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Journal of Chromatography A, 984 (2003) 121–134
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Exploration of the electrophoretic behaviour of borane cluster anions and of the capability of capillary electrophoresis to separate them chirally

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Received 17 June 2002; received in revised form 1 October 2002; accepted 31 October 2002

Abstract

Mobilities of investigated boron cluster compounds in 3-(*N*-morpholino)propanesulfonic and phosphate buffers adjusted to pH 7 either with sodium hydroxide or with tris(hydroxymethyl)aminomethane depend on both buffer ions. The zone width and zone asymmetry, which are usually markedly higher than those of organic or common inorganic ions of comparable size, depend on the type of the borane cluster anion. Unusual shapes of zones of two investigated compounds have been found in tris phosphate buffer. Acetonitrile was superior to methanol as an organic additive to separation systems from the viewpoint of the zone symmetry and separation speed. Narrow trigonal zones, typical of organic ions non-interacting with the capillary wall, have been observed for some bridged sandwich cobalt complexes in run buffers with the addition of acetonitrile. The interaction of borane cluster anions with β-cyclodextrin cavity is excessively strong in purely aqueous solutions. Methanol and acetonitrile, which generally weaken the interaction, sometimes affect the separation enantioselectivity of various compounds in different ways in addition to the weakening effect. Chiral discrimination was reached for all ten investigated anions, which belong to four different structural types of cluster boranes. Stability constants estimated for some analyte–β-cyclodextrin complexes range between 100 and 1800 l/mol in acceptable separations. The relative difference of the constants was from 3 to 20%.

Keywords: Enantiomer separations; Buffer composition; Cyclodextrins; Boranes; Cluster boranes

1. Introduction

Cluster boranes (Fig. 1) and their derivatives are synthetic compounds of molecular architecture different from that of naturally occurring compounds [1]. They exhibit exceptional properties that are frequently of practical interest. Cluster boranes and their derivatives to date have found use, for example, as extracting agents in the treatment of high-level radioactive waste [2], in neutron capture therapy of

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)01816-2

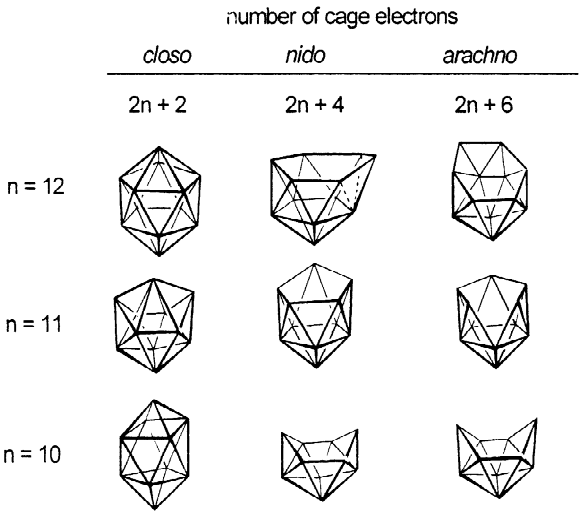


Fig. 1. Expected structures of ten to 12 vertex boron clusters. Hydrogen atoms are not given for the sake of simplicity, as is common.

brain tumours [2,3] and in the synthesis of polymers with enhanced thermal stability including, for example, stationary phases for gas chromatography [2]. The investigation of non-linear optical properties of cluster boranes [4] and their study as homogeneous stereoselective catalysts [5] are examples of highly promising topics of contemporary boron chemistry.

Most of the known boranes and their analogues, heteroboranes and metallaboranes, are symmetric species. However, an appropriate substitution of a hydrogen atom in their symmetrical three-dimensional cage by an *exo*-skeletal substituent (see, for example, compounds 1–5 and 9 in Table 1) or in some instances by an *exo*-skeletal bridging group (compounds 6–8 in Table 1), the replacement of a cage boron atom for a heteroatom or even the vertex removal impair the cluster symmetry [11,12]. Borane cluster compounds usually do not contain discrete atomic chiral centres which is different to the majority of chiral organic compounds. Their chirality is the consequence of steric asymmetry of their molecules, of their skeletal distortion or helical structure [11,12]. The term atropisomers was introduced because of their sterically different forms.

Mass spectrometry and its combinations with gas chromatography, advanced NMR techniques, X-ray diffraction analysis and IR spectrometry are used as the pilot methods in the characterization and analysis of newly synthesised boron cluster compounds. Cheap and expedient analytical methods have become necessary in the last few years for the targeted research of the utilisation of both cluster boranes and their derivatives, for the use of such compounds and, last but not least, for further development in the field [12]. Liquid chromatography proved to be effective in the achiral analysis of cluster boranes, carboranes and metallaboranes with more than approximately nine cluster atoms [13] and in the chiral analysis of zwitterionic compounds [14–19]. The charged cage borate anions, in general, and those with open cage in particular, are far more interesting than neutral species from the point of view of their reactivity and stereochemistry of their reactions [20]. Methods for the chiral analysis of charged cluster boranes and their derivatives as well as methods for the assessment of their chiral stability are therefore much more attractive than those effective in the analysis of uncharged compounds and are required urgently [12]. Despite this, only two anionic cobaltadicarboranes [*closo-commo*-6,6′-μ-R_nE(1,7-$C_2B_9H_{10}$)$_2$-2,2′-Co]$^-$ bridged with sulphur and oxygen were chirally splitted and partially resolved by liquid chromatography [18] from the large family of charged chiral boron cluster compounds. Another communication reporting the chiral separation of such compounds has not been published so far to our knowledge.

Electrophoretic methods are designed for the analysis of charged species because of their nature. The limited solubility of boron cluster species in either aqueous or organic-aqueous background electrolytes was identified as the cause of difficulties encountered in isotachophoretic separations of the compounds [21,22]. In capillary electrophoresis (CE), analytes are injected and detected in submillimolar concentrations. These concentrations are up to three orders of magnitude lower than concentrations of analytes in isotachophoresis. The simplest variant of the technique, CE in free solution, is dominant in electrophoretic methods developed for both achiral and chiral [23,24] analysis of charged organic compounds of low molecular mass within the last decade.

