

Preparative frontal size-exclusion chromatography of mineral ions on neutral hypercrosslinked polystyrene

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Available online 7 March 2005

Abstract

Hypercrosslinked polystyrene, being the first and the only one accessible for water microporous hydrophobic polymeric sorbent, was found to provide efficient separation of inorganic electrolytes under conditions of a frontal size-exclusion chromatography (SEC). The process, based on the difference in size of hydrated competing ions, permits separations of many pairs of salts, acids, and bases. The productivity of separation increases with the concentration of the feed solution rising. Remarkably, concentrations of the separated components in corresponding fractions may substantially exceed those in the initial mixture, which can be explained in terms of a conception of an ideal separation process.

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Keywords: Size-exclusion chromatography; Hypercrosslinked polystyrene; Mineral electrolytes; Ideal separation process; Compression of chromatographic zones

1. Introduction

Hypercrosslinked polystyrene-type adsorbing materials developed by Davankov et al. [1,2] and commercialized (“Hypersol-Macronet”) by Purolite Int. (Pontyclun, UK) [3] have found wide application in many large-scale adsorption technologies [4,5] and also in analytical chemistry. Used as solid-phase extraction materials (“Purosep”, “LiChrolut ENV”) they efficiently adsorb and concentrate trace amounts of both non-polar and polar [6] organic compounds from aqueous media and air. Remarkably, aromatic compounds can also be concentrated from a solution in aliphatic hydrocarbons. More recently, hypercrosslinked polystyrene in the form of rigid microbeads was shown to present a promising universal HPLC column packing material (“Chromalite”) that is compatible with any type of organic or aqueous mobile phase, thus allowing several different modes of chromatography to be performed on same HPLC column [7]. Worth mentioning is also the unique affinity of the neutral unmodified material to several transition metal ions as mercury, lead, silver that form labile complexes

with the π -electronic systems of the aromatic polymer [8].

All these outstanding properties are due to the full accessibility of the aromatic elements of the hypercrosslinked polystyrene to small molecules. Indeed, a hypercrosslinked network [2] presents an extended open-work-type rigid structure that has been prepared by an extensive crosslinking of pre-formed polystyrene chains in a media that solvates the polymer and prevents any micro phase separation of the system. By applying sufficient amounts of bifunctional crosslinking reagents capable of substituting phenyl rings of the initial polystyrene, a three-dimensional system of interconnected conformationally rigid cycles is created, as visualized in Fig. 1.

The material thus prepared is a homogeneous one-phase polymer with a reduced bulk density of about $0.5\text{--}0.7\text{ g cm}^{-3}$ and an extended apparent inner surface area as high as $1000\text{--}1500\text{ m}^2\text{ g}^{-1}$. The abnormally high free volume of the polymeric sorbent presents a porosity of a new type, since the “pores” are small voids in the above macrocycles or distances between the initial polystyrene chains, rather than channels with walls built of a dense polymeric phase. The internal free volume of the hypercrosslinked polymer is easily accessible to gases and any liquid, both dissolving linear polystyrene

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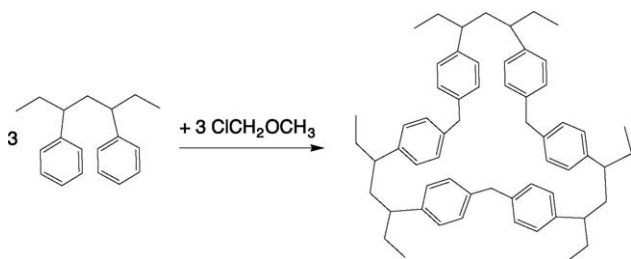


Fig. 1. Formation of hypercrosslinked polystyrene network.

(as toluene or ethylene dichloride) and precipitating it from a solution (as hexane or methanol). Even water can be placed into the interior of the hypercrosslinked polystyrene without causing a collapse of the rigid network. Thus, the interior of the hypercrosslinked sorbent bead becomes accessible to small molecules dissolved in any organic or aqueous media.

As a follow-up of the initial short communications [5,9–11], we report now in more detail on the unexpected ability of hypercrosslinked polystyrene to efficiently separate mineral salts, acids and bases, in spite of the absence of polar or ion-exchanging groups in the chemical structure of the basically aromatic and hydrophobic sorbent material. Previously, such kind of separations was thought to be possible on ion-exchange resins, only.

2. Related work by others

As early as 1950, Davankov et al. applied for and, 5 years later, received a USSR patent [12] for “A method of removing salts from aqueous solutions by using amphoteric ion-exchange resins”. For the first time, the polymeric material incorporated both cationic and anionic functional groups and, therefore, simultaneously retained both anions and cations of a dissolved salt. A real success in the removal and/or separation of electrolytes by the “ion retardation” process was achieved with amphoteric “snake-cage polyelectrolytes” suggested 1957 by Hatch et al. [13] and defined as “cross-linked polymer system containing physically trapped linear polymer”. These materials have been soon commercialized by Dow Chemical Co. under the name “Retardion” [14]. A typical snake-cage amphoteric polyelectrolyte Retardion-550WQ2 (water content 41.6%) was made by polymerizing *ar*-vinylbenzyl trimethyl ammonium chloride inside a sulphonated cation exchanger Dowex-50Wx2 and had strong basic and strong acidic functional groups. On a 100 ml column packed with this resin, a 20 ml sample 2.0 N in NH_4NO_3 and 1.6 N in HNO_3 was almost completely separated into constituents [15]. Similarly, 1.98 N FeCl_2 partially separated from 3.15 N HCl [15].

