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MODIFIED HYDROXYETHYL METHACRYLATE COPOLYMERS AS SORBENTS FOR ION CHROMATOGRAPHY

I. SYNTHESIS AND PROPERTIES OF SORBENTS

FRANTIŠEK VLÁČIL* and IVAN VINŠ*
Department of Analytical Chemistry, Prague Institute of Chemical Technology, Suchbátarova 5, 166 28 Prague 6 (Czechoslovakia)
and
JIŘÍ ČOUPEK
Laboratory Instruments Works, 162 00 Prague 6 (Czechoslovakia)

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SUMMARY

The preparation and properties of ion chromatography sorbents based on Separon hydroxyethyl methacrylate copolymers are described. Sorbents with amino, ethylamino, diethylamino, dicyclohexylamino, diethanolamino and triethylamino functional groups were prepared by reaction of the appropriate amine with a precursor gel containing epoxy groups. Sorbents with the required ion-exchange capacity and additional cross-linking on column performance were studied. Examples of applications include the determination of nitrate in milk, separation of polythionates, etc.

INTRODUCTION

The term ion chromatography was introduced by Small *et al.*[1] only a decade ago. It is a powerful, widely used analytical tool enabling selective, simple and rapid determinations of a wide variety of inorganic and organic anions and cations. Several books[2,3] and reviews[4–8] are available on this subject and the number of applications increases each year. Ion chromatography in the present sense[2] comprises a combination of classical ion-exchange methods with modern instrumentation and sorbents for high-performance liquid chromatography (HPLC).

One of the possible ways to optimize ion chromatographic separations is the development of new sorbents with better properties. New types of sorbents for separations of anions and cations by ion chromatography, *i.e.*, low capacity modified silicas[9], low capacity polystyrene–divinylbenzene resins[10] and agglomerated sorbents[11], have recently been developed. The most important properties of an ion chro-

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* Present address: Institute for Care of Mother and Child, 147 10 Prague 4, Czechoslovakia.

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matographic sorbent, *i.e.*, selectivity, retention behaviour, chemical and mechanical stability, non-specific interactions (adsorption) and kinetic properties, are influenced by the sorbent matrix, functional group character and ion-exchange capacity.

The selectivity of a sorbent can be influenced by the character of the functional group, as will be shown in Part II of this series[12]. To compare the effects of different functional groups, sorbents having the same matrix as well as ion-exchange capacity but with different functional groups were needed. For the preparation of such sorbents, Separon hydroxyethyl methacrylate (HEMA) epoxidized copolymers were chosen as the starting material. These copolymers are widely used for HPLC separations of biochemicals[13–15] and as a matrix for affinity sorbents[16–18]. They have been modified with different ion-exchange groups[19] and the preparation of derivatives with phosphoric[20], carboxylic[21], sulphuric[22] and diethylaminoethyl (DEAE)[23] groups has been described. The gels modified by epichlorhydrin are very useful as supports for affinity chromatography[24].

In this paper, the preparation of anion exchangers with different functional groups and the required ion-exchange capacity by reaction of epoxy-modified Separon HEMA with different amines is described. The properties of these sorbents, as well as of low capacity DEAE derivatives, and their application to the chromatography of inorganic anions are also presented.

EXPERIMENTAL

Materials

Separon HEMA unmodified and modified with DEAE and epoxy (E) groups were obtained from Laboratory Instruments Works (Prague, Czechoslovakia). Their properties are summarized in Tables I and II.

Distilled deionized water was used throughout this work. The chemicals used were of analytical grade (Lachema, Brno, Czechoslovakia; E. Merck, Darmstadt, F.R.G.) unless stated otherwise. Ethylamine (Lachema), diethylamine (Carlo Erba, Milan, Italy) and triethanolamine (Laborchemie, Apolda, G.D.R.), reagent grade,

TABLE I

PROPERTIES OF PRECURSOR COPOLYMERS

EDMA is ethylene dimethacrylate.

Copolymer	*d_p (μm)*		*Exclusion limit units MW (Polydextran)*	*Pore area*[***] *(m^2 g^{-1})*	*Specific volume*[***] *(cm^3 g^{-1})*	*Monomer ratio, HEMA/EDMA*
	a[*]	*b*[**]				
HEMA 1000	15–25	20	$1 \cdot 10^6$	229	1.53	61/39
HEMA 300	13–21	20	$2.9 \cdot 10^5$	142	1.13	61/39
HEMA 40	20–30	25	$4 \cdot 10^4$	240	1.11	21/79
S HEMA 1000	10–13	10	$1 \cdot 10^6$	202	1.76	61/39[§]
S HEMA 300	10–16	13	$3 \cdot 10^5$	–	–	61/39[§]

[*] As stated by the supplier.

