Chromatographic behaviour of the *closo*-$[B_{12}H_{12}]^{2-}$ derivatives on hydroxyethylmethacrylate gels

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ABSTRACT

Halo derivatives (Cl, Br, I) of the *closo*-dodecahydro-dodecaborate anion, $[B_{12}H_{12}]^{2-}$, and their positional isomers were separated on Separon HEMA hydroxyethylmetacrylate sorbents. Different effects on the separation process, including cations and anions in the mobile phase, the electrolyte concentration in the eluent, pH, addition of organic modifier and separation temperature, were studied. Optimum conditions for the separation were accomplished with Separon HEMA BIO 300 and 1000 sorbents using 0.1–0.5 M sodium perchlorate solutions in 0.01 M phosphate buffer (pH 8.5) as the mobile phase. The separated compounds were detected using direct UV detection in the range 200–210 nm.

INTRODUCTION

Over the last two decades, high-performance liquid chromatography has become an invaluable tool for the separation of non-ionic cage borane compounds [1–3]. However, an adequate chromatographic method for the separation of complex reaction mixtures produced from reactions of boron cage anionic species and purity assay of individual compounds has still been lacking. This especially applies to the family of polyhedral *closo*-hydroborate cage anions $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$ and their derivatives, where a major difficulty lies in obtaining uncontaminated crystals [4].

Salts of the $[B_{12}H_{12}]^{2-}$ anion are non-volatile compounds of reasonable chemical and thermal stability. Taking the symmetrical structure of this anion (point group *Ih*), as depicted in Fig. 1, into account, the possibility of producing an extensive range of derivatives and positional isomers via substitution reactions is evident [5]. Substitution reac-

tions proceeding via an electrophilic or radical mechanism usually produce complex mixtures of various derivatives and their positional isomers, *e.g.*, in halogenation reactions [6].

The main factors controlling the behaviour of these anions are derived from the properties of relatively highly charged small particles (dimensions comparable to the benzene molecule). The *closo*-hydroborate anions exhibit strange solution beha-
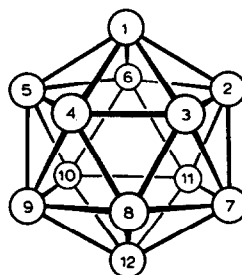

Fig. 1. Structure of the boron framework of the *closo*-$[B_{12}H_{12}]^{2-}$ anion.

viour, and seem to be borderline between inorganic and organic compounds. They behave simultaneously as strong electrolytes (a high degree of dissociation; the free acids have pK_a values comparable to those of the strongest inorganic acids), but they exhibit also hydrophobic properties comparable to those of organic aromatic molecules. The solubility of such compounds in water is therefore caused by the relatively high anionic charge. For comparison, the isostructural and isoelectronic, but uncharged, compounds of the *closo*-dicarbadodecaborane series, $C_2B_{10}H_{12}$, exhibit sufficient solubility only in non-polar solvents. The combination of both controversial properties of the *closo*-hydroborates mentioned above makes mixtures of such compounds unseparable by ordinary separation methods (crystallization, extraction and conventional adsorption and partition chromatography). The separation difficulties outlined above are the main reason for the still limited number of papers dealing with syntheses and properties of pure $[B_{12}H_{12}]^{2-}$ derivatives.

Several separation methods that have so far been developed to separate the latter derivatives are based on thin-layer chromatography [4] and ion-exchange chromatography on DEAE-cellulose [7] or on electromigration methods [8,9]. Only one high-performance liquid chromatographic (HPLC) method has been reported in this area [10], which is based on partition chromatography on silica to allow the separation of the unsubstituted anions $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$. This method seems not to be sufficiently powerful for the separation of complex mixtures of isomers produced by substitution reactions.

The purpose of this study was to employ hydroxyethylmethacrylate-based sorbents to effect the separation of polyhedral *closo*-dodecaborate analytes to investigate the range of separation conditions that could permit a more effective separation of these species.

Based on the ionic character of the compounds under study and their strong hydrophobic behaviour, two approaches can principally be considered to provide routine separations, as follows.

(1) Ion-pair and ion-exchange chromatography [11] can be considered. In ion-pair chromatography, the selection of ion-pairing agents is difficult owing to the limited solubility of alkylammonium salts of the $[B_{12}H_{12}]^{2-}$ anion and its derivatives in water. A more suitable method seems to be ion-exchange chromatography [11], which is a powerful analytical tool for the simple and rapid determination of a wide variety of common inorganic and organic anions. However, only a comparatively small number of such methods have been developed to separate inorganic anions of higher hydrophobicity. Both of these methods are characterized by a decrease in retention with increasing ionic strength of the eluent.