In the year 1958, in a theoretical study on activity coefficients of electrolytes in the resin phase, Nelson and Kraus [16] arrived at a conclusion that, “because of the relatively low activity coefficients of HCl in the examined anion exchanger Dowex-1 \times 10”, separation of HCl from concentrated halide

solutions is possible. Remarkably, they report that on that resin the break-through curves of 0.1 N HCl shift from ca 1.5 column volumes in 5 N LiCl to more than eight column volumes in the concentrated (16 N) solution of LiCl. A similar strong retention of HCl (because of the “reduced activity coefficient of HCl”) was also characteristic of the 10 N solution of MgCl_2 .

Hatch and Dillon re-discovered 5 years later [15] the fact that conventional ion-exchange resins efficiently separate acids from their salts under conditions that exclude normal ion exchange. This finding contradicted the concept of “ion exclusion” according to which all strong electrolytes should be effectively excluded from absorption into ion-exchange resins, because of the Donnan equilibrium effect [17,18]. The strong basic anion-exchange resin Dowex-1 \times 8 (8% divinylbenzene, 40% water content) was found to function especially well and authors decided to introduce a new term, “acid retardation”. “Such separations can be defined as “acid retardation” separations, since they are based on a preferential absorption of strong acids, which causes the movement of the acid on the bed to be retarded – i.e., slowed down – relative to the movement of the salt”. Acid retardation, like ion retardation can be done at high flow rates, especially at elevated temperatures. These processes have been optimized and since 1976 widely exploited by Eco-Tec, Canada on the industrial scale [19].

As to the mechanism of the acid retardation authors [15] note that various ideas or their combinations may be offered to explain the absorption of strong acids on anion-exchange resins:

- Electrolyte interactions, including “salting effects”, the HCl being salted-out into the resin phase (that can be considered as analogous to a concentrated solution of strong electrolyte);
- Interaction of protons of the strong acid with the benzene rings of the resin matrix;
- Association of strong acids to form ion pairs and non-ionized molecules in the resin phase, due to the lowered dielectric constant of that phase;
- Possibly an entropy-increasing or energy-lowering effect of excess protons on the microstructure of the water inside the resin matrix.

Still, having examined many experimental data, authors [15] conclude: “Non of the ideas are proved by the data currently available At this time, any of the theoretical explanations of the acid absorption certainly are far from proved.”

During the 40 years that passed after the appearance of the above classical works, numerous research groups examined the ion and acid retardation processes, but, no new ideas have been suggested for the explanation of the non-trivial phenomenon of the electrolyte discrimination. Interestingly, a more or less adequate mathematical description of experimental data is possible both in terms of self-association of acids in the homogeneous resin phase and association of acid molecules with the functional groups of the resin [20] and

in frames of a two-phase model of the resin bead [21]. Both approaches, however, base on the above ideas that have been formulated, considered and rejected by the pioneers of the method. Indeed, mathematical models may well fit experimental data into equations, but they do not prove the reality of the conception they are based on.

An entirely different area of research present analytical-scale separations of inorganic compounds on hydrogels, like Sephadex G-15 to G-50 (crosslinked dextran) or Bio-Gel A (crosslinked agarose). From very beginning, these materials have been designed for gel permeation chromatography, so that the size-exclusion mechanism of separation of inorganic polymers, as condensed phosphates, molybdophosphates, silicic acid and the like has been early recognized. A detailed review by Yoza [22] also deals with additional effects that complicate the SEC separation mechanism, such as adsorption, ion exclusion, secondary complexation equilibria in solution. As for simple metals and anions, correlation has been noticed between the retention and the radii of hydrated ions [23–25].

Rona and Schmuckler [26] eluted Dead Sea concentrated brine on a Bio-Gel P-2 (crosslinked polyacrylamide) column with the result of obtaining a lithium-enriched fraction free from calcium and magnesium. Bio-Gel P, however, is known to retain cations and probably enter hydrogen-bond interactions between the anions and the amide hydrogen. The elution order of chlorides was thus different from that expected for SEC, namely, K^+ , Na^+ , Li^+ , Mg^{2+} , Ca^{2+} , all emerging before the hold-up (dead) volume.

3. Experimental

Majority of experiments were carried out using home-prepared neutral hydrophobic microporous hypercrosslinked polystyrene-type sorbents with the bead size between 300 and 1000 μm . Similar materials are manufactured by Purolite Int. (Pontyclun, UK) under the trade name Hypersol-Macronet [3]. Usually, specific surface area of these sorbents amounts to about 1000 m^2/g , the pore volume to 0.7–0.8 cm^3/g , and the pore size at the distribution maximum to 2–3 nm. The sorbents used have been prepared by conventional post-crosslinking of beaded styrene–divinylbenzene copolymers with monochlorodimethyl ether and did not contain any functional groups susceptible to hydration or ion exchange. (Any residual chloromethyl and hydroxymethyl groups are present in minimal amounts, <0.1 meq./g, and do not play any noticeable role in processing concentrated electrolyte solutions under investigation.)

The sorbent in which all the pores were pre-filled with water (by washing the material with ethanol and then with water) was slurry-packed under the influence of gravity into a column ca 20 cm in length and 30 ml in volume. An aqueous solution of a salt, acid or a mixture of electrolytes was passed through the column at a flow rate of 0.5–0.7 ml/min (“forward” experiment). The concentration profile of the ef-

fluent (the break-through curve) was determined. Then the electrolytes were displaced from the column by water (“reverse” experiment) and their concentration at the outlet of the column were determined, as well.

