrin concentration on the peak shape and on separation efficiency was so low that changes of separation efficiency were not measurable at comparable concentrations of  $\beta$ -cyclodextrin. For example, typical separation efficiency was 45,000 and 59,000 theoretical plates for the more and the less retained atropisomers of [closo-4,8',8,4'-(Ph)<sub>2</sub>-(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>10</sub>)<sub>2</sub>-3-Co]<sup>−</sup>, respectively. The uncoated fused silica capillary of 65 cm (53 cm separation length)  $\times$  75  $\mu$ m i.d. and millimolar  $\beta$ -cyclodextrin concentrations have been used (Fig. 3). Effective mobilities of these atropisomers differed by 0.6 mobility unit at the 3 mM  $\beta$ -cyclodextrin concentration. Resolution,  $R = 1.0$  was reached at the selectivity,  $S = 0.02$ .

Continual slow decrease of effective mobilities of boron cluster anions was found in their consecutive analyses in background electrolytes containing acetonitrile and  $\beta$ -cyclodextrin disregarding extend of their chiral splitting. For example, mobility of the chirally unsplit [nido-7-MeS-7,8-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>]<sup>−</sup>,  $-22.4 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$  in sodium phosphate buffer pH 7 containing 30% (v/v) of acetonitrile, dropped to  $-14.5$  in first run at the presence of 3 mM of  $\beta$ -cyclodextrin (Table 1). This mobility decreased slowly within eight consecutive runs. Three runs next day morning showed practically no change in electroosmosis but the carbaborane mobility was

$-8.1 \pm 0.1$  mobility unit (runs 9–11). The background electrolyte in electrode compartments was replaced with stock background electrolyte stored overnight at room temperature near the electrophoretic instrument. The carbaborane mobility transiently restored but then decreased slowly (Table 1, runs 12–14). The decrease of mobilities of boron cluster anion with time was accompanied by worsening of the baseline stability. Replacing of the freshly prepared background electrolyte of identical composition for the used one restored both electroosmosis and the baseline stability. Mobility of [nido-7-MeS-7,8-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>]<sup>−</sup> transiently rose up to  $-14.6$ , however, its mobility decrease with time was not avoided. Capillary was not rinsed with sodium hydroxide before the introduction of the freshly prepared background electrolyte.

In contrast, time-independent mobilities have been measured with investigated boron cluster anions as well as with several randomly chosen organic anions specified in Experimental, in sodium phosphate–acetonitrile mixtures at the absence of  $\beta$ -cyclodextrin. Mobility of organic anions decreased slightly, roughly by one mobility unit, if 3 mM  $\beta$ -cyclodextrin was in sodium phosphate with 30% of acetonitrile. This mobility drop did not change measurably with time within one working day.

It is common practice in electrophoresis to prepare daily background electrolyte, which contains a cyclodextrin or a mixture of cyclodextrins [18]. The use of a cyclodextrin stock solution next days causes experimental difficulties, which increase with time even if the solution is stored in a fridge. This experience shows that aqueous solutions of cyclodextrins are not stable disregarding buffers controlling pH. Experiment described above may be therefore interpreted in the following way: acetonitrile speeds up spontaneous and very slow decomposition of native cyclodextrins in aqueous solution. Electric current has synergetic effect on the decomposition in the presence of acetonitrile. The cyclodextrin decomposition product interacts with borane cluster anions on forming of an interaction product whose mobility is lower than mobility of free cluster borane ions. Absence of comparable effect for organic ions shows either that their affinity to the decomposition product of  $\beta$ -cyclodextrins is much lower than affinity of borane cluster anions or that the hypothetical interaction between these species is absent at least in solutions containing 30% (v/v) of acetonitrile. Marked difference in affinity of boron cluster anions and organic anions to the decomposition product indicates that the product is uncharged. Presented experiments do not allow speculation on mechanisms of processes that affect mobility of boron cluster anions. It is evident that stability constants reported in [19] for several boron cluster compounds migrating in background electrolytes with acetonitrile are distorted by discussed  $\beta$ -cyclodextrin decomposition.