(2) Ion-exclusion chromatography [11] and hydrophobic interaction chromatography [12,13] have been used effectively to separate mixtures of hydrophobic organic anions. In this type of chromatography, the solute retention is enhanced with increasing mobile phase ionic strength.

The broad range of Separon HEMA hydroxyethylmethacrylate sorbents available enabled us to study both types of separation mechanisms on one type of material. The Separon HEMA sorbents have been extensively used for the separation of biochemicals and organic molecules by hydrophobic interaction chromatography [14–16]. They can be modified for ion-exchange chromatographic purposes by including appropriate ion-exchange groups [17–19]. As already demonstrated, unmodified hydroxyethylmethacrylate gels can also serve as a support for the separation of small inorganic anions [20].

For both ion-exchange and hydrophobic interaction chromatographic methods, UV detection should be preferred because of the expected need for a high electrolyte concentration in the eluent, associated with a very high background conductivity preventing conductivity detection. The use of electrochemical detection is also restricted owing to the high effective oxidation potentials of $[B_{12}H_{12}]^{2-}$-type anions. The $[B_{12}H_{12}]^{2-}$ anion absorbs in the UV region below 210 nm, thus limiting separations with direct UV detection to the use of eluents that are sufficiently transparent in this range (aqueous solutions of salts, *e.g.*, perchlorates, sulphates, phosphates and chlorides).

EXPERIMENTAL

Columns

A wide range of CGC (cartridge glass columns)

TABLE I

SURFACE PROPERTIES (FUNCTIONALITY) AND RETENTION MODES OF THE COLUMNS USED

Stationary phase	Particle size (μm)	Functional groups	Ion-exchange capacity (mequiv./g)	Mode[a]
Separon HEMA-S 1000 Q-L	10,12	Quaternary ammonium	0.03–0.1	IEC
Separon HEMA-BIO 1000 CM	7	Carboxy	0.05	IE
Separon HEMA-S 1000	10,12	None	–	HIC
Separon HEMA-BIO 300[b]	10,12,15	None	–	HIC
Separon HEMA-BIO 1000[b]	10	None	–	HIC
Separon SIX C$_{18}$	5	Octadecyl	–	HIC
Separon SIX CN	5	Cyanopropyl	–	HIC

[a] IEC = ion-exchange, IE = ion-exclusion, HIC = hydrophobic interaction chromatography.
[b] Separon HEMA-BIO 300 and 1000 materials differ in pore size; exclusion limits for dextran are 250 000–800 000 and 800 000–2 · 10^6 dalton, respectively. The Separon HEMA-BIO and -S materials differ in the surface content of hydroxy groups.

(150 × 3.3 mm I.D.) packed with Separon HEMA hydroxyethylmethacrylate supports (provided by Tessek, Prague, Czechoslovakia) were used. The columns were packed with (1) low-capacity (0.03–0.1 mequiv./g) sorbents for ion-exchange or ion-exclusion chromatography, including Separon HEMA-S 1000 Q-L (quaternary ammonium groups) and HEMA-BIO 1000 CM (carboxy groups), and (2) supports for hydrophobic interaction chromatography or for the separation of biomolecules of the Separon HEMA-S 1000, HEMA-BIO 300 and HEMA-BIO 1000 types.

Commercial cartridge CGC columns packed with the silica-based materials Separon SIX-CN and Spheron SIX-C$_{18}$, both 5 μm (supplied by Laboratory Instruments, Prague, Czechoslovakia), were also used.

The surface characteristics and retention modes of the columns used are summarized in Table I.

Eluents

Deionized water was used throughout. HPLC-grade acetonitrile was obtained from Fluka (Buchs, Switzerland); and all other reagents were of analytical-reagent grade from Lachema (Brno, Czechoslovakia); and Laborchemie (Apolda, Germany). The mobile phase was prepared by dissolving the calculated amount of the electrolyte either in water or in 0.01 M phosphate buffer with the pH adjusted with aqueous NaOH, or alternatively in a mixed acetonitrile–water solvent prepared in the required vol-

ume ratios. Eluents were filtered through a 0.45-μm filter and degassed under vacuum before use.

Apparatus

A simple isocratic HPLC system was used. The chromatographic equipment consisted of a VCR 40 pulseless dual-piston high-pressure pump, a K 1 six-port sampling valve (with 20- or 50-μl loops) (Development Workshops of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia), an LCD 2040 variable-wavelength (190–360 nm) UV spectrophotometric detector (Laboratory Instruments), a PKS-1 column holder (Tessek) equipped with a heating glass jacket with circulating water from a thermostated bath, a Servogor 2s line recorder (Brown Boveri, Germany) and a CI 100 integrator (Laboratory Instruments).