The initial sample solution and then the eluent (pure water) were supplied from a reservoir under hydrostatic conditions, and the effluent was collected in ca. 1.2 ml fractions using a fraction collector. Since no pumps were available that would tolerate aggressive concentrated solutions of acids, salts and bases, it was difficult to maintain a constant flow of supplied liquids. For this reason, every collected fraction was weighted, so that the weight of the effluent, not its volume, was plotted on the horizontal axes of all chromatograms. They cannot be directly converted into volume, since the density of effluents may change by about 20% within the few fractions that incorporate the zone fronts.

Concentration of electrolytes in each fraction was determined by titration. Generally, the acid or the base were directly titrated in an aliquot of each fraction and then the metal cation or the anion were selectively determined by titration. In order to determine concentration of chloride anions, a 200 μl aliquot of the effluent fraction were supplied with 10 ml water, 10 ml EtOH, 4 ml 0.5 N HNO_3 and small amounts of Bromphenol Blue and diphenylcarbazone and then titrated with a 0.2 N solution of $\text{Hg}(\text{AcO})_2$ that contained 3 ml of conc. HNO_3 per 1.0 l of the mercury solution. At the equivalence point yellow color of the solution changed to violet. For the determination of calcium ions concentration a 200 μl aliquot was provided with 20 ml water and 1 ml of 5.0 N NaOH and then titrated with a 0.05 M EDTA solution in the presence of murexide. The latter changed the color of solution from crimson to lilac at the point of equivalence. In other cases, with selective indicators not available, the total concentration of electrolytes in the fraction was found by passing another aliquot of the collected fraction through a bed of Dowex-50Wx8, hydrogen form, washing the resin with water, and titrating the total electrolyte with a NaOH solution. Metal ion concentration equaled the total electrolyte concentration minus the acid concentration.

EDTA disodium salt dihydrate, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were purchased from Acros Organics (Belgium); $\text{Hg}(\text{OAc})_2$ from Aldrich Chemicals (Poole, UK); all other reagents from Reakhim (Moscow, Russia).

4. Results and discussion

4.1. New findings on neutral hypercrosslinked polystyrene

Due to the rigidity of the open-work-type structure, the hydrophobic hypercrosslinked polystyrene remains expanded and fully accessible to small molecules in any organic as well as aqueous media. This property made it possible to examine the behavior of inorganic electrolytes in the hydrophobic network environment. We first registered the break-through

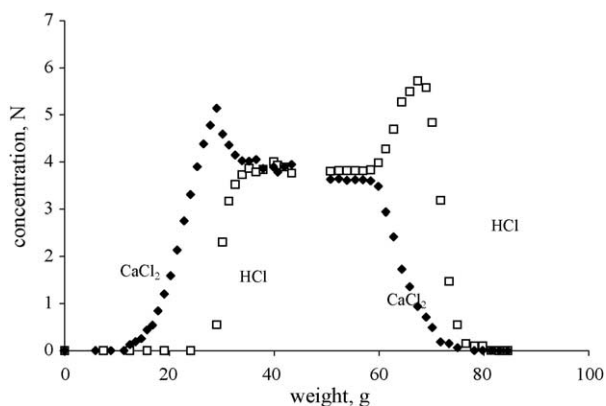


Fig. 2. Separation of 4N CaCl_2 from 4N HCl on hypercrosslinked polystyrene.

curve of an aqueous 2N LiCl solution from a 30 ml column packed with the beaded material (forward experiment). Then, the elution curve of the salt from the column with pure water was also determined (reverse experiment). Analogous breakthrough and elution curves were further obtained for a 2N HCl solution. The resulting concentration profiles in combined forward and reverse experiments for each electrolyte show a typical trapezoidal shape. However, it was impossible not to note the fact that in both the forward and reverse experiments, LiCl emerges from the column with smaller elution volumes than does the acid.

If a mixed solution of a salt and an acid, for example, a 4N solution of CaCl_2 in 4N HCl, is passed through the same column, in this case, too, the salt elutes ahead of the acid, which allows one to obtain a fraction of pure CaCl_2 solution in the forward experiment and a fraction of the acid in the reverse experiment (Fig. 2). A substantial degree of separation is confirmed by the mere fact that the salt and acid fronts can diverge by almost half of the column volume. Similar remarkable separations were also obtained by passing rather concentrated solutions of LiCl, KCl or NaCl in solutions of hydrochloric acid.

First question to be answered is why the hypercrosslinked polystyrene discriminates salts and acids.

Due to the local electroneutrality principle, the cations move along the column together with their anions. Since the salts and the acid in the above systems have the same chloride anion, the divergence of the salt and acid fronts is due to different behaviors of the proton and the metal cations in the hypercrosslinked sorbent phase. This difference may be due to one of the two reasons: either polystyrene retains the proton stronger than it retains the alkali or alkaline-earth metal cation or different pore volumes are accessible for the proton and these cations; i.e., they are excluded to different extents from the sorbent phase. Positive interactions between cationic species, including protons, with aromatic structures comprise an intensively examined and already well-documented phenomenon [27,28]. In the hypercrosslinked polystyrene, these interactions may well be enhanced by possible presence of

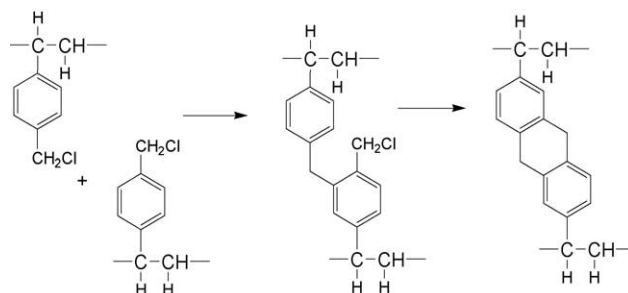


Fig. 3. Possible route of formation of condensed aromatic structures.

condensed aromatic systems. Thus, anthracene-type structures may easily form by condensation of two chloromethylated styrene repeat units, as shown in Fig. 3, with a subsequent oxidation. Size-exclusion phenomena have to be also considered, since, with the maximum of the pore size distribution curve of the material at about 2–3 nm, one has to expect a substantial amount of channels or pores to be comparable in their size with diameters of hydrated mineral ions.