However, application of CE for the analysis of electrophoretically charged cluster species and their derivatives has not yet been reported, so currently

Table 1
List of investigated compounds

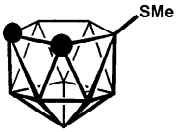
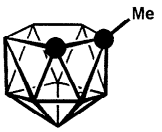
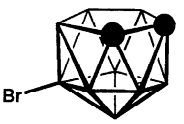
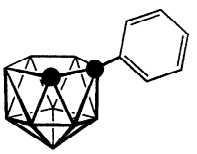
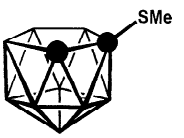
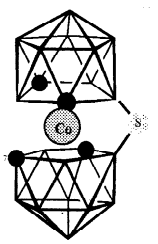
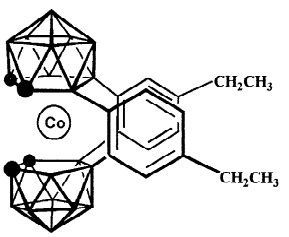
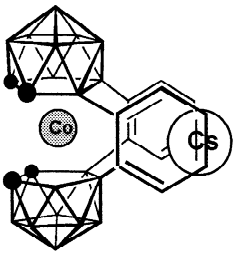
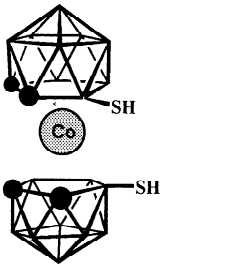
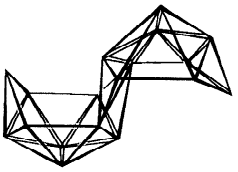
Compound No.	Systematic formula	Structural formula	μ^a	Ref.
1	[*nido*-9-Me-S-7,8-$C_2B_9H_{11}$]$^-$		−29.7	[6]
2	[*nido*-7-Me-7,8-$C_2B_9H_{11}$]$^-$		−32.1	[6]
3	[*nido*-5-Br-7,8-$C_2B_9H_{11}$]$^-$		−31.4	[6]
4	[*nido*-7-Ph-7,8-$C_2B_9H_{11}$]$^-$		−27.8	[6]
5	[*nido*-7-MeS-7,8-$C_2B_9H_{11}$]$^-$		−30.1	[6,7]
6	[*closo*-6,6′-μ-S< $(1,7\text{-}C_2B_9H_{10})_2$-2-Co]$^-$		−22.6	[8]
7	[*closo*-4,8′;8,4′-$(EtPh)_2$-$(1,2\text{-}C_2B_9H_{10})_2$-3-Co]$^-$		−15.5	[9]

Table 1. Continued

Compound No.	Systematic formula	Structural formula	μ^a	Ref.
8	[*closo*-4,8′;8,4′-$(Ph)_2$-$(1,2\text{-}C_2B_9H_{10})_2$-3-Co]Cs		−17.3	[9]
9	[*closo*-4,4′-$(HS)_2$-$(1,2\text{-}C_2B_9H_{10})_2$-3-Co]-		−21.2	[7]
10	[*i*-$B_{18}H_{21}$]$^-$		−29.2	[10]

[a] Mobility in 20 m*M* sodium phosphate buffer pH 7.0 at 25 °C, given in 10^{-9} m^2 V^{-1} s^{-1} units.

there is no information on the electrophoretic behaviour of the compounds in CE systems. Some deviations in the behaviour of boron cluster species in electrophoretic separation systems from that of organic ions have to be expected with respect to the unusual architecture of boron cluster species and to their properties, which result from the architecture. The behaviour of compounds chosen for this study was therefore tested briefly in several common achiral separation CE systems. The minimal capability of liquid chromatography to chirally separate borate cluster anions is documented with a single published communication on the successful chiral splitting of two compounds of the type [18] only and with the failure of several other attempts to separate such anions [25]. This is surprising and, with respect to the existing experience from chiral separations of organic compounds [23,24], hard to understand if the pronounced capability of liquid chromatography to chirally separate zwitterionic cluster boranes is considered.

The main aim of the study was therefore to ascertain the capability of capillary electrophoresis to chirally separate cluster borate anions. A total of nine representatives of three structural types of monovalent anionic cluster dicarbolide anions, namely five derivatives of [*nido*-7,8-$C_2B_9H_{11}$]$^-$, one unbridged Co complex and three bridged Co complexes, and one binary fused-cage borane deprotonized in situ have been chosen for the study (Table 1). The supplementary aim was to obtain information either necessary for or helpful to the proposal of systematic research of chiral separations of borate cluster anions by means of capillary electrophoresis.

This aim was included after the principal capability of capillary electrophoresis to chirally separate borate cluster anions was evidenced.

2. Experimental

2.1. Instrumentation

The 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) for liquid chromatography, adapted for CZE experiments and allowing thermostating of the capillary with circulating water with precision better than ±0.1 °C, was utilised in the study. The wavelength of 254 nm was used for detection. The electrode compartments as well as the paths for filling the compartments, for injecting samples and for flushing of separation capillary were drilled in polyetheretherketon (PEEK) block (DSM Engineering Plastic Products, Tielt, Belgium). The sandwiching of the capillary, placed in a stainless-steel holder, with fused-silica lenses increased the intensity of the measuring light beam to the intensity of the beam that passes through the standard liquid chromatography cell. The concentration of injected analytes was 0.3 m*M* at the most. Uncoated and polyacrylamide coated [26] thin-wall fused-silica capillaries (Capillary Columns, Bratislava, Slovak Republic) of 75 μm I.D. (150 μm O.D.) were thermostated to 24.0 °C. Their separation lengths varied around 50 cm; total lengths were 10.1 cm longer than the separation length. The temperature of the outer capillary wall ensured the temperature inside the capillary was equal to ~25 °C at the Joule heat input of ~0.2 W [27]. This standard temperature was used in the majority of experiments. A temperature of 30 °C was used in tests on the influence of temperature on the course of the chiral discrimination process. The intelligent integrator CSW 1.7 (DataApex, Prague, Czech Republic) served for the measurement of migration times, and for the calculation of peak characteristics common in chromatography.