Sample preparation

Pure derivatives and positional isomers of substituted *closo*-dodecaborate anions were prepared by published methods [7,21,22]. Salts of the unsubstituted $[B_{12}H_{12}]^{2-}$ and $[B_{10}H_{10}]^{2-}$ anions were prepared by conventional methods [23,24]. Free acids or sufficiently water-soluble salts of the $[B_{12}H_{12}]^{2-}$ derivatives, *e.g.*, salts with Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, NH$_4^+$ and N(CH$_3$)$_4^+$ counter cations, were directly injected as aqueous solutions at concentrations ranging from 0.01 to 7 μmol/ml. Sparingly soluble compounds, such as salts with bulky cations, were converted into sufficiently solu-

ble forms by standard ion-exchange techniques or by suitable metathetical reactions before injection. All samples were filtered through a 0.45-μm nylon microfilter (Tessek) before injection.

RESULTS AND DISCUSSION

To optimize the chromatographic procedures, we investigated the effects of the following factors that might affect the separation: (1) surface properties of the supports used; (2) dependence of retention on the concentration of strong electrolytes in the mobile phase; (3) influence of cations and anions present in the mobile phase on the retention of solutes and the separation selectivity of the isomeric dihalo derivatives; (4) influence of pH; (5) effect of the organic modifier (acetonitrile) in the mobile phase; and (6) influence of the separation temperature.

We found that the retentions of the *closo*-borate anions $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$ and their derivatives on differently surface modified sorbents under identical conditions increased in the order Separon HEMA-BIO 1000 CM $\ll$ HEMA-S 1000 $<$ HEMA-BIO 300 $\leqslant$ HEMA-BIO 1000 $\ll$ HEMA-S 1000 Q-L. The very weak retention on Separon HEMA-BIO 1000 CM was apparently affected by electrostatic exclusion of *closo*-borate anions by the carboxy group of the same charge from the polymer surface. The very strong retention was observed on materials with quaternary ammonium group, indicating that an ion-exchange mechanism plays an important role in this particular instance.

The best column efficiency and separation selectivity for the disubstituted 1,2- and 1,7-isomers of the *closo*-$[B_{12}H_{12}]^{2-}$ anion was achieved on Separon HEMA-BIO 300 and 1000. The use of these types of materials enabled us to separate, under appropriately selected conditions, a wide variety of $[B_{12}H_{12}]^{2-}$ derivatives. The separation of a model mixture of monosubstituted species (Fig. 2) demonstrates the efficiency and scope of the method.

The capacity factors of various *closo*-$[B_{12}H_{12}]^{2-}$ derivatives for a series of materials employed for the separation either in pure water or in the presence of different strong electrolytes were also measured and are discussed below.

Except for the material for ion-exchange chromatography (Separon HEMA-S 1000 Q-L), the retention of the *closo*-$[B_{12}H_{12}]^{2-}$ derivatives in pure wa-
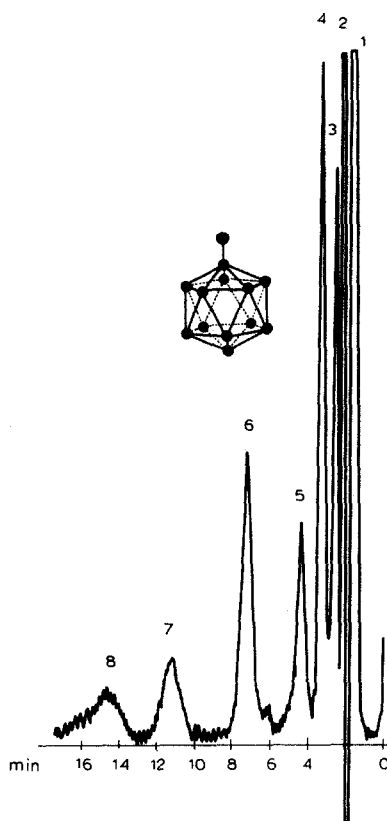

Fig. 2. Separation of *closo*-$[B_{12}H_{12}]^{2-}$ and its monosubstituted derivatives. Column, CGC (150 × 3.3 mm I.D.), Separon HEMA-BIO 300 (12 μm); mobile phase, 0.1 M NaClO$_4$ in acetonitrile–water (10:90); flow-rate, 0.5 ml/min; detection, UV at 205 nm; sensitivity, 0.16 a.u.f.s.; injection volume, 20 μl; temperature of separation, 55°C. Peaks: 1 = $[B_{12}H_{11}OH]^{2-}$; 2 = $[B_{12}H_{12}]^{2-}$; 3 = $[B_{12}H_{11}SH]^{2-}$; 4 = $[B_{12}H_{11}Cl]^{2-}$; 5 = $[B_{12}H_{11}Br]^{2-}$; 6 = $[B_{12}H_{11}I]^{2-}$; 7 = $[B_{12}H_{11}SCN]^{2-}$; 8 = $[B_{12}H_{11}SMe_2]^{-}$.

