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Ion-exchange properties of hypercrosslinked polystyrene impregnated with methyl orange

Maria G. Kiseleva, Lubov' V. Radchenko, Pavel N. Nesterenko*

Division of Analytical Chemistry, Lomonosov Moscow State University, Vorob'evy Gory GSP-3, Moscow 119899, Russian Federation

Abstract

A novel bipolar stationary phase (HCPS–MO) was prepared by impregnation of hypercrosslinked polystyrene (HCPS) with methyl orange (MO; 4-dimethylamino-4′-sulfoazobenzene) and its ion-exchange properties were studied. Simultaneous separation of cations and anions on HCPS–MO is possible, although it behaves preferentially as a cation-exchanger. Unusual selectivity of HCPS-MO for alkali and alkaline-earth metal cations: $Na^+ < Li^+ \sim K^+ < Rb^+ < NH_4^+ < Cs^+$ and $Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+}$ was observed. The effect of temperature on retention of alkali and alkaline-earth metal cations was studied. Separation of Na^+, K^+, Rb^+, NH_4^+, Cs^+, Mg^{2+} and Ca^{2+} on HCPS–MO with diluted cerium(III) nitrate solution as an eluent in single run is presented. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Polystyrene–methyl orange stationary phase; Methyl orange–polystyrene stationary phase; Stationary phases, LC; Alkali metals; Alkaline–earth metals; Metal cations; Inorganic anions; Inorganic cations

1. Introduction

The presence of oppositely charged ion-exchange cites at the surface of stationary phase results in ion-exchangers with several advantageous properties. First of all, mass transfer characteristics of such stationary phases are usually superior to those of mono charged ion-exchangers. Second, bipolar stationary phases provide additional possibility to vary ion-exchange separation selectivity. Sample ion is subjected to simultaneous attraction and repulsion from bipolar stationary phase, the ratio of these forces determines separation selectivity. Simultaneous influence of opposite forces results in weaker retention of sample ions on bipolar stationary phases in comparison with traditional monofunctional stationary phases. It means, that very diluted or even pure water can be used as eluent providing not only efficient elution and separation of ions but also a reasonable enhancement of conductimetric detection sensitivity.

Different types of bipolar stationary phases were well reviewed recently by Nesterenko and Haddad [1]. According to the authors the most efficient way to realize the benefits of bipolar stationary phases is to combine oppositely charged functional groups in a single molecule which is immobilized onto the surface of stationary phase. Covalent attachment of bipolar molecules to matrix is a very complex task. Yu and Hartwick were the first to synthesize silica-bonded sulfobetaine [2] and used it for selective separation of ampholyte molecules such as peptides,

*Corresponding author. Division of Analytical Chemistry, Lomonosov Moscow State University, Lenin Hills, Moscow, 109028, Russian Federation. Tel.: +7-095-9394416; fax: +7-095-9394675.

E-mail address: paveln@analyt.chem.msu.ru (P.N. Nesterenko).

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nucleosides, and amino acids in accordance with so-called «quadrupolar retention mechanism». Tramposh and Weber studied the chromatographic properties of silica-bonded sulfobetaine under normal-phase HPLC conditions [3]. However, there was no evidence for zwitter-ionic behavior of prepared stationary phase. Jiang and Irgum studied retention of inorganic anions and cations on polymer bonded sulfobetaine [4]. Silica bonded amino acids were also demonstrated as bipolar stationary phases [5] unless the charge of functional groups is pH dependent.

Dynamic modification of ODS with bipolar surfactants is a simple method to obtain variety of bipolar stationary phases. Suitable surfactants are CHAPS {3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate}, CHAPSO {3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate}, Zwittergents (*N*-alkyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate), sodium taurodeoxycholate and sodium taurocholate [6]. To increase stability of dynamically modified stationary phases it is desirable to use additionally hydrophobized ODS columns. Ability of surfactant molecules to move in the bonded layer of ODS could result in formation of intermolecular associates and inner salt structures, which commit some kind of ambiguity to results.