If the hypercrosslinked polystyrene displays an enhanced proton affinity, it would be manifested not only under chromatographic (dynamic) conditions, i.e., as the acid front lagging behind the front of the mobile phase and behind the salt front, but also under static conditions, i.e., as an increase in the acid concentration in the sorbent phase when the latter is brought in equilibrium with the acid solution. In the latter case, the HCl concentration in the outer equilibrium solution is expected to decrease. Table 1 presents some data obtained in a series of experiments under such static conditions. The data indicate that the sorbent does not retain the proton, since equilibration with the sorbent does not change the HCl concentration in the solution. Conversely, the measured concentrations of NaCl and NaOH solutions exceed the calculated values after the contact with the sorbent. This is only possible if a larger volume in the sorbent phase is accessible for water and HCl than for the salt or the base; i.e., NaCl and NaOH are partially excluded from the sorbent phase. This result points out to an exclusion of Na^+ cations and to exclusion mechanism of separations observed in the above systems.

Table 1
Sorption of HCl, NaCl and NaOH on hypercrosslinked polystyrene under static conditions^a

Sorbate	Initial ^b concentration (N)	Equilibrium concentration in solution (N)
HCl	1.364	1.360
HCl	1.417	1.425
NaOH	1.568	1.682
NaCl	2.050	2.108

^a 50 g of wet sorbent, 100 ml of solution, periodic stirring for 3 days at room temperature.

^b Calculated concentration of the sorbate in the initial solution with allowance for the moisture added with the polymer.

Indeed, alkali and alkaline-earth cations are known to be strongly hydrated in aqueous media and exceed in their size dimensions of hydrated chlorine anions. Actually, the hydroxonium cation H_3O^+ must be also strongly hydrated. However, in an aqueous solution, it can appear at any point that accommodates a water molecule, provided the latter is connected to the bulk aqueous phase by chains of hydrogen bridges. Therefore, a proton does not need to move, at all, since a very rapid movement of electrons along the chains of hydrogen bonds would easily provide any required relocation of charges within the aqueous phase, thus simulating rapid movement of protons or hydroxyl anions (Fig. 4). This is the reason for the extremely high values of electrophoretic mobility that are constantly found for these two ions in aqueous solutions as well as for the high electric conductivity of aqueous solutions of acids and bases. We can thus assume that in the resin phase protons and hydroxyls virtually have no proper size, whereas the placing of hydrated metal cations requires larger pores.

Above considerations fully explain, in terms of size-exclusion chromatography, the facts of the effective separation of various metal halide from hydrochloric acid on microporous hypercrosslinked polystyrene. However, they do not explain the unexpected and remarkable increase in concentrations of the salt and acid in those fractions of the effluent where the two electrolytes appear separated from each other (Fig. 2). Explanation of this unprecedented for conventional chromatography phenomenon requires a more detailed consideration of peculiarities of the SEC process.

4.2. Remarkable features of size-exclusion chromatography

Size-exclusion chromatography has been mainly used for the separation of large species on the analytical scale. Applying this technique to preparative separations requires considering closer some of peculiar features of that technique, which may have escaped the attention of most chromatog-

raphy specialists. Some of these features may even sound paradoxically.

Species separated in the exclusion chromatography process are transported along the column by the mobile phase, but move faster than that mobile phase. To explain this paradox, one has to remember that only the liquid situated in the interstitial space (ca. 40% of the bed volume [29]) really moves through the column. The other part of the mobile phase remains immobilized in the pores. Large particles have limited possibilities to visit pores of the packing, and they mostly stay in the main stream of the mobile phase (in the “central channel” of the column). Smaller particles enter the stagnant zones of the mobile phase more often and are transported by the moving part of the latter less frequently, thus advancing along the column at a reduced velocity. Molecules of the mobile phase explore all the stagnant zones in all smallest accessible pores of the packing and move at a velocity that is determined by the pumping rate and the ratio of interstitial volume and total volume of the mobile phase in the column. Particles that are (partially) excluded from the column packing thus quickly separate from their “mother sample solvent” and start exploiting the movement of those molecules of the previously existed in the column mobile phase which, in the given moment, happen to enter the interstitial space of the column.

Consider situation when a pump starts delivering a sample solution into a column packed with porous material. Large particles arrive at the column outlet and emerge in the effluent before smaller particles and before the sample solvent. At the moment the front of the sample solvent arrives at the outlet, the column becomes equilibrated with the feed mixture. At that moment, certain portions of excluded particles have already left the column with the effluent. Therefore, a size-exclusion column, being equilibrated with the mixture under separation, always incorporates liquid with reduced concentrations of excluded species. Even at a prolonged pumping, concentration of these species within the column will never reach that of the feed. This fully corresponds to the name of the process: species are *excluded* from the packing, i.e., from