ter is slightly higher than the column void volume. Nevertheless, the addition of a strong electrolyte to the mobile phase significantly increases the retention of both substituted and unsubstituted $[B_{12}H_{12}]^{2-}$ anions to make their separation possible. The retention of individual derivatives also increases in the order of increasing hydrophobicity of the substituent groups. The retention of $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$ anions and their chloro derivatives increased in the following order of salts used for the preparation of the eluent: perchlorate $<$ chloride $<$ sulphate $<$ sulphosalicylate; Na$^+$ $<$

TABLE II

CAPACITY FACTORS (k') FOR SOME $closo$-$[B_{12}H_{12}]^{2-}$ DERIVATIVES IN THE PRESENCE OF LOW CONCENTRATIONS OF ELECTROLYTES IN AQUEOUS MOBILE PHASES

Column, Separon HEMA-BIO 300 (12 μm); detection, UV at 200 nm (indirect at 289 nm for sulphosalicylate).

Anion	Electrolyte concentration (mmol/l)													
	NaCl				KCl		NH$_4$Cl				Na$_2$SO$_4$		NaSul[a]	
	5	10	20	100	5	10	1	2.5	5	10	2.5	5	2.5	5
$[B_{12}H_{12}]^{2-}$	0.88	1.33	2.50	5.50	1.33	1.60	2.17	5.50	5.83	7.33	1.17	1.30	1.08	1.16
$[B_{12}H_{11}Cl]^{2-}$	2.08	3.66	8.83	—	4.50	6.67	8.00	12.7	—	—	2.67	3.50	2.50	3.66
$[B_{12}H_{11}Br]^{2-}$	5.33	8.67	—	—	—	—								
$[B_{12}H_{10}Cl_2]^{2-}$	9.0	23.5	—	—	13.0	—	15.0	—	—	—	8.17	20.5	9.33	16.0

[a] Sodium sulphosalicylate.

TABLE III

CAPACITY FACTORS (k') OF $closo$-BORATE ANIONS $[B_{10}H_{10}]^{2-}$ AND $[B_{12}H_{12}]^{2-}$ AND HALOGENO DERIVATIVES WITH VARIOUS CONCENTRATIONS OF SODIUM PERCHLORATE IN THE MOBILE PHASE

Column, Separon HEMA-BIO 300 (12 μm); detection, UV at 200 nm.

Anion	Concentration of NaClO$_4$ (mmol/l)							
	0	5	10	20	50	100	250	500
$[B_{10}H_{10}]^{2-}$	0.17	0.17	0.17	0.17	0.25	0.33	0.25	0.25
$[B_{12}H_{12}]^{2-}$	0.58	0.75	1.17	1.38	1.62	1.66	—	1.17
$[B_{12}H_{11}Cl]^{2-}$	1.00	1.83	4.33	4.83	5.16	5.25	—	3.33
$[B_{12}H_{11}Br]^{2-}$	1.33	3.16	6.33	7.66	8.45	8.63	—	4.38
1,7-$[B_{12}H_{10}Cl_2]^{2-}$	2.00	8.83	14.5	21.2	24.7	22.3	21.0	10.54
1,7-$[B_{12}H_{10}Br_2]^{2-}$	2.66	21.0	—	—	—	—	—	29.6

TABLE IV

CAPACITY FACTORS (k') OF $closo$-BORATE ANIONS $[B_{10}H_{10}]^{2-}$ AND $[B_{12}H_{12}]^{2-}$ AND CHLORO DERIVATIVES FOR VARIOUS CONCENTRATIONS OF SODIUM PERCHLORATE IN THE MOBILE PHASE

Column, Separon HEMA-S 1000 Q-L (12 μm), capacity 0.05 mequiv./g; pH of the eluent, adjusted to 6.1 with 0.01 M phosphate buffer; detection, UV at 200 nm.

Anion	Concentration of NaClO$_4$ (mmol/l)				
	5	50	250	500	750
$[B_{10}H_{10}]^{2-}$	2.43	1.10	1.00	0.29	0.29
$[B_{12}H_{12}]^{2-}$	7.28	2.57	1.29	0.71	0.71
$[B_{12}H_{11}Cl]^{2-}$	—	9.00	3.60	1.57	1.43
1,7-$[B_{12}H_{10}Cl_2]^{2-}$	—	—	7.00	4.57	4.43

$K^+ < NH_4^+$. It was also found that sodium salts generally tend to exhibit a better selectivity than potassium and ammonium salts. The capacity factors of some solutes for low-concentration electrolytes (except for $NaClO_4$) for the Separon HEMA-BIO 300 column are summarized in Table II. It should be noted that concentrations of electrolytes below 0.1 M caused the peaks to be highly asymmetric.