Another class of bipolar molecules suitable for dynamic modifiers is aromatic dyes, containing acidic (sulfo- or carboxy-) group and amino group. Aromatic rings in the dye molecule provide their strong fixation on a surface of neutral polystyrene resins that makes polystyrene–divinylbenzene (PS–DVB) substrates more suitable support for dynamic modification than ODS. Dye impregnated PS–DVB resins were used in anion chromatography [7] and in chelation ion chromatography of trace metals [8,9].

Methyl orange (MO) dye can be used for preparation of bipolar stationary phase. MO molecule contains strong acidic sulfo-group, tertiary amino group protonated at pH below 3 and two benzene rings, providing high affinity to polystyrene resin. Jones and Schwedt found MO impregnated resin unstable due to continuous leaking of dye from column [10]. A novel support for impregnation — hypercross-linked polystyrene (HCPS) — is proposed in this paper. HCPS was found to have extremely high affinity to aromatic compounds. Retention increment for a one phenyl group for HCPS was calculated in 2.5 times higher then for poly(styrene–divinylbenzene) resin with 60% crosslinking degree and in 3.6 times higher then for alkylsilica [11].

The aim of this paper is to present the study of ion-exchange properties of a novel bipolar stationary phase prepared by an impregnation of hypercross-linked polystyrene with methyl orange.

2. Experimental

2.1. Apparatus

The isocratic HPLC system consisted of model 101A pump, model 210 injection valve (both from Altex, Berkeley, CA, USA) equipped with a 100-μl sample loop, HPLC column oven (CBO Industrial Electronics, Vienna, Austria) and model 2238 Uvicord photometric detector (LKB, Bromma, Sweden) set at 254 nm for indirect detection of cations and at 405 nm for detection of methyl orange. Two stainless steel columns (150×4.6 mm I.D. and 250×4.6 mm I.D.) packed with 5 μm hypercrosslinked polystyrene (Purolite, Pontyclun, UK) were used. In accordance with chromatographic data [11] HCPS has biporous structure consisting of micropores of diameter around 1.1 nm and macropores of an average diameter 100 nm. The micropores dominate in sorbent structure resulting in very high specific surface area of about 1500 m^2/g as it was measured by mercury porosimetry.

2.2. Reagents

Acetonitrile (HPLC grade, E. Merck, Darmshtadt, Germany), solutions of cerium (III) nitrate (analytical grade, Reakhim, Novosibirsk, Russia), nitric acid (pure for analysis, Reakhim, Russia) in distilled water were used for preparation of mobile phases. For stationary phase impregnation was used methyl orange (pure, Reakhim, Moscow, Russia) aqueous solution. All tested compounds were of analytical grade.

2.3. Preparation of HCPS–MO

Methyl orange aqueous solution (16 m*M*) was run through the column until saturation detected as

constant absorbance at 405 nm. The amount of methyl orange adsorbed by the column under these conditions was calculated by monitoring the absorbance of the coating streams and measuring 50% breakthrough point. It proved to be 1.54 mmol of MO per 150×4.6 mm I.D. column. This value is above real ion-exchange capacity of the column. Excess of MO was washed by 1 m*M* $Ce(NO_3)_3$ and with eluent to be used further. Ion-exchange capacity was calculated as quantity of MO washed from the column after all experiments were finished, its concentration was found to be 0.31 mmol per 150×4.6 mm I.D. column.

3. Results and discussion

3.1. Retention of MO on HCPS

Stability of dynamic coating of HCPS by MO can be characterized with the hypothetical value of capacity factor of MO retention on HCPS in pure water as a mobile phase (k'_w). The effect of acetonitrile concentration in the eluent on retention of MO on the HCPS was studied to evaluate k'_w. The dependence of retention ($\log k'$) on the concentration of MeCN (C, vol. %) is well described by the following equation:

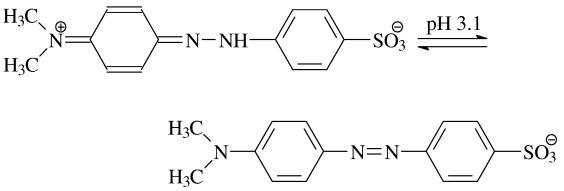
$$\log k' = 3.143 - 0.204C + 0.003C^2$$

$$r = 0.984 \quad n = 6, \; P = 0.95$$

According to Jandera [12], when $C=0$ $\log k' = \log k'_w$, hence capacity factor of MO retention on HCPS with water as a mobile phase is equal to $k'_w = 1.3\times10^3$. The affinity of HCPS to MO is very high and hence the dynamic coating of polymeric phase is expected to be enough stable. So, it is not necessary to add reagent into the eluent for stabilization of coating.