From analytical point of view, this finding disqualifies acetonitrile as the background electrolyte constituent in electrophoretic experiments with boron cluster compounds if  $\beta$ -cyclodextrin is present in the system. It cannot be a priori excluded that instability of mobilities of cluster boranes will

Table 1

Decrease in mobilities of the chirally unsplit [nido-7-MeS-7,8-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>]<sup>−</sup> in 5 mM sodium phosphate of pH 7–acetonitrile (7:3, v/v) and containing 3 mM of  $\beta$ -cyclodextrin

Run number	Fresh BGE		Run number	Stored BGE	
	$\mu_{eo}$	$\mu_{eff}$		$\mu_{eo}$	$\mu_{eff}$
1	55.7	−14.5	12	58.6	−12.1
2	55.5	−14.0	13	58.2	−11.5
3	55.4	−13.6	14	58.0	−11.3
4	55.5	−13.1	–	–	–
5	55.4	−12.8	–	–	–
6	55.1	−12.3	–	–	–
7	54.8	−11.8	–	–	–
8 <sup>a</sup>	54.8	−11.6	–	–	–

<sup>a</sup> Effective mobilities of [nido-7-MeS-7,8-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>]<sup>−</sup> were  $-8.1 \pm 0.1$  in runs 9–11 next day morning. For other information, see text.

Table 2

Comparison of chiral separations of  $[\text{closo-4,8',8,4'-(Ph)}_2\text{-(1,2-C}_2\text{B}_9\text{H}_{10})_2\text{-3-Co}]^-$  in background electrolyte (BGE) containing either (A) 5 mM sodium phosphate of pH 7 and ionic strength,  $I = 11.1$  mM or (B) 30 mM sodium borate of pH 9 and ionic strength,  $I = 11.1$  mM methanol (1:1, v/v) and containing 2.7 mM of  $\beta$ -cyclodextrin as a chiral selector

BGE	Peak number	$N$	As	$ \Delta\mu ^a$	S	R
A	1 <sup>b</sup>	76,000	4.0	0.40	0.01	1.4
	2	79,000	3.8	–	–	–
B	1 <sup>b</sup>	64,000	1.8	0.41	0.13	1.9
	2	100,000	3.6	–	–	–

<sup>a</sup> Absolute value of the mobility difference of peaks 1 and 2.

<sup>b</sup> Less retained, faster migrating peak.

be found in separations with other cyclodextrins and with their derivatives at the presence of acetonitrile, too.

### 3.4. Methanol as the competing agent, sodium borate pH 9 as the buffer

Time-independent effective mobilities have been obtained with boron cluster anions in mixtures of sodium phosphate buffer pH 7 with methanol as a competing agent disregarding the presence or the absence of  $\beta$ -cyclodextrin. Unavoidable drawback of separations with methanol as a competing agent is lower separation speed, which was caused by higher viscosity of methanol–water mixtures compared with viscosity of acetonitrile–water mixtures. Another drawback is asymmetry of zones of boron cluster anions, which deteriorates the separation efficiency. Fast divalent phosphate anions in phosphate buffer of pH 7 may contribute to asymmetry of zones of boron cluster anions [24].

We checked therefore sodium borate of pH 9, which also does not absorb UV-light above 200 nm, as an alternative to sodium phosphate (Figs. 4–6). Peak symmetry of zones of cluster boron anions and separation efficiency improved a little in sodium borate at the presence of  $\beta$ -cyclodextrin. Electroosmosis was lower in borate buffer even if its pH was higher by 2 pH units than that of sodium phosphate. The mobility difference between chirally splitted enantiomers was independent of the used buffer. Higher separation selectivity in sodium borate was therefore caused only by lower electroosmosis. This selectivity increase and slightly better symmetry of zones are the probable causes of better resolution of splitted atropisomers as illustrate data for  $[\text{closo-4,8',8,4'-(Ph)}_2\text{-(1,2-C}_2\text{B}_9\text{H}_{10})_2\text{-3-Co}]^-$  (Fig. 6, Table 2). However, stabilization of effective mobilities of chirally splitted atropisomers, accompanied by slow increase in their mobility difference, was slower in borate than in phosphate buffer in consecutive runs after a buffer change. Explanation of this mobility decrease cannot be identical with the explanation proposed for separation systems with acetonitrile and  $\beta$ -cyclodextrin. The reason is time-independence of stabilized effective mobilities of anionic cluster boranes in background electrolytes with sodium phosphate and methanol, which has been found both at the

absence of  $\beta$ -cyclodextrin in the system as well as at its two different concentrations.