The best separation selectivity for the 1,2- and 1,7-positional isomers of $[B_{12}H_{10}X_2]^{2-}$ (X = halogen) on all the materials used was achieved with $NaClO_4$ in the mobile phase. The capacity factors measured in mobile phases containing different molarities of this salt on Separon HEMA-BIO 300 and HEMA-S 1000 Q-L columns are shown in Tables III and IV. As already outlined above, concentrations below *ca.* 0.1 M caused an asymmetric shape of the peaks. The retention on Separon HEMA-BIO 300, 1000 and HEMA-S 1000, which were not modified by ion-exchange groups, increased with increasing concentration of $NaClO_4$ in the mobile phase in the approximate range 0.15–0.20 M. Further increases in the concentration caused only a slight decrease in retention and a slight increase in column efficiency.

The capacity factors on Separon HEMA S 1000 Q-L decreased monotonously as the $NaClO_4$ concentration in the eluent increased. However, the decrease in retention was unexpectedly small enough to be explained by an ion-exchange mechanism alone. From this fact together with the above-mentioned retention behaviour on the parent material Separon HEMA-S 1000 having no ion-exchange capacity, it was concluded that a mixed ion-exchange and hydrophobic interaction mechanism is probably involved in the separation on Separon HEMA S 1000 Q-L; the electrolyte concentration of *ca.* 0.1 M represents a boundary for a predominant ion-exchange mechanism.

Changes in the eluent pH in the range 2–10 had only a slight effect on the retention of *closo*-$[B_{12}H_{12}]^{2-}$ derivatives.

Mobile phases containing $NaClO_4$ at a constant concentration of 0.1 M were used to study the influence of the acetonitrile concentration in the mobile phase on the capacity factors of *closo*-$[B_{12}H_{12}]^{2-}$ derivatives on Separon HEMA-BIO 300. As shown in Fig. 3, the log k' values decrease approximately
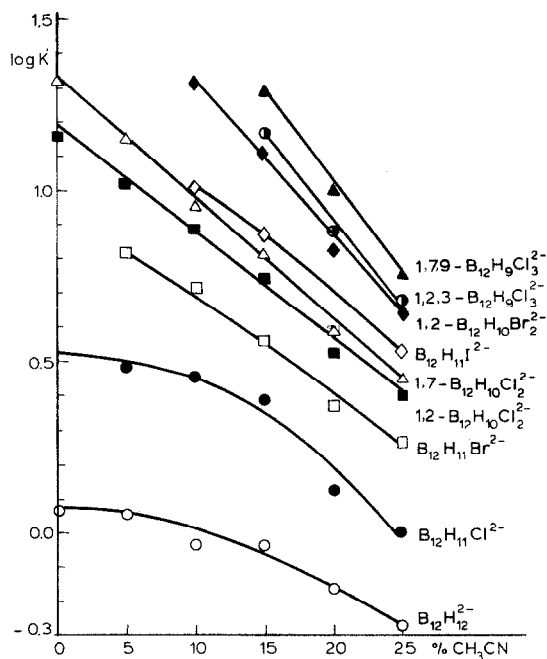

Fig. 3. Dependence of the capacity factors of the anions on the content of the organic modifier (acetonitrile) in the mobile phase (0.1 M $NaClO_4$). Column, Separon HEMA-BIO 300 (12 μm); detection, UV at 205 nm.

linearly with increasing acetonitrile concentration in the mobile phase, except for some species with log k' values below *ca.* 0.5. A significant decrease in k' values was observed for strongly retained species. Higher acetonitrile contents cause a slight decrease in the resolution of positional isomers, namely of the isomeric chlorinated anions $[B_{12}H_{10}Cl_2]^{2-}$ and $[B_{12}H_9Cl_3]^{2-}$. On the other hand, the presence of this organic modifier increased the peak symmetry for strongly retained species.