2.2. Methods and chemicals

Stock buffers (20 m*M*) were prepared by dissolving calculated amounts of orthophosphoric acid or 3-(*N*-morpholino)propanesulfonic acid (MOPS) in freshly boiled distilled water. Sodium hydroxide or tris(hydroxymethyl)aminomethane (Tris) were used to adjust the buffer pH to 7.0. Run buffers were prepared daily. A stock buffer, stored in the fridge, was mixed with an organic solvent (acetonitrile or methanol) if necessary, and β-cyclodextrin was dissolved in the mixture to declared concentration. The same procedure was used if the composition of the run buffer varied. Run buffers were filtered with 0.45-μm microfilter and degassed by sonication before use. Chemicals for buffers, β-cyclodextrin and acetonitrile were from Sigma–Aldrich (Prague, Czech Republic), while methanol was from Lachema (Brno, Czech Republic) and Sigma–Aldrich. Mesityloxide (Sigma–Aldrich) served as the electroosmosis marker in runs in uncoated capillaries. Phtalic acid was the anionic mobility standard if the polyacrylamide coated capillary was used for separations. All chemicals, including those used for other purposes from various sources, were of analytical grade purity.

Boron compounds used for the study were synthesised using generally known procedures [7–10] mostly employing methods developed in Department of Boron Chemistry at the Institute of Inorganic Chemistry [6–9] (Table 1). The synthesised borane *i*-$B_{18}H_{22}$ [10] dissociates in solutions to the anion *i*-$B_{18}H_{21}^-$; borane derivatives were crystallised as potassium or caesium salts. Boron compounds insoluble in water or in aqueous buffers were dissolved in a few drops of acetonitrile and diluted with run buffer to concentrations of ~0.3 m*M* at the most. Samples were injected by the difference in the hydrostatic pressure between the capillary inlet and outlet. The length of the injected zone in the separation capillary, controlled by the injection time, was 2–10 mm.

2.3. Calculation procedures

The asymmetry of migrating zones was calculated as the ratio of rear and front half of the peak read in

0.1 of its height. The direction of the peak migration against the solvent was decisive for the determination of the peak front. The standard equation [28]:

$$\mu_{\text{eff}} = \left(\frac{1}{t_{\text{m}}} - \frac{1}{t_0}\right) \cdot \frac{l_{\text{c}} l_{\text{d}}}{V} \quad (1)$$

served for the conversion of the migration time of an analyte, t_{m}, to its effective mobility, u_{eff}; t_0 is the migration time of the mesityloxide serving as electroosmosis marker, V is applied voltage, l_{c} and l_{d} are the total length of the capillary and its migration distance, respectively. Mobilities and electroosmotic coefficients are given as signed values [24] in 10^{-9} $\text{m}^2\ \text{V}^{-1}\ \text{s}^{-1}$ units. Selectivity of chiral separations, S, carried out in coated capillaries at the effective absence of electroosmosis, was calculated from effective mobilities of the faster and slower migrating forms, μ_1 and μ_2, respectively, using the equation [24,28,29].

$$S = \left| \frac{\mu_1 - \mu_2}{\frac{1}{2}(\mu_1 + \mu_2)} \right| \quad (2)$$

Stoichiometric stability constant for the complexion of an atropisomer of a compound A with β-cyclodextrin, K_{A}, was calculated from effective mobilities of the atropisomer, u_{eff}, measured as the function of the cyclodextrin concentration in run buffer [C], using the equation [30,31],

$$\mu_{\text{A,eff}} = \frac{\mu_{\text{A}} + \mu_{\text{AC}} K_{\text{A}}[C]}{1 + K_{\text{A}}[C]} \quad (3)$$

where μ_{A} and μ_{AC} are mobilities of the free and complexed forms of A in the system, respectively. The CSW 1.7 integrator supplied resolution, R, and separation efficiency, N, calculated in the standard chromatographic way. The respective calculation routines are the constituents of the calculation software.

3. Results and discussion

3.1. Achiral separations

Uncoated fused-silica capillaries have been used in the exploration of the behaviour of various borane cluster anions in separation systems of capillary electrophoresis. Mesityloxide was injected as the electroosmosis marker. Before a change in the composition of the run buffer, the capillary was flushed with 0.1 M NaOH for 30 min and with run buffer for 15 min. Mobility data have been measured after the stabilisation of electroosmosis, which was indicated by the fluctuation of electroosmosis around a mean value.

The compounds listed in Table 1 are such strong acids that they are fully ionised at pH 7.0. Identical mobilities of the compounds at pH 7.0 and 9.0 evidence this. Two commonly used buffers, phosphate and MOPS, were chosen for the test of the influence of the buffer composition on mobilities of investigated boron specie. Both sodium hydroxide and Tris served to adjust their pH to 7.0 because of the reported tendency of boron cluster anions to form ion pairs with bulkier organic cation [2]. Systematically higher mobilities of investigated anions in buffers adjusted with sodium hydroxide in comparison to those in buffers adjusted to the same pH with Tris (Table 2) may be explained by the absence of their ion pairing with sodium cations and by slight ion pairing with the Tris cation; the latter may be expected from Ref. [2]. The dependence of mobility of boron cluster anions on the anion of the run buffer (Table 2) can be explained neither by standard electrophoretic arguments nor from our exploration experiments. The same holds for systematically higher mobilities of borate cluster anions in MOPS buffers compared to those in phosphate buffers, for the marked influence of the buffer composition on the peak shape and on the peak asymmetry (Table 2), for the dependence of the peak shape and peak width of compounds 2 and 3 on the buffer cation in phosphate buffer, as illustrated in Fig. 2 for the case of compound 2, and for other unexpected findings in our study. In MOPS buffers, all analytes exhibited identical shape type disregarding the buffer cation. MOPS buffers have been therefore chosen for the next achiral and chiral experiments.