[**] Mean value from the measurement of *ca.* 100 particles using an optical microscope.

[***] Determined by mercury porosimetry.

[§] Additionally cross-linked.

TABLE II

EPOXY AND DEAE DERIVATIVES

Sorbent	*Functional group*	*Functional group content* (mmol g^{-1})*
HEMA 300 DEAE-1	DEAE	1.53
HEMA 300 DEAE-2	DEAE	0.47
HEMA 300 DEAE-3	DEAE	0.14
HEMA 300 DEAE-4	DEAE	0.07
S HEMA 100 DEAE	DEAE	0.16
S HEMA 300 DEAE	DEAE	0.33
HEMA 1000 E_{med}	Epoxy	0.39**
HEMA 40 E_{med}	Epoxy	0.58**
HEMA 1000 E_{max}	Epoxy	1.00**

* Determined by elemental analysis except where indicated otherwise.

** Determined by titration [40].

were used for sorbent modification as received. Dicyclohexylamine (Moravian Chemical Works, Valašské Meziříčí, Czechoslovakia), distilled, was used without purification; triethylamine (Lachema), reagent grade, was purified by distillation and then twice distilled with phenyl-isocyanate[25].

Instrumentation

The chromatographic equipment consisted of a high-pressure membrane pump VCM 300, a six-port sampling valve (Developing Workshops, Prague, Czechoslovakia), a micropump MMC (Mikrotechna, Prague, Czechoslovakia), a septum injector LCI 02, an on-line recorder TZ 4200 or TZ 4100, an integrator CI 100 (Laboratory Instruments Works), a photometric detector operated at 254 or 280 nm (Varian, Palo Alto, CA, U.S.A.) and a spectrophotometric detector, Model 87.00 (Knauer, Oberursel, F.R.G.). Empty compact glass columns CGC, 150 mm × 3.3 mm, and a column holder PKS-1 were obtained from Laboratory Instruments Works.

Modification of sorbents

Sorbents with DEAE functional groups were prepared by Laboratory Instruments Works as described[23]. Those with amino (A), ethylamino (EA), diethylamino (DEA), dicyclohexylamino (DCHA), diethanolamino (DEtA) and triethylamino (TEA) groups were synthesized by treating the precursor copolymer containing epoxy groups with an aqueous solution of the appropriate amine. The required ion-exchange capacity was controlled by the amine concentration (for A, EA, DEA and DEtA sorbents) or by the reaction time (for DCHA and TEA sorbents). Unreacted epoxy groups were hydrolyzed by perchloric acid[24].

The following procedure was used for sorbents with A, EA, DEA, and DEtA functional groups. A 1-g amount of the precursor copolymer was mixed with 10 ml of an aqueous amine solution of appropriate concentration and allowed to react for 72 h at room temperature, with mixing of the suspension on a rotary platform shaker for 16 h. The sorbent was then washed with water, mixed with an excess of 0.1 *M* perchloric acid and stirred for 3 h at room temperature. It was transferred to a glass frit and washed with water, 0.1 *M* sodium hydroxide, water, 0.1 *M* hydrochloric acid, water and acetone (50 ml of each), dried by suction and then *in vacuo.*

For the preparation of sorbents with DCHA and TEA functional groups, 1 g of the precursor copolymer was mixed with (a) 2 ml of dicyclohexylamine and 18 ml of water, (b) 10 ml of triethylamine and 0.1 ml of water and gently boiled under reflux for the appropriate time.

The amine concentration, c_A, or the reaction time, t, necessary for obtaining a sorbent with the desired ion-exchange capacity, Q_g (0.1 mmol g^{-1}), was found by interpolation from the dependences of Q_g on log c_A or on log t. These dependences were linear, thus only three experimental points were sufficient for satisfactory prediction of the two parameters (Figs. 1–3).

The sorbents used for comparison of the basicity of the functional groups and for some applications were synthesized by heating the precursor copolymer having a high content (1 mmol g^{-1}) of epoxy groups with an excess of aqueous amine solution under reflux for 8 h.

Column packing

Chromatographic columns were packed by an ascending slurry method. The slurry was prepared by mixing 0.8 g of a dry sorbent, 4 ml of 1 *M* perchloric acid and 1 ml of ethanol and the mixture was left to stand overnight to swell the sorbent. It was then sonicated for 3 min in an ultrasonic bath and transferred to a reservoir coupled to a glass column.