Plots of log k' *versus* $1/T$ in the temperature range of 25–80°C on colums packed with Separon HEMA-S 1000 Q-L and HEMA-BIO 300 are depicted in Figs. 4 and 5. The results suggest an apparent decrease in the retention of solutes with increase in temperature. Temperature has a negative effect on the resolution of isomers; at elevated temperatures, however, the increase in column efficiency due to the lower mobile phase viscosity competes with this effect. The appropriate k' values for the most strongly retained *closo*-$[B_{12}H_{12}]^{2-}$ anion derivatives, *e.g.*, iodo and rhodano derivatives, or deriv-
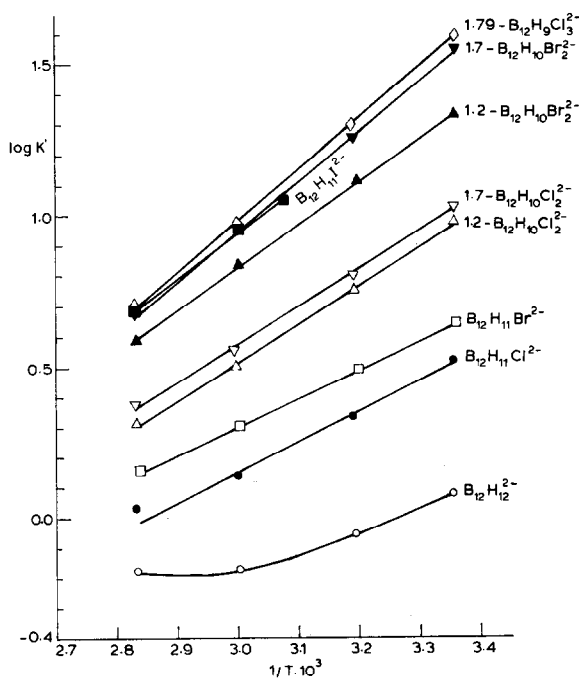
Fig. 4. Plots of the capacity factors (k') of the closo-[B$_{12}$H$_{12}$]$^{2-}$ derivatives as a function of 1/T. Column, Separon HEMA-BIO 300 (12 μm); mobile phase, 0.5 M NaClO$_4$ in 0.01 M phosphate buffer (pH 8.5); detection, UV at 205 nm.
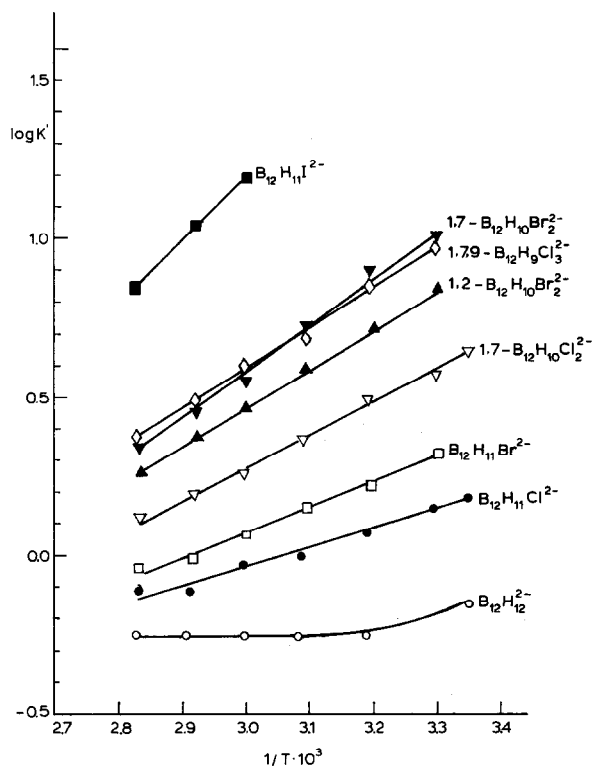

Fig. 5. Plots of the capacity factors (k') of the closo-[B$_{12}$H$_{12}$]$^{2-}$ derivatives as a function of 1/T. Column, Separon HEMA-S 1000 Q-L (12 μm); mobile phase, 0.5 M NaClO$_4$ in 0.01 M phosphate buffer (pH 8.5); detection, UV at 205 nm.

atives with a greater number of substituents, can be adjusted either by increasing the temperature or by adding an organic modifier.

The results of the study of the dependence of the retention properties of the [B$_{12}$H$_{12}$]$^{2-}$-type anions on Separon HEMA materials on both the concentration of salts and the organic modifier in the mobile phase, together with the temperature dependence of the capacity factors, suggest that the separations on materials that do not contain ion-exchange groups were controlled by hydrophobic interactions of the solutes with the polymer surface. This behaviour seems to parallel, to some extent, that of aromatic sulphonic acids in chromatography on octadecylsilica in the presence of an electrolyte in aqueous or aqueous–organic mobile phases [12,13]. In contrast, both the retention and the selectivity are strongly dependent on the nature of the electrolyte used.

In order to understand this factor better, a study of the retention behaviour of the closo-[B$_{12}$H$_{12}$]$^{2-}$ derivatives on the ordinary revesed-phase silica-