Explanation of findings listed above requires investigation of the behaviour of boron cluster species in solutions at conditions relevant to electrophoretic analysis. Tailed peaks in Figs. 3–6 (see below) imply that interactions of the borane cluster anions with the capillary wall cannot be a priori

Table 2
The dependence of mobility, μ, given in 10^{-9} m^2 V^{-1} s^{-1} units, and of zone asymmetry, A_s, of investigated compounds on the composition of 20 m*M* run buffers pH 7 at 25 °C

Compound	$Na–H_3PO_4$		$Tris–H_3PO_4$		Na–MOPS		Tris–MOPS	
	μ	A_s	μ	A_s	μ	A_s	μ	A_s
1	−29.7	2.600	−28.0	3.250	−29.9	0.222	−28.3	0.143
2	−32.1	0.094	−32.6	8.000	−33.0	0.091	−30.5	0.060
3	−31.4	0.167	−32.0	6.990	−32.4	0.158	−30.9	0.143
4	−27.8	6.990	−27.0	4.260	−28.1	0.600	−26.2	0.375
5	−30.1	5.320	−30.7	6.490	−30.5	0.133	−29.1	0.121
6	−22.6	0.833	−22.0	6.250	−23.7	1.000	−22.5	0.667
7	−15.5	1.000	−14.8	0.750	−16.8	0.267	−15.6	0.128
8	−17.3	1.500	−16.9	0.890	−19.0	0.667	−17.8	1.000
9	−21.2	2.750	−20.7	2.090	−22.7	2.330	−22.0	1.000
10	−29.2	4.410	−27.7	3.000	−29.0	0.286	−28.0	0.261

$Na–H_3PO_4$: 20 m*M* orthophosphoric acid adjusted with NaOH; $Tris–H_3PO_4$: 20 m*M* orthophosphoric acid adjusted with Tris; Na–MOPS: 20 m*M* MOPS adjusted with NaOH; Tris–MOPS: 20 m*M* MOPS adjusted with Tris.

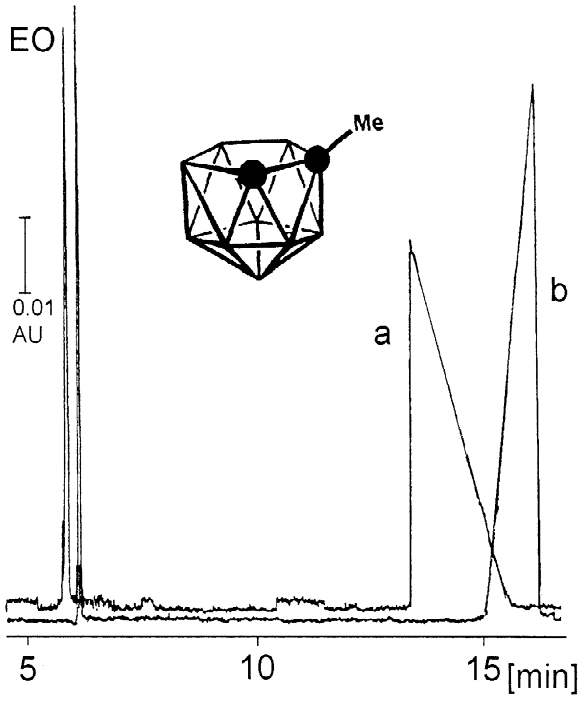


Fig. 2. Standard peak shape of [*nido*-9-MeS-7,8-$C_2B_9H_{11}$]$^-$ in 20 m*M* sodium phosphate buffer pH 7.0 (a) and unusual peak shape of the compound in 20 m*M* Tris–phosphate buffer of the same pH (b). Experimental details: uncoated fused-silica capillary, 401 mm (300 mm separation length)×75 μm I.D.; voltage 12 kV; temperature 30 °C. Electroosmosis marker: mesityloxide.

excluded as a process which participates in the transport of zones of investigated anions in fused-silica capillaries.

The width and asymmetry of zones of single-cage substituted dicarbolide anions (carborates) markedly exceeded those of organic anions of comparable size and charge in purely aqueous solutions, as illustrated in Table 3. Asymmetry of zones of water soluble carborates Nos. 1–3 analysed in aqueous run buffers was independent of the solvent used for the dissolution of these carborates (water, buffer, acetonitrile). The zone of the water-insoluble substituted cobalt bis(dicarbollide) No. 6, dissolved in acetonitrile, was much more symmetric in the aqueous buffers (Table 2). The addition of acetonitrile or methanol to the background electrolyte generally decreased the asymmetry of zones of investigated compounds. Their mobilities in run buffers with acetonitrile or methanol correlate reasonably with the viscosity of water-organic buffers. Acetonitrile proved to be a better additive from the viewpoint of the analysis speed than methanol. For example, migration times of the highly hydrophobic cobalt bis(dicarbollide) 8 were 13.1 and 53.3 min in Tris–MOPS buffer mixed in a 1:1 (v/v) ratio with acetonitrile and methanol, respectively. The reasons are the markedly lower viscosity of the liquid medium with acetonitrile, 0.83 cP, compared to that with methanol, 1.78 cP, and higher electroosmotic coefficient in the acetonitrile-containing background electrolyte, $37.3 \cdot 10^{-9}$ m^2