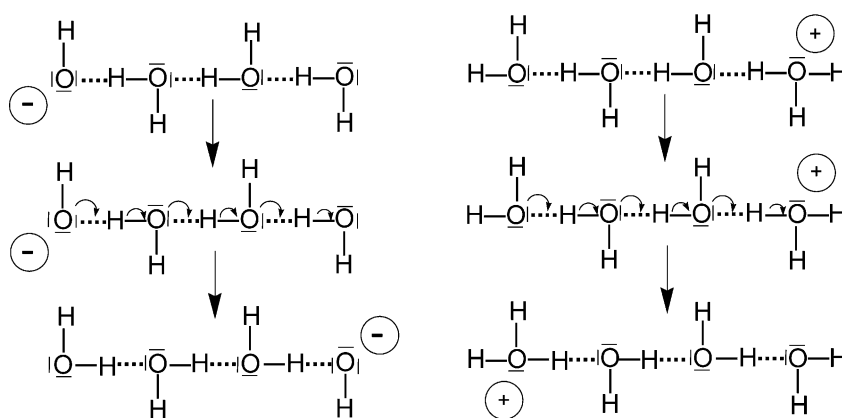


Fig. 4. Movement of electrons along chains of hydrogen bonds results in the relocation of protons or hydroxyl groups.

the column. The larger the particles, the lower their concentration within the column.

Nevertheless, the situation fully repairs at the column outlet, i.e., in the effluent. The effluent receives larger species at an enhanced rate, since they move through the central channel of the column with the velocity higher than the velocity of the mobile phase. The larger the species, the lower their concentration in the column, and the faster they are delivered into the effluent. As a result, *in frontal size-exclusion chromatography concentration of all species under separation in all corresponding fractions of the effluent is equal to their concentration in the initial mixture*. Important is that *frontal size-exclusion chromatography does not cause dilution of solutes*. Of course, we assume here that all fronts are sharp and there is no noticeable dispersion at the borders of rectangular or slightly trapezoidal chromatographic zones.

4.3. Conception of “ideal separation process”

Chromatography is generally recognized as an unrivaled analytical tool, whereas preparative and industrial potentials of chromatography have been mainly exploited in the field of ion exchange. Only recently, these potentials attracted general attention of chromatographic community in connection with chiral separations in pharmaceutical industry. Benefits of chromatography in the large-scale processes still remain to be fully recognized in its hard competition with classical separation techniques, as crystallization, distillation, flotation, sedimentation, sieving, etc.

From the viewpoint of separation science, there must be some common features characteristic of all or several separation techniques, as well as distinguishing features of each of them. In order to explain the above unexpected effect of the electrolyte self-concentration in the SEC procedure (Fig. 2), we would like to point out that preparative chromatography is just one of many approaches to separation and it must be also considered from the point of view of involvement of additional auxiliaries in the separation process. Let us use term “ideal separation process” to define any separation that does not add any supplementary matter to the separated fractions of the initial mixture under separation. An automatic consequence of separating one component from the initial mixture in such an ideal separation process would be the inevitable rise of concentrations of all other components in the remaining mixture. When applied to distillation, crystallization, sieving and the like, this statement is so simple and self-evident, that it never needed to be formulated. In chromatography, on the contrary, one usually dilutes the initial sample with large amounts of the mobile phase, so that the very idea of observing a self-concentration effect *for all analytes because of their separation* seems unrealistic, at the first sight.

Indeed, one usually deals with *retention* of analytes on the stationary phase, which reduces concentration of the analyte in the moving zone of the mobile phase and requires

additional amounts of the mobile phase to elute the retained portion of the analyte from the stationary phase. Cases of peak compression in chromatography are mostly coupled with the displacement of the adsorbed portion of the analyte (or analytes) by an auxiliary component of the mobile phase (displacer, mobile phase modifier). In order to act like this, the latter must adsorb on the stationary phase even stronger than the analytes. In frontal analysis, only, several weaker retained components can be obtained with an enhanced concentration, but never the stronger retained components of the same mixture.

With this respect, exclusion chromatography basically differs from all other modes of chromatography in that the analytes are *not retained* by the column packing and, therefore, do not need any special displacer or additional portions of the mobile phase, in order to be eluted from the column. Dilution of fractions separated in accordance with the size-exclusion mechanism is no more unavoidable. (Dilution can be minimized to the diffusion effects at the front and tail of the analyte zone.) The absence of any supplementary matter in the frontal exclusion chromatography process relates SEC to the above defined ideal separation process.

A starting solution that is 1 M in component **A** and 1 M in component **B** should be resolvable, in a hypothetical ideal separation process, in many different ways, e.g., giving, from 1.01 of the initial mixture, two fractions, 0.51 each, one of them being 2 M in component **A** and the other 2 M in analyte **B**, i.e., resulting in the enhancement of concentration of each of the components of the initial mixture.

In our typical experiment (Fig. 2), the initial solution was 4 N in CaCl_2 and 4 N in HCl. A portion of 100 ml of that solution had a density of 1.332 g/cm^3 and was composed of 22.20 g CaCl_2 , 14.58 g HCl and 96.42 g H_2O . Removing HCl from that mixture would automatically enhance concentration of the remaining CaCl_2 by 12%, whereas removing CaCl_2 from the mixture would enhance concentration of HCl by 20%. In reality, the concentrations of the resolved fractions peaked up to 30% for CaCl_2 and up to 50% for HCl. This is fully logical, since the faster moving CaCl_2 molecules have also removed a sufficient amount of hydration water from the slower moving zone of HCl.