3.2. The effect of pH on retention

Depending on the eluent pH molecules of MO adsorbed on the surface of HCPS can exist in zwitterionic or anionic form. In homogeneous solution the equilibrium transition from one form to another takes place at pH about 3.1:



Thus at pH below 3 HCPS–MO can be both cation and anion-exchanger, while at pH over 3 it is cation-exchanger mainly.

Several acids, such as perchloric, sulfosalicylic and citric, were tried as eluents for separation of inorganic anions and cations on HCPS–MO. Organic acids caused visible bleeding of MO from the column, conductimetric detection with perchloric acid eluent was not satisfactory due to appearance of system peak which caused distortion of adjacent chromatographic peaks. Utilization of Ce(III) nitrate–nitric acid eluents with photometric detection ($\lambda=254$ nm) appeared to be the most appropriate. Leaking is minimized and has no effect on column reproducibility, as it is shown below. Ce(III) is a strong eluting cation and makes possible simultaneous separation of alkali and alkaline-earth metal cations [13].

The effect of eluent pH on retention was studied for ammonium, alkali metal cations and several anions (Fig. 1A) and alkaline-earth metal cations (Fig. 1B). As it was expected, retention of cations increases and anions decreases with pH. At pH below 3 HCPS–MO was expected to retain both cations and anions. However, retention of anions, excluding SCN^- is low and even in zwitterionic form HCPS–MO is preferentially a cation-exchanger. Retention of cations, especially of alkaline-earth metals, increases in pH range from 2 to 4, but at pH over 4 the increase becomes less pronounced, as far as adsorbed reagent does not change its ionic state.

3.3. Effect of eluent concentration

Effect of eluent concentration on retention of metal cations was studied at pH 5, when HCPS-MO behaves as cation-exchanger. Uncommon elution

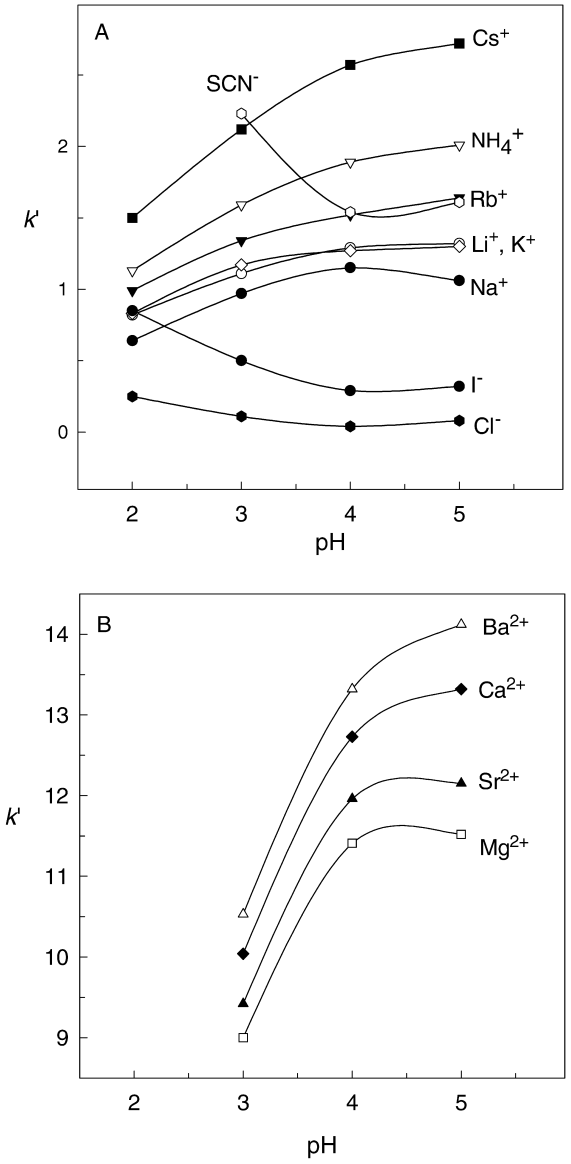


Fig. 1. The effect of mobile phase pH on retention of Cl^-, I^- and SCN^- anions, ammonium and alkali metal cations (A) and alkaline-earth metal cations (B) on HCPS–MO. Eluent: 0.3 m*M* $Ce(NO_3)_3$, HNO_3.