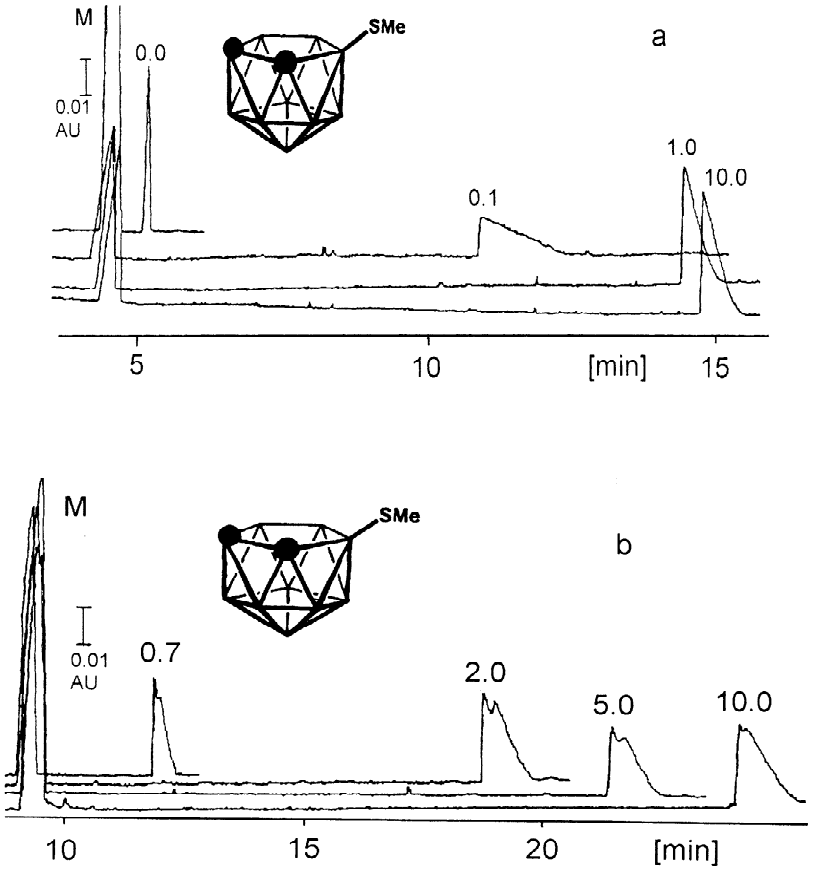


Fig. 3. Chiral separation of [*nido*-9-MeS-7,8-$C_2B_9H_{11}$]$^-$ in 20 m*M* aqueous Tris–MOPS buffer of pH 7 (a) and in the same buffer mixed with methanol in 55:45 (v/v) ratio (b). Concentration of β-cyclodextrin in run buffer in m*M* is given as parameter. M, mobility standard (phtalic acid). Experimental details: polyacrylamide coated capillary 401 mm (300 mm separation length)×75 μm I.D.; voltage 12 kV; temperature 30 °C.

$V^{-1}\ s^{-1}$, compared to $20.4\cdot10^{-9}\ m^2\ V^{-1}\ s^{-1}$ in the methanol-containing one.

The drop in electroosmosis caused by the addition of an organic solvent to the run buffer increases with the solvent concentration. This drop and the opposite directions of cationic electroosmosis and of the migration of anions cause experimental difficulties. Therefore, capillaries coated with chemically bonded polyacrylamide [26], which eliminates electroosmotic flow [26,28], have been preferred in chiral separations.

3.2. Chiral separations

The chiral discrimination process provoked by a chiral selector is principally identical in chromatography and in electrophoresis [24]. The possibility of applying the knowledge from chromatographic experiments to electrophoresis and vice versa was demonstrated with organic analytes several times [32–34]. Water-organic liquids have been used exclusively in chromatographic chiral separations of zwitterionic cluster boranes with chemically bonded β-cyclodextrin [14–19]. This chiral selector is the most popular in electrophoresis, and was therefore chosen for our study. Water-soluble [*nido*-9-MeS-7,8-$C_2B_9H_{11}$]$^-$ (compound 1, Table 1) was chosen for the first check of the capability of capillary electrophoresis to chirally separate boron cluster anions. Its mobility dropped by more than 50% if