based Spheron SIX-C$_{18}$ and SIX-CN was also performed. A similar influence of the nature of the electrolyte in the eluent was also observed. However, in contrast to Separon HEMA sorbents, concentrations of NaClO$_4$ in the range 0.1–0.5 M are low enough to effect a sufficient retention of hydroborate anions on this type of material. A mobile phase consisting of 0.1–0.5 M Na$_2$SO$_4$ with a higher salting-out effect must be used to achieve appropriate values of the capacity factors and a higher resolution of positional isomers. However, poor selectivity and peak shapes were observed on the octadecylsilica material. The selectivity achieved on the cyanopropylsilica with Na$_2$SO$_4$ solutions as eluent for mixtures of positional isomers exhibiting similar properties (such as positional isomers of chloro derivatives) is better but still poorer than that obtained using Separon HEMA systems with NaClO$_4$ as eluent.
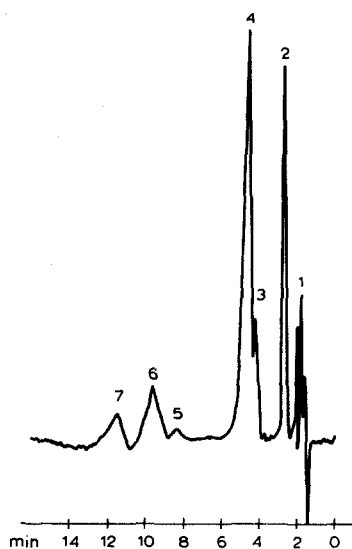
Fig. 6. Separation of the products of the chlorination of $Na_2B_{12}H_{12}$ in aqueous solution with chlorine–nitrogen mixture (2 vol.% of chlorine). Column, CGC (150 × 3.3 mm I.D.), Separon HEMA-BIO 300 (12 μm); mobile phase, 0.5 M NaClO$_4$ in 0.01 M phosphate buffer (pH 8.5); detection, UV at 205 nm; sensitivity, 0.16 a.u.f.s.; flow-rate, 0.5 ml/min; injection volume, 20 μl; temperature of separation, 60°C. Peaks: 1 = $[B_{12}H_{12}]^{2-}$; 2 = $[B_{12}H_{11}Cl]^{2-}$; 3 = 1,2-$[B_{12}H_{10}Cl_2]^{2-}$; 4 = 1,7-$[B_{12}H_{10}Cl_2]^{2-}$; 5 = isomer of $[B_{12}H_9Cl_3]^{-}$, unknown structure; 6 = 1,2,3-$[B_{12}H_9Cl_3]^{2-}$; 7 = 1,7,9-$[B_{12}H_9Cl_3]^{2-}$.

The selectivity of the system with Separon HEMA-BIO 300 using 0.1–0.5 M NaClO$_4$ is sufficient to achieve the separation of a number of *closo* derivatives of the general formula $[B_{12}H_{12-n}X_n]^{2-}$ (for the halo derivatives, n = 1–6 for chloro, 1–4 for bromo and 1–3 for iodo derivatives). The separation efficiency of the method described is sufficient, at least for resolving the positional isomers of di- and trisubstituted derivatives of the $[B_{12}H_{12}]^{2-}$ anion.

Figs. 6–8 summarize practical examples of the separation of real mixtures of isomeric halo derivatives of the *closo*-$[B_{12}H_{12}]^{2-}$ anion resulting from various substitution reactions. Fig. 6 shows the separation of a mixture of derivatives and positional isomers of di- and trisubstituted anions prepared by electrophilic chlorination under mild conditions. Fig. 7 is a chromatogram of a mixture that is more abundant in positional isomers of chloro derivatives arising from the high-temperature reaction of
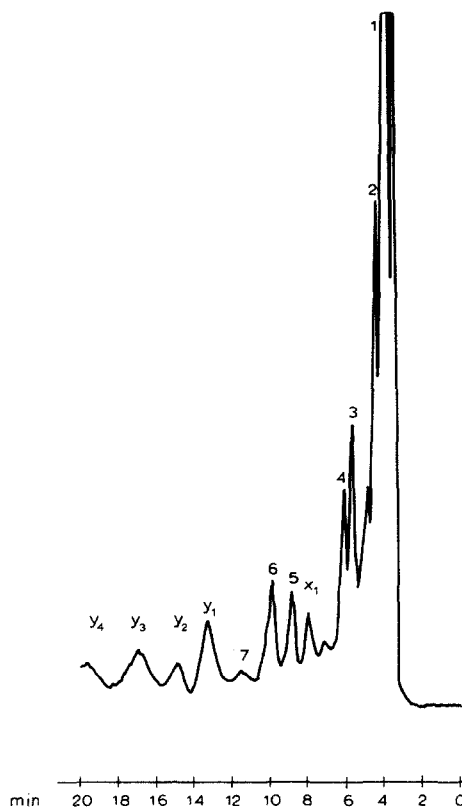

Fig. 7. Separation of the products of the high-temperature chlorination of solid $Na_2B_{12}H_{12}$ with gaseous BCl_3-H_2. Chromatographic conditions as in Fig. 6, except temperature of separation (80°C). Peaks: 1 = $[B_{12}H_{12}]^{2-}$; 2 = $[B_{12}H_{11}Cl]^{2-}$; 3 = 1,2-$[B_{12}H_{10}Cl_2]^{2-}$; 4 = 1,7-$[B_{12}H_{10}Cl_2]^{2-}$; 6 = 1,2,3-$[B_{12}H_9Cl_3]^{2-}$; 7 = 1,7,9-$[B_{12}H_9Cl_3]^{2-}$; 5, x_1 = $[B_{12}H_9Cl_3]^{2-}$, undetermined structure; y_1-y_4 = anions of formula $[B_{12}H_{12-n}Cl_n]^{2-}$ with $n > 3$.