Determination of the column ion-exchange capacity

This measurement was based on the exchange of sulphosalicylate anion for the perchlorate anion. The column-exchange capacity, Q (μmol), was calculated from the registered breakthrough curve. The specific ion-exchange capacity, Q_g, was calculated from the column capacity and the sorbent mass (dry) in the column. The latter was determined by emptying the column on a glass frit, washing with water, 0.1 *M* hydrochloric acid, water, acetone and drying to constant weight *in vacuo* over phosphorus pentoxide. The column plate count was calculated from the peak width at half peak height.

RESULTS AND DISCUSSION

Modification of sorbents

For evaluating the effects of the functional groups on sorbent selectivity[12], sorbents differing only in the type of functional group were necessary. To prepare such modified sorbents, we started from Separon HEMA, *i.e.* an hydroxyethyl methacrylate copolymer modified with epoxy groups. This gel, having excellent chemical stability and good mechanical strength, intended as a support for affinity chromatography, reacts readily with a variety of amines[24].

From previous results[24], it could be concluded that the amino-group content in the modified sorbent depends on the amine concentration and/or the reaction time. In most of our experiments we found that such dependences are linear after logarithmic transformation (Figs. 1–3). Different slopes were found with the sorbent HEMA

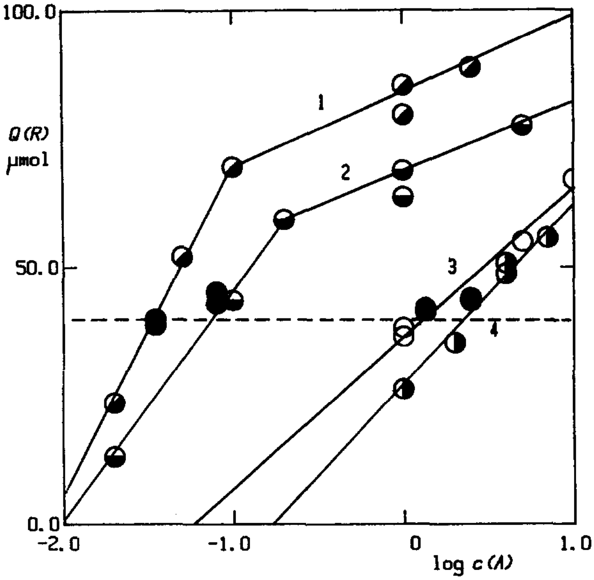


Fig. 1. Dependence of the column ion-exchange capacity, *Q(R)* on the amine concentration, *c* (mol l^{-1}). Precursor copolymer: HEMA 1000 E_{med}. Reaction conditions as given in the text. The required ion-exchange capacity is indicated by the dashed line; ●, points corresponding to sorbents prepared under controlled conditions with diethylamine (1), ethylamine (2), ammonia (3) and triethanolamine (4).

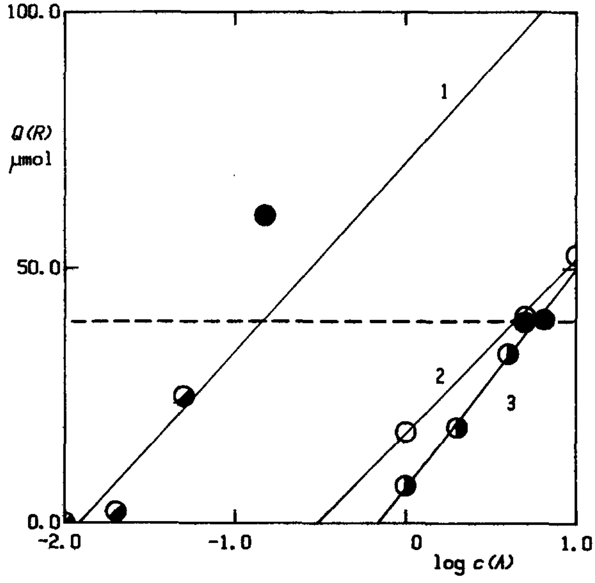


Fig. 2. Dependence as in Fig. 1, but for copolymer HEMA 40 E_{med}. ●, Points corresponding to sorbents prepared under controlled conditions with diethylamine (1), ammonia (2) and triethanolamine (3).