order was observed for ammonium, alkali and alkaline-earth metal cations:

$$Na^+ < Li^+ \sim K^+ < Rb^+ < NH_4^+ < Cs^+ \text{ and}$$

$$Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+}$$

Usually elution order of these cations on sulfonated polystyrene type resins is as following [14]:

$$Li^+ < Na^+ < NH_4^+ < K^+ < Rb^+ < Cs^+ \text{ and}$$

$$Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$$

The orders of elution are the orders of decreasing hydration.

Specific matrix properties could be the reason for the increased retention of Li^+, NH_4^+ and Ca^{2+} cations on HCPS–MO. Several hypotheses can be proposed. First, retention of cations can be governed by two different mechanisms: electrostatic attraction and size exclusion. HCPS has a biporous structure with approximately 1 nm micropores dominating [11]. Electrostatic attraction force increases with the decrease of hydrated ion radii in the rows of alkali and alkaline-earth metal cations from Li^+ to Cs^+ and from Mg^{2+} to Ba^{2+}. On the contrary, ability to penetrate in pores decreases in the above rows. The sum of these two trends results in unusual elution order. Hydrated radius for ammonium cation is not known the reason for its elution between Rb^+ and Cs^+ can be size exclusion also. Presence of complexing metals as impurities in the matrix could also increase ammonium retention due to complex forming.

Another hypothesis explaining strong retention of Li^+ and NH_4^+ cations could be their specific affinity to matrix. It is known, that HCPS contains about 6 mass % of oxygen atoms which can form crown ether-like structures in the micro pores. These structures may cause uncommonly strong retention of Li^+ and NH_4^+. If any of the above hypotheses is correct, unmodified HCPS should also retain Li^+ and NH_4^+ stronger than other cations. However, experiment did not verify this supposition, all cations eluted with void volume. It should be noted, that ion-exchange selectivity of sulfonated HCPS is very similar to that of HCPS–MO. Elution order of alkali metal cations on sulfonated HCPS with diluted nitric acid as eluent was following: $Na^+ < Li^+ < K^+ < NH_4^+ < Rb^+ < Cs^+$ [15].

Thus, uncommon elution order can not be attributed to the properties of impregnated molecule. From the other hand, the bare matrix does not retain cations. The only way out is to assume cumulative effect of cation-exchange groups and matrix. First,

crown-ether like structures can contribute into cation retention only if sulfo group attracts the latter to the resin surface. Also, HCPS matrix can change properties of attached sulfo group.

Increase of eluting ion concentration causes decrease of cation retention. Dependencies of retention of studied cations on the eluting ion concentration in the log–log coordinates are linear, the slopes are listed in the Table 1. The slope values are close to the theoretical ones (0.33 for alkali and 0.66 for alkaline-earth metal cations).

3.4. Effect of temperature

Thermodynamic characterization of sorption process can be helpful instrument for retention mechanism elucidation [16]. For example, ion-exchange heat values do not exceed 8–13 kJ/mol [17], while in case of chelation the temperature effect is more pronounced. Hence, adsorption heats values reflect any noticeable change in retention mechanism. Temperature increase causes decrease of cation retention on HCPS–MO (Fig. 2). Sorption heat is connected with capacity factor and temperature by a simple equation:

$$\ln k' = -\Delta H/RT + \Delta S/R + \ln \phi$$

where ΔH and ΔS are sorption enthalpy and entropy, respectively; ϕ is the phase volume ratio, a constant characteristic for a particular column. ΔS is usually supposed to be temperature independent value, thus the slope of $\ln k'$ plotted vs. the reciprocal temperature would be equal to $\Delta H/R$ [18]. The values of ion-exchange heats ($-\Delta H$, kJ/mol) of ammonium, alkali and alkaline-earth metal cations on HCPS–MO in 0.3 m*M* $Ce(NO_3)_3$–HNO_3, pH 5 are calculated:

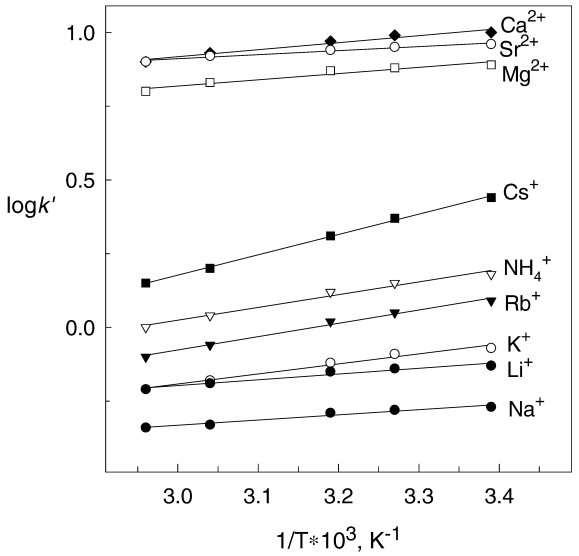


Fig. 2. Temperature effect on retention of ammonium, alkali and alkaline-earth metal cations on HCPS–MO. Eluent: 0.3 m*M* $Ce(NO_3)_3$, HNO_3, pH 5.

Na^+	Li^+	K^+	Rb^+	NH_4^+	Cs^+	Mg^{2+}	Sr^{2+}	Ca^{2+}
3.4±0.4	3.7±0.5	6.5±0.5	8.6±0.5	8.3±0.7	13.2±0.3	4.0±0.6	2.6±0.3	4.6±0.6

Two facts should be pointed out. At first, ion-exchange heat values of monovalent cations, excluding ammonium, increase in accordance with their retention. At second, enthalpies for ion-exchange of Li^+, NH_4^+ and Sr^{2+} are less, than it could be expected.

3.5. Stability of HCPS–MO

Strong retention of MO on HCPS with pure water eluents provides high stability of HCPS–MO phase. Nevertheless, MO leak from the column was noticed, the effluent was slightly colored, especially under

Table 1
The effect of mobile phase concentration (C_{Ce3+}, *M*) on the retention of ammonium and alkali and alkaline-earth metal cations on HCPS–MO, presented in the form of corresponding equations $\log k' = a + b \log C$

Cation	*a*	*b*	*r*
Na^+	−1.470	0.42±0.01	0.999
Li^+	−1.122	0.35±0.01	0.997
K^+	−1.25	0.39±0.01	0.999
Rb^+	−1.086	0.36±0.02	0.991
NH_4^+	−0.949	0.35±0.01	0.997
Cs^+	−0.766	0.34±0.01	0.997
Mg^{2+}	−1.061	0.60±0.01	0.999
Sr^{2+}	−1.140	0.63±0.01	0.999
Ca^{2+}	−1.013	0.61±0.01	1.000
Ba^{2+}	−1.210	0.67±0.04	0.993

Eluent: $Ce(NO_3)_3$, pH 5 adjusted with HNO_3.

Table 2
The stability of the column loading evaluated on the as a reproducibility of retention times (t_R) of cations on HCPS–MO with different volumes of 0.3 m*M* $Ce(NO_3)_3$ (pH 5, HNO_3) passed through the column

Cation	Eluent volume (l)		
	3	11	21
Na^+	2.92	3.06	3.21
K^+	3.30	3.47	3.58
NH_4^+	4.27	4.38	4.72
Mg^{2+}	17.78	18.90	19.10
Ca^{2+}	20.33	21.00	21.32

eluent composition change. To prove the column coating stability retention of several cations was studied under equal conditions from time to time during the whole experiment. The volume of eluent passed through the column had a little effect on cation retention (Table 2).

3.6. Separation of cations and anions

As it was already mentioned, HCPS–MO exhibits cation-exchange properties preferentially. Retention of anions is weak, only strongly polarizable anions, such as I^- and SCN^-, which are inclined to sorption at the hydrophobic HCPS surface are retained comparatively strong. A potential ability of simultaneous separation of cations and anions on HCPS–MO was demonstrated (Fig. 3).