solid $Na_2B_{12}H_{12}$ with boron trichloride–hydrogen mixture. Fig. 8 exemplifies a separation of a mixture of iodo derivatives, the retention of which is considerably higher than that of chloroderivatives. This can be, simply separated, however, with an aceto-nitrile-containing mobile phase. The chromatograms in Figs. 2 and 6–8 demonstrate that both the separation efficiency and peak symmetry are acceptable.

CONCLUSIONS

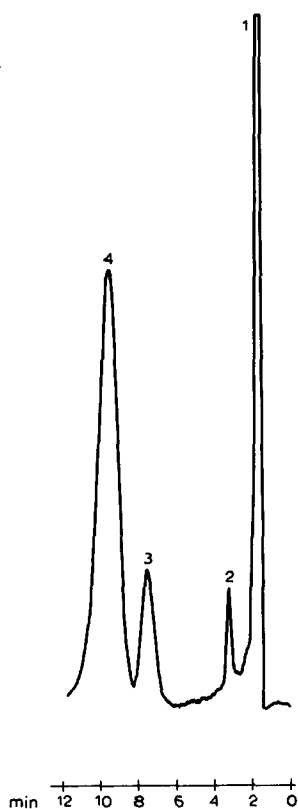The chromatographic separation of a wide range of *closo*-$[B_{12}H_{12}]^{2-}$ derivatives can be performed

Fig. 8. Separation of the mixture of products of iodination of $Na_2B_{12}H_{12}$ in aqueous solution with iodine in CCl_4. Chromatographic conditions as in Fig. 2, except acetonitrile content in the mobile phase (25 vol.%) and temperature of separation (45°C). Peaks: 1 = I^-; 2 = $[B_{12}H_{11}I]^{2-}$; 3 = 1,2-$[B_{12}H_{10}I_2]^{2-}$; 4 = 1,7-$[B_{12}H_{10}I_2]^{2-}$.

Fig. 9. Separation of the the closo-hydroborate anions $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$. Column, Separon HEMA-S 1000 Q-L (12 μm) (capacity 0.1 mequiv./g); mobile phase, 0.1 M $NaClO_4$ in 0.01 M phosphate buffer (pH 8.5); flow-rate, 0.55 ml/min; detection, UV at 205 nm; sensitivity, 0.32 a.u.f.s.; injection volume, 20 μl. Peaks: 1 = $[B_{10}H_{10}]^{2-}$; 2 = $[B_{12}H_{12}]^{2-}$.

using Separon HEMA-BIO 300 hydroxyethylmethacrylate material and aqueous solutions of sodium perchlorate as the mobile phase. The retention and selectivity can be controlled to some extent by adjusting the concentration of the salt, separation temperature and, in some instances, the acetonitrile content in the mobile phase. The mechanism of separation is governed by the hydrophobic interaction of the closo-$[B_{12}H_{12}]^{2-}$ framework and its substituted groups with the polymer surface when a strong electrolyte is present in the mobile phase. The influence of the nature of the electrolyte used was observed.

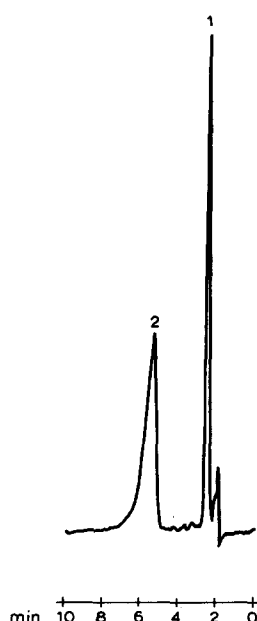Ion-exchange chromatography on materials modified with quaternary ammonium groups (Separon HEMA-S 1000 Q-L) was found to be less suitable for the separation of halo derivatives because of their very strong retention and the involvement of a mixed separation mechanism causing peak tailing. In contrast, such a method seems to be more suitable than hydrophobic interaction chromatography for the separation of less hydrophobic anions, e.g., the unsubstituted anions $[B_{12}H_{12}]^{2-}$ and $[B_{10}H_{10}]^{2-}$ (Fig. 9) and hydroxy and fluoro derivatives of the $[B_{12}H_{12}]^{2-}$ anion. The method presented seems to offer a very simple and powerful tool for the analysis of a number of the mixtures of closo-hydroborate anions arising from synthetic work, and for purity assay of closo-hydroborate species.

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