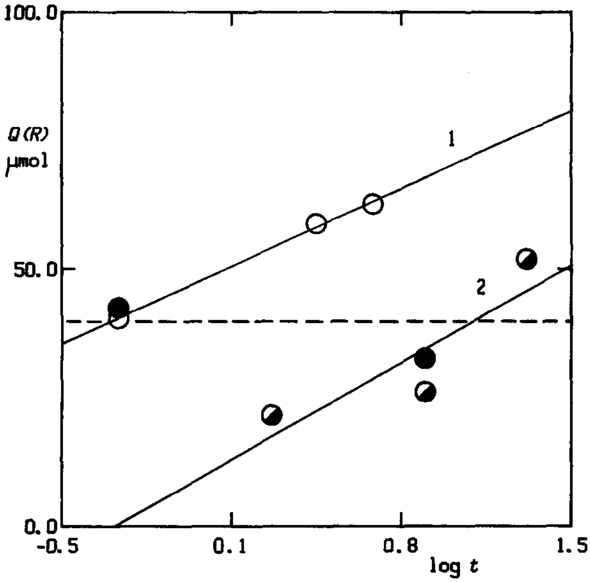


Fig. 3. Dependence of the column ion-exchange capacity, $Q(R)$, on the reaction time, t, in hours. Precursor copolymer: HEMA 1000 E_{med}. Reaction conditions as described in the text. The required ion-exchange capacity is indicated by the dashed line. ●, Points corresponding to sorbents prepared under controlled conditions with dicyclohexylamine (1) and triethylamine (2).

1000 E and high ethylamine or diethylamine concentrations probably due to some other factor such as the epoxy group content or availability. Good agreement between the desired and achieved capacity has been found for all sorbents, except for H 40 DEA, where extrapolation instead of interpolation had to be used and gave incorrect results.

As is seen from Fig. 1 and 2 the reactivity of amines with the precursor copolymer increases with increasing amine hydrophobicity, *i.e.*, in the order ammonia < ethylamine < diethylamine, in agreement with previous results[24].

The reactivity of triethylamine is very low and only 30% of the ion-exchange capacity achieved was due to quaternary amine groups, according to the strong base capacity of the ion exchanger measured by exchange of the nitrate anion of a neutral salt for the hydroxide ion of a sorbent in hydroxide form. Medium basic functional groups were thus formed, even after triethylamine purified with phenylisocyanate had been used.

The ion-exchange capacity of modified sorbents was determined from the breakthrough curve (sorption isoplane) for the exchange of sulphosalicylate anion for perchlorate anion. For comparison, the nitrogen content of the sorbents was determined by elemental analysis. These results are presented in Table III. The nitrogen content of sorbents prepared from HEMA 1000 and HEMA 300 is slightly higher than the ion-exchange capacity, which is true of all ion exchangers. This is probably due to the inaccessibility of some of the functional groups caused by sterical hindrance in the gel. The large difference between the nitrogen content and the capacity found for sorbents with the matrix HEMA 40 is due to a similar effect.

TABLE III

ION-EXCHANGE CAPACITY AND NITROGEN CONTENT OF SORBENTS

Sorbent	*Capacity (mmol g^{-1})*	*Nitrogen content (mmol g^{-1})*
H 1000 A	0.109	0.150
H 1000 EA	0.118	0.129
H 1000 DEA	0.110	0.164
H 1000 DEtA	0.114	0.164
H 1000 DCHA	0.099	0.125
H 1000 TEA	0.091	0.282
HEMA 300 DEAE-1	–	1.53
HEMA 300 DEAE-2	0.560	0.471
HEMA 300 DEAE-3	0.109	0.136
HEMA 300 DEAE-4	0.060	0.068
H 40 A	0.095	0.257
H 40 DEA	0.132	0.268
H 40 DEtA	0.086	0.275
S HEMA 1000 DEAE	0.098	0.175
S HEMA 300 DEAE	0.111	0.332

Column packing and performance

The column packing method used was similar to methods used for silica-based sorbents[26]. In contrast to the high pressure and pressure pulse used form packing silica sorbents, relatively low pressures (5–8 MPa) and a slow increase in flow-rate proved to be necessary for packing Separon HEMA sorbents. Further optimization of this method should give better results; however, reproducible and satisfactory column performance were obtained. The results of the column testing are presented in Table IV.

TABLE IV

COLUMN PERFORMANCE FOR AN UNRETAINED SOLUTE AT HIGH MOBILE PHASE VELOCITY

For conditions of testing see the text. *N* is the number of theoretical plates; *h* is the reduced plate height.