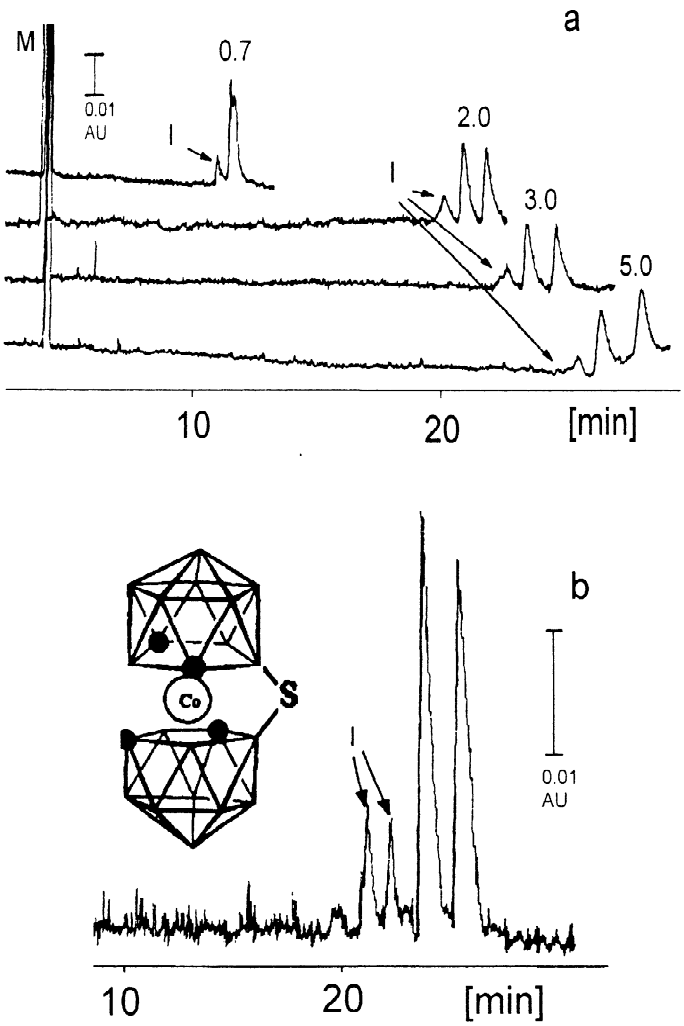


Fig. 4. Chiral separation of [*closo*-6,6′-μ-S<(1,7-$C_2B_9H_{10})_2$-2-Co]$^-$ in 20 m*M* aqueous Tris–MOPS buffer of pH 7 mixed with methanol in 55:45 (v/v) ratio (a) and in the same buffer mixed with acetonitrile in 75:25 (v/v) ratio if 5 m*M* β-cyclodextrin serves as chiral selector (b). Concentration of β-cyclodextrin in run buffer in m*M* is given as parameter in (a). M, mobility standard (phtalic acid). Other experimental details: (a) polyacrylamide coated fused-silica capillary of 401 mm (300 mm separation length)×75 μm I.D.; voltage 12 kV; temperature 30 °C; (b) polyacrylamide coated capillary fused-silica capillary 612 mm (500 mm separation length)×75 μm I.D.; voltage 20 kV; temperature 25 °C.

purely aqueous run buffer contained 0.1 m*M* of β-cyclodextrin. However, no chiral discrimination was observed. Still higher mobility drops have been obtained with β-cyclodextrin concentrations ten and hundred times higher, however, no indication of the expected chiral discrimination of atropisomers of the compound was observed. Mobility drops measured with various concentrations of β-cyclodextrin in run buffer decrease if they are normalized to 1 m*M* concentration of β-cyclodextrin in run buffer. A qualitatively identical result was obtained at an elevated temperature of 30 °C (Fig. 3a), which acts against spontaneous inclusion of analytes in the cavity of cyclodextrins and, consequently, weakens it [24]. These findings imply that the interaction of [*nido*-9-MeS-7,8-$C_2B_9H_{11}$]$^-$ with the β-cyclodextrin cavity is too strong [24]. Organic solvents effectively weaken the hydrophobic interaction of included

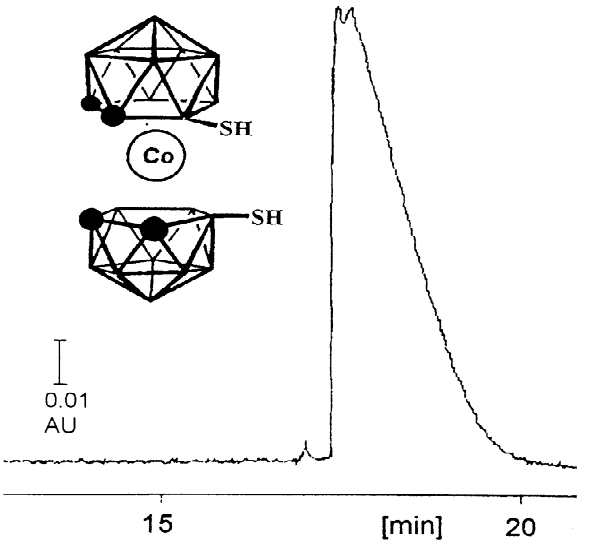


Fig. 5. Chiral separation of [*closo*-4,4′-$(HS)_2$-(1,2-$C_2B_9H_{10})_2$-3-Co]$^-$ with 1 m*M* β-cyclodextrin in 20 m*M* Tris–MOPS buffer of pH 7 mixed with acetonitrile in 75:25 (v/v) ratio. For experimental details, see Fig. 4b.

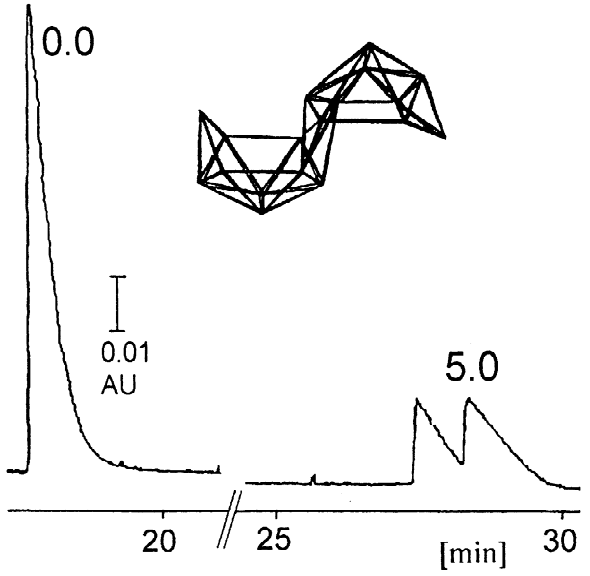


Fig. 6. Chiral separation of [*i*-$B_{18}H_{21}$]$^-$ in 20 m*M* Tris–MOPS buffer of pH 7 mixed with acetonitrile in 70:30 (v/v) ratio. Concentration of β-cyclodextrin (0 or 5 m*M*) given as parameter. For other experimental details, see Fig. 4b.

compounds with the cavity of cyclodextrins [24]. Methanol, which is a good solvent for both β-cyclodextrin and boron cluster species, was tested as such a competing agent first. The retardation of [*nido*-9-MeS-7,8-$C_2B_9H_{11}$]$^-$ caused by β-cyclodextrin was substantially lower if methanol was present in run buffer (Fig. 3b). Its chiral splitting observable with both submillimolar and millimolar β-cyclodextrin indicates that the optimal β-cyclodextrin concentration, which causes the maximum possible difference in effective mobilities of sterically different forms of the analyte, is close to 2 m*M* at 30 °C in 45% methanol. Chiral discrimination of the compound was reached with other concentrations of methanol and with acetonitrile as the competing agent, too (Table 4). This result indicates that methanol or acetonitrile may serve as the constituents of run buffer, which reasonably weakens the interaction of boron cluster anions with β-cyclodextrin cavity. These indications were verified by the chiral splitting of compounds 2–5. These separations, not presented here, and Fig. 3 evidenced that a competing agent is necessary for reaching the chiral discrimination of water-soluble borate cluster anions with analytically reasonable concentrations of β-cyclodextrin.