## 4. Conclusions

The causes of low sensitivity of boron cluster compounds detection in experiments reported in ref. [19] have been found and requirements for its maximization have been specified. Effective method for the elimination of enormous tailing of zones of boron cluster anions was proposed and successfully checked. Some knowledge from presented research give hints for the search for buffers, which allow separations of boron cluster anions in coated fused silica capillaries in addition to sodium phosphate. Unexpected finding is the slow decrease of mobilities of anionic boron cluster compounds in background electrolytes containing acetonitrile and  $\beta$ -cyclodextrin. Analogous decrease was not observed with organic anions. This observation is additional evidence that different behavior of anionic boron cluster compounds and of organic anions in electrophoretic separation systems is not exceptional. This fact supports the necessity for systematic checking of experimental conditions and of other variables on electrophoretic separations of boron cluster compounds at present. It holds even if their effects on the course or on results of separations of organic compounds have not been found.

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## References

- [1] R.E. Williams, Chem. Rev. 92 (1992) 177.
- [2] J. Plešek, Chem. Listy (in Czech) 90 (1996) 273.
- [3] J. Plešek, Chem. Rev. 92 (1992) 269.
- [4] J. Plešek, B. Grüner, P. Maloň, Collect. Czech. Chem. Commun. 58 (1993) 1087.
- [5] V. Ščasník, V. Koprda, J. Radioanal. Chem. 71 (1983) 77.
- [6] V. Ščasník, Anal. Chem. 56 (1984) 605.
- [7] D.M. Goldberg, P.M. Sharkey, Proc. Natl. Acad. Sci. 81 (1984) 560.
- [8] R.F. Barth, A.H. Soloway, J.H. Goodman, R.A. Gahbauer, N. Gupta, T.E. Blue, W.L. Yang, W. Tjarks, Neurosurgery 44 (1999) 433.
- [9] Y. Endo, T. Yoshimi, C. Miyaura, in: Yu.N. Bubnov (Ed.), Boron Chemistry at the Beginning of the 21st Century, Proceedings of the 11th International Conference on the Chemistry of Boron, IME-BORON XI, Moscow, 2002, Editorial URSS Scientific Literature and Textbooks, Moscow, 2003, pp. 298–306.
- [10] F. Teixidor, M.A. Flores, C. Viñas, Organometallics 18 (1999) 5409.
- [11] J. Plešek, S. Heřmánek, B. Štíbr, Pure Appl. Chem. 63 (1991) 399.
- [12] J. Plešek, Inorg. Chim. Acta 289 (1999) 45.
- [13] B. Grüner, Z. Plzák, J. Chromatogr. A 789 (1997) 497.
- [14] B. Grüner, J. Holub, J. Plešek, T. Vaněk, H. Votavová, J. Chromatogr. A 793 (1998) 249.



- [15] M. Brown, B. Grüner, B. Štíbr, X.L.R. Fontaine, M. Thornton-Pett, J.D.J. Kennedy, *Organometal. Chem.* 614 (2000) 269.
- [16] B. Štíbr, *Chem. Rev.* 92 (1992) 225.
- [17] K.D. Altria, *Analysis of Pharmaceuticals by Capillary Electrophoresis*, Vieweg, Wiesbaden, 1998.
- [18] R. Vespalec, P. Boček, *Chem. Rev.* 100 (2000) 3751.
- [19] V. Slavíček, B. Grüner, R. Vespalec, *J. Chromatogr. A* 984 (2003) 121.
- [20] R. Vespalec, M. Vlčková, H. Horáková, *J. Chromatogr. A.* (2004) in press.
- [21] M. Vespalcová, D. Gregorová, *J. Sep. Sci.* 26 (2003) 727.
- [22] M. Koval, D. Kanianský, L. Mátel, F. Macásek, *J. Chromatogr.* 243 (1982) 144.
- [23] E. Šimunovičová, D. Kanianský, *J. Chromatogr.* 390 (1987) 121.
- [24] J.L. Beckers, P. Boček, *Electrophoresis* 24 (2003) 518.