Thus, the observed phenomena of self-concentration of the both resolved CaCl_2 and HCl fractions are becoming self-evident from the close relations between the frontal exclusion chromatography and the above defined ideal separation process. Indeed, hydrated ions of calcium chloride, which move in the reverse experiment faster than the acid ions, depart from the mixed-solution zone and liberate a substantial portion of space in this zone. In the SEC process, this portion of space is not filled with additional water. Instead, the zone of hydrochloric acid, lagging behind the CaCl_2 zone, narrows down, resulting in the corresponding rise in the acid concentration. In its turn, calcium chloride, which passes (in the forward experiment) along large pores at a higher rate than does HCl along both large and small pores, gets rid of HCl and becomes concentrated, too.

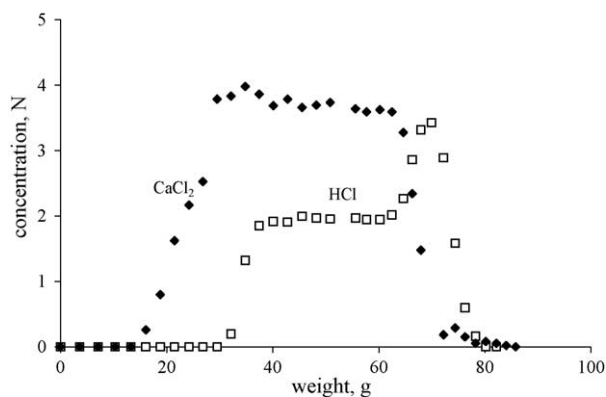


Fig. 5. Separation of 3.5 N CaCl_2 from 2 N HCl on hypercrosslinked polystyrene.

From the ideal separation concept it also logically follows that the greater the molar volume and the higher the concentration of the component that is being removed from the initial mixture, i.e., the larger the space liberated upon its removal, the more pronounced the self-concentrating effect for the other component. All experimental results that have been observed thus far fully correspond to this expectation. An example is presented in Fig. 5.

4.4. Size-exclusion chromatography—a general approach to differentiation of electrolytes

If size-exclusion presents the main reason for the differentiation of electrolytes on microporous hypercrosslinked polystyrene, the extent of separation must mainly depend on the difference in the size of competing hydrated ions, regardless their charge or other properties. If, in addition, we also accept the above assumption that, in the chromatographic systems with aqueous mobile phases under consideration, the protons and hydroxyl ions have but a minimal effective volume, the efficiency of resolutions of a salt and the corresponding acid must mainly depend on the size of the metal cation. Similarly, efficiency of differentiation between a salt and corresponding base must mainly depend on the size of the anion. The larger the cation and anion of a salt, i.e., the bigger their difference from the “infinitely small” proton and hydroxyl group, respectively, the more effective the front of the salt should diverge from the lagging front of the corresponding acid or base.

Relative information on the size of ions in aqueous media can be obtained from data on the electrophoretic mobility of these ions [30], since the velocity of their movement in an electric field is directly proportional to their charge and inversely proportional to their hydrodynamic radius. According to these estimations (Table 2) the size of hydrated cations and anions decreases in the following series: $\text{Tris}^+ \gg \text{Li}^+ > \text{Na}^+ \sim \text{EtNH}_3^+ > \text{NH}_4^+ \sim \text{K}^+ \gg \text{H}_3\text{O}^+$ and $\text{SO}_4^{2-} \sim \text{acetate} > \text{formate} \sim \text{F}^- \gg \text{NO}_3^- > \text{Cl}^- \gg \text{OH}^-$. There is not much of information available on the real size of hydrated ions. For a few simple alkali

Table 2
Electrophoretic mobility of ions, μ , $10^5 \text{ cm}^2 \text{ v}^{-1} \text{ s}^{-1}$

H_3O^+	362.5	OH^-	205.5
K^+	76.0	Cl^-	79.1
NH_4^+	72.2	NO_3^-	74.1
EtNH_3^+	53.1	F^-	57.4
Na^+	51.9	Formate	56.6
Li^+	40.1	Acetate	42.4
Tris^+	29.5	SO_4^{2-}	41.5×2

Table 3
Ion radius, Å [31]

Cation	Li^+	Mg^{2+}	Zn^{2+}	Na^+	Ca^{2+}	Sr^{2+}	La^{3+}	Ba^{2+}
Crystal	0.60	0.65	0.74	0.97	0.99	1.13	1.15	1.35
Hydrate	3.7	4.4	4.4	3.3	4.2	4.2	4.6	4.1

and alkaline-earth cations, the Stokes' radius decreases from about 8.8 to about 6.6 Å in the following series: $\text{Mg}^{2+} \sim \text{Zn}^{2+} > \text{Ca}^{2+} \sim \text{Sr}^{2+} > \text{Ba}^{2+} > \text{Li}^+ > \text{Na}^+$. Noteworthy, this sequence is largely opposite to that of the van der Waals radius (Table 3) of the cations themselves [31]. Obviously, smaller cations exhibit larger charge density on their surface and stronger bind water molecules, compared with larger ions.

In good agreement with the above considerations, for mixtures of HCl with the examined metal halides better separations have been observed for stronger hydrated CaCl_2 and LiCl as compared to weaker hydrated NaCl and KCl .

The initial hypothesis about the exclusion mechanism of separation of electrolytes is strongly supported by the fact that when a 4 N solution of H_2SO_4 in 4 N HCl is applied to the column, sulfuric acid emerges in the effluent before hydrochloric acid (Fig. 6). This order of emergence of the acids is predictable in view of the fact that the hydrated sulfate anion is much larger than the chloride ion.