Sandwich cobalt bis(dicarbollides) are more hydrophobic as evidenced by their insolubility in water. If they are bridged with large hydrocarbonic groups, e.g. compounds 7 and 8 in Table 1, their hydrophobicity is very high. Nevertheless, the content of 25% of acetonitrile in run buffer, which was estimated by the rule of thumb, was sufficient for chiral splitting both the compounds with millimolar concentrations of β-cyclodextrin. An impurity was separated from the compounds with selectivity dependent on the β-cyclodextrin concentration. Cobalt bis-(dicarbollide) complex bridged with sulphur (compound 6 in Table 1) is one of two compounds whose chromatographic chiral splitting is reported [18]. Chiral resolution of sterically different forms of the complex by CE was easy in run buffer containing 45% (v/v) of methanol (Fig. 4a). Faster separation was reached if methanol was replaced by 25% (v/v) of acetonitrile (Fig. 4b). Enantioselectivity of the complex separation decreases slightly in the acetonitrile-containing run buffer (Table 5), however, impurity which behaved as achiral in the run buffer

Table 3
Comparison of the peak asymmetry, A_s, of the migrating zone of [*nido*-7-Me-7,8-$C_2B_9H_{11}$]$^-$ (compound 2 in Table 1) with those of β-indolylacetic acid (IAA) and 2,3-dibenzoyltartatric acid (DBTA) at 30 °C

Buffer	μ_{eof} [a]	IAA		No. 2		DBTA	
		μ	A_s	μ	A_s	μ	A_s
Na–MOPS	56.7	−28.2	0.628	−35.0	0.098	−37.5	0.205
Tris–MOPS	54.7	−27.5	0.500	−34.1	0.093	−36.2	0.345

[a] Electroosmostic coefficient in the run buffer given in 10^{-9} m^2 V^{-1} s^{-1} units.

with methanol, was chirally splitted to baseline with 5 m*M* β-cyclodextrin also (Fig. 4b). This indicates that methanol and acetonitrile affect the separation selectivity of the complex and of its impurity in different ways in addition to the general weakening of their interactions with the β-cyclodextrin cavity.

Rotation of carborane cages along the complex axis cannot be a priori excluded in sandwich complexes without a bridge, which are represented by compound 9. The rotation is expected to eliminate the energetic barrier of the spontaneous racemization and, in this way, to preclude the formation of discrete sterically different forms. The finding if the expectation is correct or not for compound 9 was the main reason for including the compound in the set of investigated compounds. Different from bridged sandwich complexes, low enantioselectivity and, mainly, pronounced peak tailing which causes resolution to deteriorate, have been found with the sandwich complex 9 (Table 1, Fig. 5). Nevertheless, the existence of separable sterically different forms of the compound, evidenced by its observable chiral splitting, reveals that the mutual rotation of carborane cages of the compound is either markedly hindered or even impossible at the used conditions of separation. Moreover, this separation indicates that any bridge markedly increases the separation enantioselectivity of cobalt bis(dicarbollides) and improves their peak shape.

Table 4
Influence of the concentration of β-cyclodextrin, *c*, on separation characteristics of [*nido*-9-Me-S-7,8-$C_2B_9H_{11}$]$^-$ at various concentrations of organic solvents in run buffer

Solvent	°C	*c*	μ	*S*	R_s	*N*
45% MeOH	30	0.0	−18.8	0.000	0.00	298 000
		1.5	−11.1	0.014	0.61	89 000
		3.0	−9.26	0.013	0.47	38 000
		5.0	−8.02	0.015	0.54	42 000
		10	−6.92	0.000	0.00	23 000
60% MeOH	30	1.0	−12.3	0.000	0.00	40 000
		3.0	−11.1	0.013	0.64	106 000
		5.0	−9.53	0.017	0.72	62 000
		7.5	−8.29	0.017	0.54	34 000
25% ACN	25	0.0	−23.5	0.000	0.00	180 000
		1.0	−17.5	0.005	0.27	27 000
		2.0	−15.6	0.008	0.35	25 000
		5.0	−16.1	0.008	0.29	17 000
		10	−12.0	0.006	0.26	24 000

ACN, acetonitrile; μ, electrophoretic mobility in 10^{-9} m^2 V^{-1} s^{-1} units; MeOH, methanol; *N*, number of theoretical plates per meter of capillary for the faster migrating atropisomer; R_s, resolution; *S*, separation selectivity.

Table 5
Influence of the concentration of β-cyclodextrin, *c*, on separation characteristics of [*closo*-6,6′-μ-S<(1,7-$C_2B_9H_{10})_2$-2-Co]$^-$ at various concentrations of organic solvents in run buffer at 25 °C

Solvent	*c*	μ	*S*	*R*	*N*
45% MeOH	0.0	−15.3	0.000	0.00	69 000
	0.7	−10.9	0.009	0.54	107 000
	3.0	−5.98	0.041	2.36	153 000
	5.0	−5.45	0.053	2.15	77 000
	10	−5.40	0.034	1.59	131 000
60% MeOH	1.0	−8.68	0.000	0.00	56 000
	3.0	−7.74	0.014	0.55	69 000
	5.0	−5.60	0.029	1.55	142 000
	7.5	−5.36	0.034	1.08	165 000
25% ACN	0.0	−17.1	0.000	0.00	47 000
	1.0	−12.0	0.023	1.15	72 000
	2.0	−11.7	0.036	2.40	204 000
	5.0	−11.0	0.035	1.62	520 000
	10	−8.29	0.043	1.98	63 000

ACN, acetonitrile; μ, electrophoretic mobility given in 10^{-9} m^2 V^{-1} s^{-1} units; MeOH, methanol; *N*, number of theoretical plates per meter of capillary for the faster migrating atropisomer; R_s, resolution; *S*, separation selectivity.