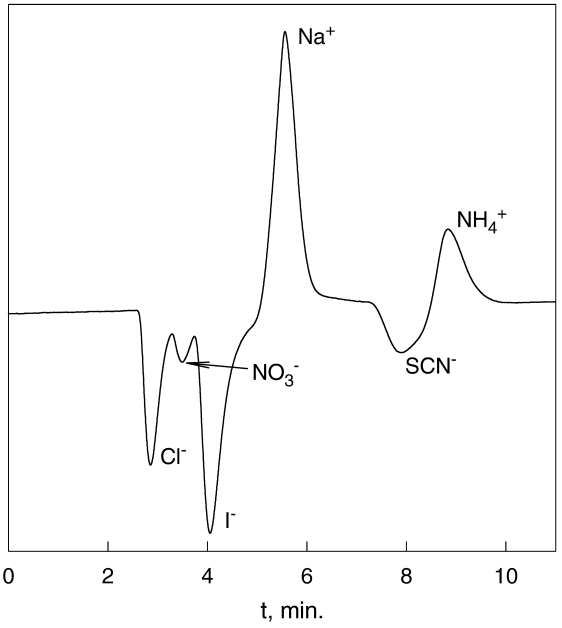


Fig. 3. Simultaneous separation of cations and anions. Column: HCPS–MO, 250×4.6 mm I.D. Eluent: 1 m*M* $Ce(NO_3)_3$, HNO_3, pH 5; flow-rate 0.6 ml/min; UV detection 254 nm.

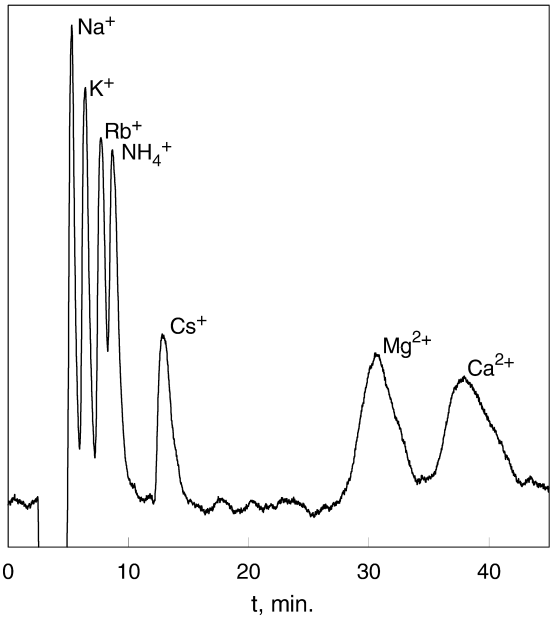


Fig. 4. Separation of ammonium, alkali and alkaline earth metal cations. Column: HCPS–MO, 250×4.6 mm I.D. Eluent: 1 m*M* $Ce(NO_3)_3$, HNO_3, pH 5; flow-rate 0.6 ml/min; UV detection 254 nm.

HCPS–MO with Ce^{3+} as eluting ion was found to be useful for simultaneous separation of alkali and alkaline-earth metal cations. Na^+, K^+, Rb^+, NH_4^+, Cs^+, Mg^{2+} and Ca^{2+} were separated in the single run for 40 min (Fig. 4).

4. Conclusions

A novel approach for synthesis of bipolar stationary phases is proposed namely impregnation of HCPS with bipolar aromatic molecules. High affinity of the support for phenyl group provides coating stability.

HCPS impregnated with methyl orange dye is a novel bipolar stationary phase, synthesized according to this technique. It is capable to separate both

cations and anions simultaneously, though cation-exchange dominates. Special properties of the matrix cause unusual selectivity of HCPS-MO for the separation of alkali and alkaline-earth metal cations: $Na^+ < Li^+ \sim K^+ < Rb^+ < NH_4^+ < Cs^+$ and $Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+}$.

HCPS–MO with Ce^{3+} as eluting ion provided simultaneous separation of Na^+, K^+, Rb^+, NH_4^+, Cs^+, Mg^{2+} and Ca^{2+} cations for 40 min.

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