Column	*Particle size (μm)*	*N*	*h*
H 1000 A	20	590	13
H 1000 EA	20	556	14
H 1000 DEA	20	535	14
H 1000 DEtA	20	516	15
H 1000 DCHA	20	627	12
HEMA 300 DEAE-2	20	288	26
HEMA 300 DEAE-3	20	289	26
HEMA 300 DEAE-4	20	269	28
H 40 A	25	101	60
H 40 DEA	25	144	42
H 40 DEtA	25	162	37
S HEMA 1000 DEAE	10	1150	13
S HEMA 300 DEAE	13	429	27

The column performance depends strongly on the sorbent porosity (exclusion limit). Under the test conditions, *i.e.*, a low capacity factor of the solute and a high mobile phase velocity, the height equivalent to a theoretical plate (HETP) is usually determined by the mass transfer. To demonstrate that mass transfer is hindered in sorbents with lower exclusion limits, the dependence of the reduced plate height, h, on the reduced mobile phase velocity, was measured for unmodified gels. Acetone (nearly unretained) was used as the solute to reduce the influence of the sorption kinetics. The steep lines for sorbents HEMA 40 and HEMA 40 E (Fig. 4) confirm the above hypothesis.

Increasing slopes of the dependence of the plate height, H, on the linear mobile phase velocity, u, were observed for DEAE derivatives with increasing ion-exchange capacities (Fig. 5). This may be due to the poor accessibility of some functional groups in sorbents with higher ion-exchange capacities.

Macroporous gels are supposed to have a nodular structure[19]. Porosity studies by gel permeation chromatography[27–30] suggest that no sharp interphase exists between intraporous mobile and gel phases; on the contrary, the polymeric chains can move freely in the mobile phase. The macropores are thus gradually changing into micropores. The micropore area (thickness 0.5–10 nm) will have the greatest influence on the kinetics of small inorganic anions.

Additional cross-linking of free polymeric chains on the surface of the individual nodules, (sorbents S HEMA), which was supposed to sharpen the phase boundary and to improve the mechanical stability of the gel structure, did not significantly change the mass transfer as can be seen in Table IV and Fig. 4, curves 3

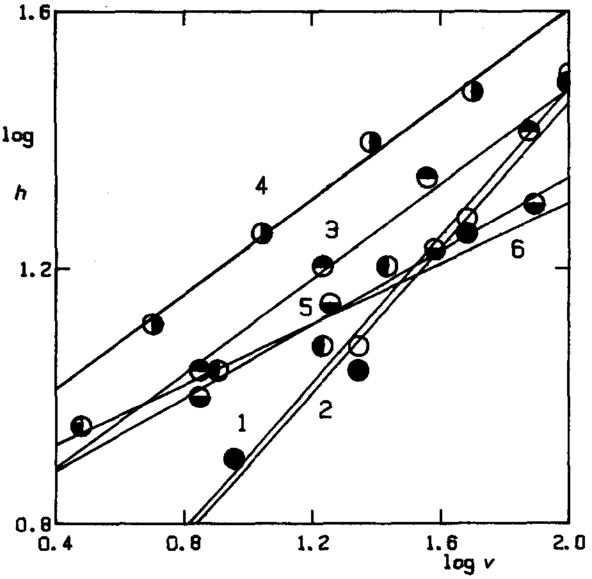


Fig. 4. Plots of log h against log v for different unmodified gels and an unretained solute. Eluent: water, flow-rate 0.5–2 ml min^{-1}. Solute: acetone, 0.01% aqueous solution. Injected volume: 5 μl. Detection: at 254 nm, sensitivity 0.16 a.u.f.s. D_m (acetone) (where D_m is the diffusion coefficient in the mobile phase) = 1.28 m^2 s^{-1} (ref. 41). Sorbents: HEDMA 40 (1); HEMA 40 E (2); HEMA 300 (3); S HEMA 300 (4); HEMA 1000 (5); S HEMA 1000 (6).

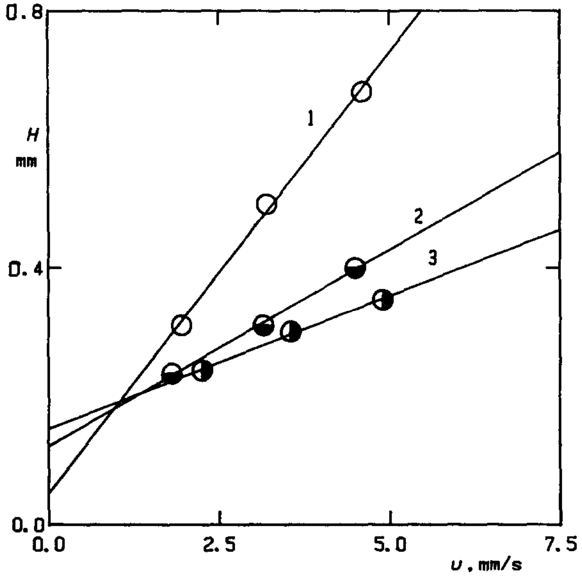


Fig. 5. Plots of H against u for HEMA 300 DEAE sorbents with different ion-exchange capacities. Eluent: sodium perchlorate, 0.5–20 mM. Solute: NO_3^-, k_{NO_3} = 2–4. Q_g = 1.5 (1), 0.56 (2) and 0.11 mmol g^{-1} (3).