Another convincing argument is the efficient separation of Na_2SO_4 from NaOH (Fig. 7). The size of sulfate anion is definitely larger than that of OH^- . Moreover, the latter does not need to move through the polymer pores, at all, so its

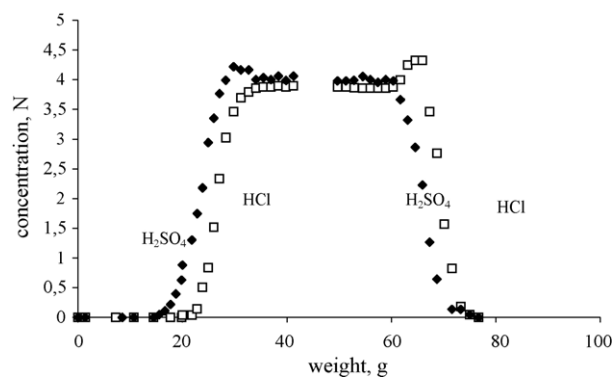


Fig. 6. Separation of 4 N H_2SO_4 from 4 N HCl on hypercrosslinked polystyrene.

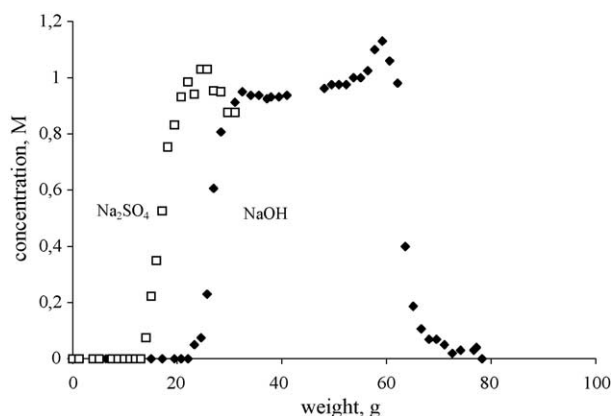


Fig. 7. Separation of 1 M Na₂SO₄ from 1 M NaOH on hypercrosslinked polystyrene.

effective size can be considered close to zero. Exclusion and movement of NaOH are mainly determined by the size of sodium cations.

An additional strong argument in favor of the idea on infinitely small effective volumes of protons and hydroxyl ions in systems considered is the fact that, whereas Ca²⁺ easily separates from H⁺ (Fig. 2), the separation of Ca²⁺ from K⁺, all taken in the form of chlorides, is not impressive, at all, though the diameter of hydrated Ca²⁺ can be estimated to be almost twice as large as that of K⁺. Calcium indeed emerges from the column ahead of the front of potassium, but the break-through curve of Ca²⁺ is more diffuse, so that the two curves cross at about half of their heights. Obviously, the larger calcium cation displays a smaller diffusion rate in the system. Of course, one can expect that proper optimization of the porous structure of the packing material, reducing the particle size or elution rates will enhance the separation efficiency of that pair of cations.

Domination of the size-exclusion mechanism of separation does not imply that other types of interactions between the ions and the stationary phase may not contribute to the retention volumes of electrolytes. Thus, large and polarizable anions of bromine, iodine, as well as cations like silver, copper, etc. may enter intensive interactions with aromatic π -systems of polystyrene matrix, which will retard the movement of the ions. These interactions may contribute to their separation from still larger competing ions, but, they may be counterproductive to the size-exclusion effect and deteriorate their separation from smaller species.

The exclusion mechanism of separation of electrolytes permits enhancing the column productivity by simply increasing the concentration of the initial mixture, which could be considered a strong argument for preparative and industrial-scale applications of the new process. This situation favorably distinguishes the new technique from all other types of adsorption chromatography and ion exchange, where the threshold of column loadability sets a limit to the concentration of the injected sample.

On the other hand, one has to take into account the effect of concentration and temperature on the extent of hydration of ions and on their effective size in solution. Some rather unpredictable phenomena may emerge with highly concentrated solutions. Thus, for instance, by examining a mixture 0.06 N in HCl and 12 N in LiCl, we suddenly observed a rather strong retention of HCl on the hypercrosslinked polystyrene. In the forward experiment, the break-through curve of HCl was found to start after five bed volumes, only, and to drag over additional two bed volumes of the effluent. Hydrochloric acid was obviously retarded in the column packing from that extremely concentrated solution. On the contrary, the elution peak of HCl in the reverse experiment was found to be sharp, so that the concentration of HCl peaked out to about 0.9 N, i.e., it increased by a factor of 15 compared to HCl concentration in the initial mixture. The influence of concentration and temperature on retention and separation of electrolytes still requires a more detailed examination by using a more precise measurement technique. Chromatography may thus provide unique information on the process of hydration of ions in concentrated solutions.

4.5. Use of other microporous column packings

Above concepts of ideal separation process and size-exclusion mechanism of observed differentiation of electrolytes should be applicable to separations on all microporous column packing materials, regardless their chemical nature. Of course, chemistry of the material and pore walls can contribute, in one way or another, to the retention of ions. Indeed, we were able to support the general validity of basic ideas developed for neutral hypercrosslinked polystyrene with two additionally examined materials that can be expected to possess nano-sized pores. One is porous carbon prepared by pyrolysis of hypercrosslinked polystyrene beads under protective atmosphere, the other—commercially available sulphonated hypercrosslinked polystyrene cation exchanger, MN-500 (Purolite Int., UK).

Interestingly, the carbon material, distinct from its parent hypercrosslinked polystyrene, can be wetted by water directly. Therefore, for the preliminary experiments under static conditions this material was taken in dry state. After equilibration of the carbon with a 2 N solution of HCl, concentration of the acid in the supernatant was found to slightly decrease, pointing out to some adsorption of HCl on the carbon. On the contrary, concentration of a 2 N CaCl₂ solution was observed to slightly increase, implying that calcium cannot penetrate all the pores that are accessible to water. The same tendency of discriminating protons and calcium ions was also observed for similar solutions in aqueous methanol (1/1, v/v) that was taken as the solvent that eventually penetrates all pores of dry carbon even better than does water.