The fused cage borane (compound 10 in Table 1) differs from the other compounds chosen for our introductory study by the absence of heteroatoms in its molecule. Twisted structure of the compound cage is therefore the only possible source of its asymmetry. Nevertheless, the chiral separation of the compound was easy with 5 m*M* of β-cyclodextrin (Fig. 6). High asymmetry and the tailing of the peak of compound 10 were observed during its analysis in the absence of β-cyclodextrin. This asymmetry survived in the presence of β-cyclodextrin. The same behaviour was observed with substituted single-cage carborates (Fig. 3) and with the cobalt complex without a bridge (Fig. 5). Strictly triangular, narrow peaks free of tailing (Fig. 4b), as required by electrophoretic theory [28,34], have been either obtained or approached if a bridged cobalt sandwich complex was separated in run buffers containing acetonitrile. This implies that the peak symmetry, peak tailing and, consequently, the separation efficiency markedly depend on the compound type and, probably, on the organic solvent used as competing agent (Tables 4 and 5). It is reasonable to expect that solvation effects underly these dependencies.

4. Concluding remarks

The principal aim of the study was to discover the possibility of achieving chiral splitting of expected atropisomers of investigated compounds by means of CE as separation technique. Effects of the separation system composition and of experimental conditions on the separation result have been selectively tested in order to obtain either qualitative and introductory insight in their role in the chiral discrimination process or an indication of in their influence on the process. Research for rigorous comparisons or for generalizations is planned in the next steps. The presented separations are not optimised analytically. Optimisation requires systematic investigation of separations of individual compounds, which is out of scope of the study.

All compounds chosen for the introductory study from readily accessible compounds have been splitted chirally. The splitting was achieved for some compounds in separation systems of different composition and at two temperatures. This evidences that free solution CE is a powerful and highly promising experimental technique for chiral separations of borane cluster anions and of their derivatives. β-Cyclodextrin proved to be widely effective as chiral selector in such separations. These findings are stimulating if the hitherto low success of liquid chromatography in the chiral splitting of cluster boron anions is considered.

Interactions of boron cluster anions with the cavity of β-cyclodextrin are excessively strong in purely aqueous solutions disregarding their solubility in water. A competing agent, which weakens these interactions, must therefore be present in run buffer for the shifting of the optimum concentration of β-cyclodextrin [24,30,31] into the analytically reasonable range of the order of millimoles. Acetonitrile is recommended for this purpose for its general dissolving capability for boranes and their derivatives and for better characteristics of separations in systems with acetonitrile compared to those with methanol.

Equilibrium constants and their differences given in Table 6, which characterise these interactions quantitatively, are of primary importance from both the theoretical and practical points of view. They were calculated from raw data obtained at conditions meeting standard requirements [35,36] as much as possible. The values of constants and their differences listed in Table 6 are comparable with those reported for chiral separations of organic compounds in purely aqueous background electrolytes with cyclodextrins [24,37]. Some trends in listed constants have never been reported with chiral organic compounds, and are seemingly incompatible with existing, generally accepted theoretical concepts. For example, the increase of stability constants for [*nido*-7-Me-7,8-$C_2B_9H_{11}]^-$, [*nido*-5-Br-7,8-$C_2B_9H_{11}]^-$ and [*closo*-4,8′,8′,-$(EtPh)_2$-1,2-$(C_2B_9H_{10})_2$-3-Co]$^-$ with increasing concentration of methanol in background electrolyte has no analogy in published stability constants for organic compounds. The same holds for the differences of stability constants for the slower and faster migrating forms of [*nido*-7-Me-7,8-$C_2B_9H_{11}]^-$ and [*nido*-5-Br-7,8-$C_2B_9H_{11}]^-$. Therefore, constants in Table 6 have to be considered conditional and used with care until these unexpected trends are explained.

Table 6
Stability constants

Compound	Solvent	°C	K_A [a]	ΔK [b]	ΔK (%) [c]
[*nido*-9-Me-S-7,8-$C_2B_9H_{11}$]$^-$	45% MeOH	30	410	28	6.8
	60% MeOH	30	370	18	4.9
	25% ACN	25	670	26	3.9
[*nido*-7-Me-7,8-$C_2B_9H_{11}$]$^-$	45% MeOH	30	490	25	5.1
	60% MeOH	30	860	56	6.5
	25% ACN	25	170	10	5.9
[*nido*-5-Br-7,8-$C_2B_9H_{11}$]$^-$	45% MeOH	30	600	0	0.0
	60% MeOH	30	630	20	3.2
	25% ACN	25	470	0	0.0
[*closo*-6,6′-μ-S<	45% MeOH	30	1820	360	19.8
$(1,7\text{-}C_2B_9H_{10})_2$-2-Co]$^-$	60% MeOH	30	340	16	4.7
	25% ACN	25	760	33	4.3
[*closo*-4,8′;8,4′-$(EtPh)_2$-	45% MeOH	30	430	24	5.6
$(1,2\text{-}C_2B_9H_{10})_2$-3-Co]$^-$	60% MeOH	30	1850	0	0.0
	25% ACN	25	1150	210	18.3

[a] Stability constant for the faster migrating form given in l/mol units.
[b] The difference in stability constants for the slower and faster migrating forms.
[c] The difference in stability constants related to the stability constant of the faster migrating form.

Unusual properties of boron cluster compounds and their unknown interactions with constituents of separation systems are probably behind the unexpected effects and dependencies found in our study. Recognising these properties and interactions is necessary for their explanation. Recognition is also the only way to judge whether and how standards may be reached, which are established in both achiral and chiral CE analysis of organic and common inorganic ions, in the analysis of boron cluster ions also.

Acknowledgements

The research was supported by the Grant Agency of the Czech Republic, grants Nos. 203/99/0044, 203/00/1042, 203/02/0023 and by the Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A-4031703. The article subject was presented in part at the 2nd European Symposium on Boron Chemistry, EUROBORON 2, Dinard, France, 2–6 September, 2001, and at the 2nd International Symposium on Separation in BioSciences SBS 2001, Prague, Czech Republic, 17–20 September, 2001.

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