and 4, 5 and 6. The best reduced plate heights achieved, 5–10 at a flow-rate of 0.1 ml min^{-1}, were 3–5 times worse than those achieved for silica-based sorbents with the same particle diameter. This is quite common for polymeric ion exchangers and it is probably caused by the presence of functional groups in highly cross-linked areas of the gel. The performance is even worse at higher flow-rates due to the poor mass transfer. However, for S HEMA 1000 DEAE sorbents with 10-μm particles, columns (lenght 150 mm) having more than 1000 theoretical plates at a flow-rate of 1 ml min^{-1} were prepared, which is quite sufficient for most ion chromatographic applications. The lifetime of columns produced according to the Experimental was relatively short, the performance usually being halved after passage of 6–8 l of mobile phase. This was due to a void volume formed at the top of the column; probably, better eluent filtering and pulse damping will partially solve this problem. The column capacity remained unchanged during the whole testing cycle, *i.e.*, *ca.* 6 l of different mobile phases and more than 100 injections, and did not decrease as described for silica-based sorbents[31,32].

Applications

Although the sorbents were prepared mainly for checking the effects of the functional groups on the selectivity, they were successfully applied to a variety of practical problems.

DEAE-modified sorbents with a low ion-exchange capacity, in combination with indirect photometric detection, enable the separation of many common inorganic anions (Fig. 6). For a direct photometric detection, especially when the ionic strength of the sample is high, sorbents with higher capacity are advantageous.

Using powerful eluting anions, successful methods for the determination of

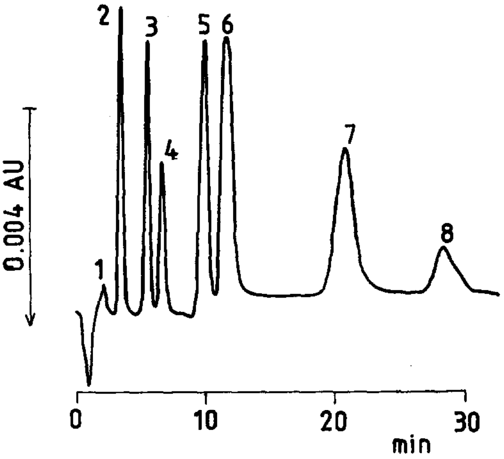


Fig. 6. Separation of common inorganic anions on S HEMA 1000 DEAE. Eluent: 0.2 m*M* potassium hydrogenphthalate, pH 6.0, adjusted by sodium hydroxide. Flow-rate: 1 ml min^{-1}. Pressure: 3.5 MPa. Indirect photometric detection at 254 nm, sensitivity 0.08 a.u.f.s. Injected volume: 30 μl. Peaks: 1 = HCO_3^-, 10 mg l^{-1}; 2 = Cl^-, 10 mg l^{-1}; 3 = NO_2^-, 20 mg l^{-1}; 4 = Br^-, 20 mg l^{-1}; 5 = NO_3^-, 40 mg l^{-1}; 6 = SO_4^{2-}, 20 mg l^{-1}; 7 = $S_2O_3^{2-}$, 100 mg l^{-1}; 8 = I^-, 100 mg l^{-1}.

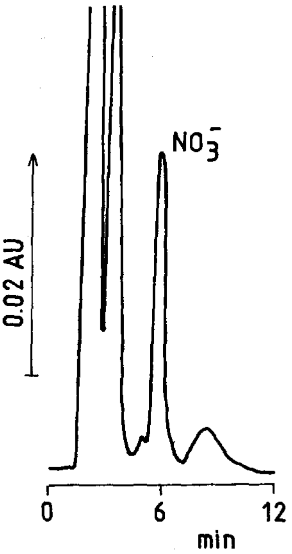


Fig. 7. Determination of nitrate in milk[33]. Sorbent: Spheron DEAE 1000 (Lachema), d_p = 25 μm, nitrogen content 1.3 mmol g^{-1}. Eluent: 40 m*M* sodium perchlorate, 5 m*M* phosphoric acid; flow-rate 0.5 ml min^{-1}. Pressure: 0.35 MPa. Detection at 205 nm, sensitivity 0.04 a.u.f.s. Injected volume: 5 μl. Sample: milk containing 13.5 mg l^{-1} of nitrate. Proteins and fats were removed by the Carrez reagent and filtered off before injection.