In dynamic forward and reverse experiments on a mixture 3 N in HCl and 7 N in CaCl₂, acceptable separation of the two electrolytes was observed, as shown in Fig. 8. Note that concentrations of the electrolytes in the both separated zones

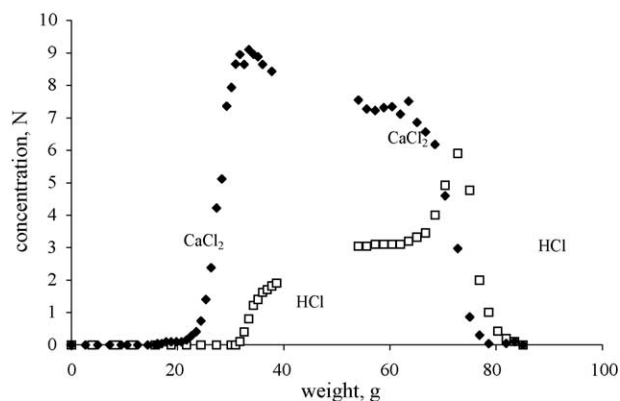


Fig. 8. Separation of 7 N CaCl_2 from 3 N HCl on a pyrolysed hypercrosslinked polystyrene.

substantially increased compared to their concentrations in the initial mixture, with the increase being more pronounced for the minor component (HCl), in full agreement with the requirements of the ideal separation concept. Similar results, with even sharper break-through fronts and elution curves, were observed for the mixed solution 4 N in LiCl and 5 N in HCl.

On the sulfonated cation exchanger MN-500, a slight separation of H_2SO_4 from HCl was observed, with the acids emerging in the given sequence, which implies that sulfate and chloride anions can be recognized and discriminated. However, no noticeable separation of CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ could be achieved. Obviously, the difference between nitrate and chloride is smaller than that between sulfate and chloride.

As mentioned in section “Related work by others” ion retardation and acid retardation phenomena have been known for circa 50 years, but did not find an acceptable explanation, thus far. We are convinced now that the size-exclusion effect as main reason for separations in these systems has been overseen. The experimentally selected best separating media, anion exchanger Dowex-1 \times 8, is a homogeneous gel with a reduced water uptake (40%) that may well have “pores and channels” of most suitable size for the purpose of differentiating inorganic ions

4.6. Alternating up-and-down process

Since in size-exclusion chromatography all species elute from the column before the hold-up volume, relatively small sample volumes can be processed in each chromatography cycle. Fortunately, SEC does not prevent using really concentrated initial solutions. The latter, however, have a density and viscosity that strongly differ from that of the eluent, pure water. Thus, the model solution (4 N CaCl_2 + 4 N HCl) used in Fig. 2 has a density of 1.332 g cm^{-3} . Density of a mixture that is 4 N in both calcium chloride and calcium nitrate is still higher, 1.357 g cm^{-3} . Even a more dilute mixture 1.5 N CaCl_2 + 1.5 N KCl is definitely heavier than water, 1.191 g cm^{-3} . In order to prevent severe broadening of the

break-through curves due to trivial convection, such samples must be better introduced into the column from the bottom to the top (forward experiment). Conversely, delivering water for the elution of remaining components from the column must be performed from the top to the bottom (reverse experiment). Fortunately, by using appropriate multi-port valves and a two pumps system, the whole alternating up-and-down process can be easily automated.

Another approach to handling automatically such concentrated samples is a pulse-wise introduction of water and sample solutions in a constant direction, as in [15]. However, columns with much better packing quality are needed in this case and much smaller bead particles with a narrow size distribution.

4.7. Application niche for frontal size-exclusion chromatography

The developed method of frontal size-exclusion chromatography on neutral hypercrosslinked polystyrene and other microporous materials has a number of substantial advantages over traditional ion-exchange processes, namely, (i) regeneration of the column is not required and, hence, no waste flows are formed; (ii) the process productivity increases with an increase in the concentration of the starting mixtures; (iii) separation is accompanied by further concentrating of the components.

In addition to the known phenomenon of acid retardation on anion exchangers, the size-exclusion approach on neutral materials opens ways for separating efficiently bases from salts, as well as different acids, bases or salts from each other. Several industry branches have severe problems of recovering strong acids or bases from aqueous mixtures of their salts. Such problems are common in ore leaching, metal pickling, electroplating of metals, chemical manufacture of alumina, phosphoric acid and many others.

5. Conclusions

A high-throughput method of separating inorganic electrolytes on microporous column packings has been developed. Recognition of size exclusion as main mechanism of the ion differentiation opens ways for designing purposefully optimal structures of nano-porous materials that can be both of hydrophobic and hydrophilic chemical nature. It also permits developing approaches to solving several practically important problems, but also to designing principally new separations, as is the resolution of a salt into the corresponding acid and base (results are going to be published elsewhere).

The unexpected fact of the substantial increase in the concentration of both the weaker and stronger retained components in the effluent, as compared to their concentrations in the initial mixture, was explained in terms of a concept of an ideal separation process. This concept sets new goals

and new criteria for the evaluation of preparative chromatographic separations.

Acknowledgements

This work was partially supported by the Russian Academy of Sciences, Department of Chemistry and Material Sciences, grant 08/131. Authors gratefully acknowledge technical assistance by N. Alexienko and Z. Blinnikova.

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