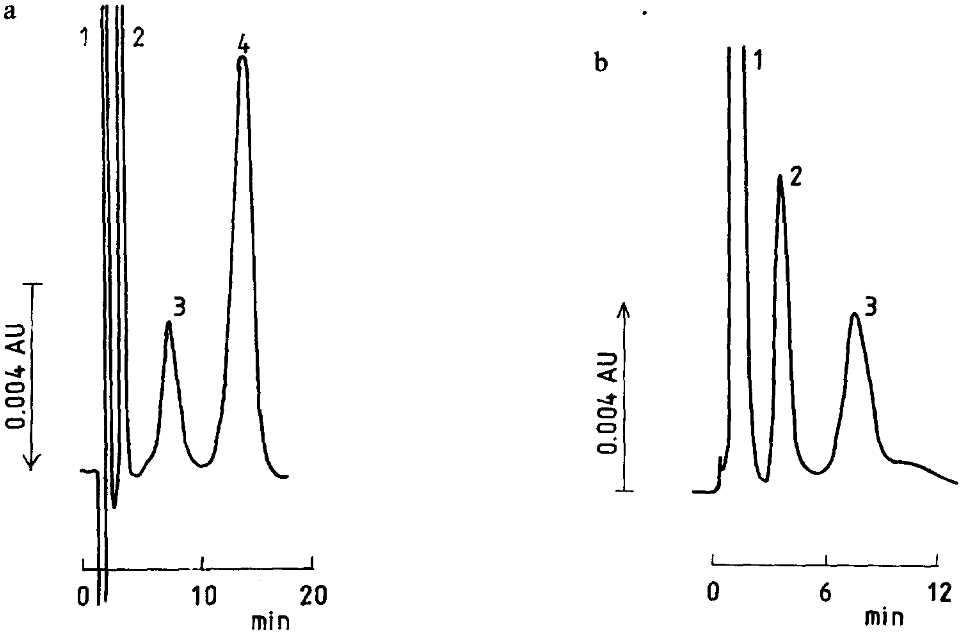


Fig. 8. Separation of sulphur oxoanions[34]. Model carbonate leaching solutions, containing 20 g l^{-1} carbonate, 4 g l^{-1} each of chloride and ammonium ions, were analyzed for sulphate, thiosulphate, trithionate and tetrathionate. Sensitivity: 0.04 a.u.f.s. A 5-μl volume of the sample was injected directly. Sorbent: Separon HEMA 300 DEAE, Q_g = 0.56 mmol g^{-1}, d_p = 20 μm. (a) Determination of sulphate and thiosulphate. Eluent: 0.5 m*M* sodium sulphosalicylate, pH 6.0; flow-rate 1.5 ml min. Pressure: 2 MPa Indirect photometric detection at 254 nm. (b) Determination of trithionate and tetrathionate. Eluent: 25 m*M* sodium perchlorate, 5 m*M* phosphate buffer, pH 6.0; flow-rate 1 ml min^{-1}. Pressure 1.5 MPa. Detection at 205 nm. Peak identification (a): 1 = HCO_3^-; 2 = Cl^-; 3 = SO_4^{2-}; 4 = $S_2O_3^{2-}$. Peak identification (b): 1 = $S_2O_3^{2-}$; 2 = $S_3O_6^{2-}$; 3 = $S_4O_6^{2-}$.

nitrates in milk[33] (Fig. 7) and for the separation of polythionates[34] (Fig. 8) were developed.

The sorbent with the DEtA functional group shows a relatively low selectivity for separation of anions of the same charge, thus anion mixtures exhibiting usually unacceptably different capacity factors can be separated isocratically, *e.g.*, polythionates (Fig. 9) or halides (Fig. 10).

In contrast to DEAE derivatives, sorbents prepared from epoxy-modified precursor copolymers showed an anomalous ion concentration peak when indirect photometric detection with phthalate as the eluent was used. Such peaks are created when the ion-exchange equilibrium on the column is disturbed by injecting the sample[35–39]. Jackson and Haddad[39] recently discussed the mechanism of formation of such peaks.

The anomalous behaviour of the ion concentration peak on modified sorbents prepared from precursor epoxy gels results in its slight retention, which interfered with the determination of chloride, and even of bromide and nitrite on sorbents with the HEMA 40 matrix (Fig. 11). The extent of retention depends on the concentration of the eluting anion. As this peak is formed by a zone with changed eluent anion concentration, it is considered to be caused by some interaction between phthalate

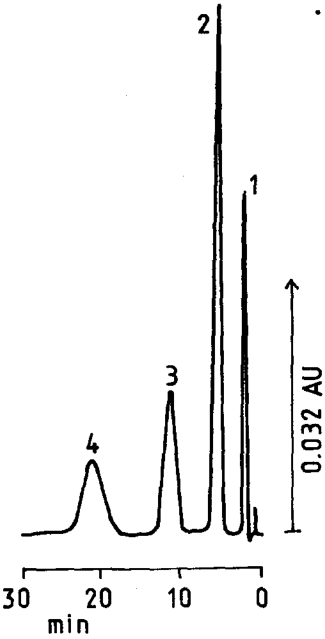
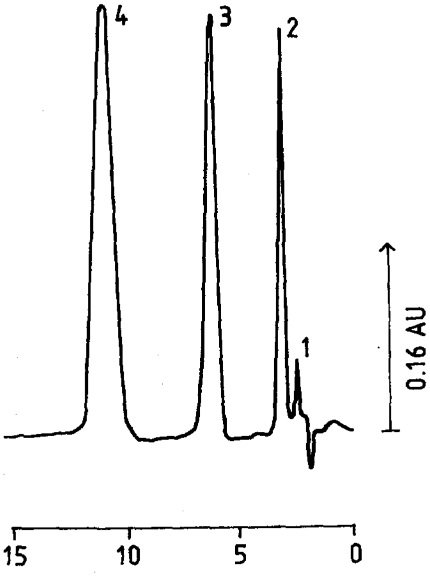


Fig. 9. Separation of polythionates formed by decomposition of trithionate. Sorbent: H 1000 DEtA, Q_s = 1 mmol g^{-1}, d_p = 20 μm. Eluent: 25 m*M* sodium perchlorate 5 m*M* phosphate buffer, pH 6.0; flow-rate 1 ml min^{-1}. Pressure: 1.5 MPa Detection at 205 nm, sensitivity 0.32 a.u.f.s. Injected volume: 5 μl Peaks: 1 = thiosulphate; 2 = trithionate; 3 = tetrathionate; 4 = pentathionate (not identified by the standard).



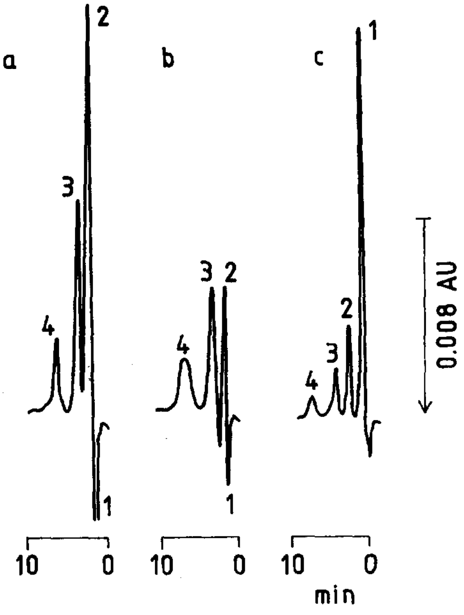


Fig. 10. Separation of halides and thiocyanate. Sorbent as in Fig. 9. Conditions as in Fig. 9 except eluent pH 3.0, detection at 185 nm and sensitivity 0.64 a.u.f.s. Peaks: 1 = Cl^-, 60 mg l^{-1}; 2 = Br^-, 60 mg l^{-1}; 3 = I^-, 240 mg l^{-1}; 4 = SCN^-, 240 mg l^{-1}.

Fig. 11. Ion concentration peak on different sorbents. Eluent: potassium hydrogenphthalate, pH 6.0; flow-rate 1 ml min^{-1}. Indirect photometric detection at 254 nm, sensitivity 0.04 a.u.f.s. Peaks: 1 = ion concentration; 2 = Cl^-; 3 = NO_2^-; 4 = NO_3^-. (a) Sorbent H 1000 A, c_{eluent} = 0.3 m*M*; (b) sorbent H 40 A, c_{eluent} = 0.3 m*M*; (c) sorbent HEMA 3000 DEAE, c_{eluent} = 1.0 m*M*.

and diol groups (formed by hydrolysis of unreacted epoxy groups). This behaviour limits the use of such sorbents for ion chromatography with indirect photometric detection.

CONCLUSION

The described modification of hydroxyethyl methacrylate copolymers enables the reproducible preparation of ion chromatography sorbents with different functional groups (with the exception of quarternary amines) and desired ion-exchange capacity. Such sorbents can be very useful for optimization of ion chromatographic separations.

An hindered mass transfer of anions was observed with sorbents having low exclusion limits and high ion-exchange capacities. Sorbents with high exclusion limits, *e.g.*, H 1000 and low ion-exchange capacities are recommended for ion chromatography. The usefulnes of such sorbents has been demonstrated by several examples of practical separations.

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