Research article

Hydrophobicity of polymer based anion-exchange columns for ion chromatography

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ABSTRACT

The regularities of the retention of alkanoic and alkanesulfonic acids homologues were investigated for the set of 36 anion-exchange columns produced by various manufacturers. The role of hydrophobic and electrostatic interactions in the retention and separation of organic anions was studied. The methylene selectivity increments $\alpha(CH_2)$ were measured for the studied columns with 10 mM sodium hydroxide eluent. The influence of matrix, surface area, polar group structure, ion-exchange capacity, the density of charged functional groups on the surface and other characteristics of anion-exchangers on resin hydrophobicity was considered.

A unified approach for the measurements of hydrophobic properties of anion-exchange resins is proposed and the ratio of chloride retention factor (k_{Cl}) to $\alpha(CH_2)$ was introduced as mixed-mode factor. The synergetic effect of electrostatic and hydrophobic interactions was observed.

1. Introduction

Hamish Small was an extraordinary and enthusiastic scientist, who had a truly broad range of research interests. His official bibliography is rather modest and amazing, only eight of his research papers [1, 2, 3, 4, 5, 6, 7, 8] were directly related to the development of ion chromatography, but without a doubt, these works dramatically changed the world of analytical chromatography.

In his early research career, Hamish studied properties of various ion-exchange resins [9] including the agglomeration of oppositely charged cation- and anion-exchanger's particles, which provided a breakthrough in the production of low-capacity composite anion-exchangers and development of a novel type of separation phase with excellent mass-transfer characteristics and extraordinary selectivity. His early work also focused on investigating the importance of various interactions between ion-exchangers and different compounds, such as the poisoning of cation-exchangers with oppositely charged cationic surfactants [10] and adsorption of nonionic compounds by ion-exchange resins and evaluation of their hydrophobicity [11].

The work presented here is dedicated to Hamish's input in the field of ion chromatography and devoted to the investigation of hydrophobicity for the comprehensive evaluation of anion-exchange resins, and its contribution to the mixed-mode separation of organic anions using IC columns.

A mixed-mode retention mechanism has been considered for many years as one of the most important principles for getting new separation selectivities in high-performance liquid chromatography (HPLC) by optimisation of various interactions between analytes and specially designed stationary phases [12, 13, 14, 15]. Particular attention has been paid to a combination of hydrophobic and electrostatic interactions, which has been widely used in the design of new reversed phase/ion-exchange chromatographic columns. These phases have received a huge popularity in HPLC of organic molecules possessing ionogenic groups, such as pharmaceuticals, various biomolecules (proteins, peptides, nucleosides etc), food additives, and others [16, 17, 18, 19, 20]. There are numerous mixed-mode stationary phases available, but for the majority of them, hydrophobic interactions are considered to be dominant for retention/separation in HPLC, while ion-exchange interactions play a secondary role and are responsible only for tuning of separation selectivity towards specific ionisable analytes [17, 20, 21, 22]. As the majority of bio- and pharmaceutical molecules separated by reversed phase HPLC (RP HPLC) are charged, mixed-mode stationary phases may be extremely useful as it was highlighted by Kelmers [23]. However, a lack of understanding of secondary electrostatic interactions for this type of stationary phases makes their practical use more difficult. Remarkably, ion-exchange capacities as a basic measure of electrostatic interactions for mixed-mode RP HPLC phases are typically not provided by researchers and manufacturers.

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Interestingly, a similar, but completely inverted situation is observed in ion-exchange and ion chromatography (IC). Here, electrostatic interactions are considered to be the only possible type of interactions for a huge number of organopolymer based ion-exchangers, while hydrophobic interactions are usually considered as insignificant by many researchers. Obviously, hydrophobic interactions in ion-exchangers depend mainly on the properties of the matrix, but these can also be regulated by varying the density of charged functional groups on the surface of the adsorbent, the length and properties of linkers, and the bulkiness and hydrophobicity of functional groups, particularly, in case of anion-exchangers. Again, the vast majority of works in the area of ion chromatography lack quantitative evaluation of the impact of secondary hydrophobic interactions on ion-exchange separations. Nevertheless, hydrophobic interactions, in line with other factors, play a significant role in IC separation selectivity of complex mixtures of organic ions for many adsorbents, thus they should be properly evaluated and considered in the development of new stationary phases and column selection for relevant analytical applications.

The role of hydrophobic interactions in adsorption of long chain alkanols, alkanoic and alkanesulfonic acids on polystyrene-divinylbenzene (PS-DVB) anion-exchange resins was systematically investigated by Small et al. [11], Janauer et al. [24, 25], Gregory and Semmens [26, 27], Sargent and Rieman [28] and Sanemasa [29, 30]. A strong correlation between distribution coefficients ($\log K_D$) and a number of carbon atoms (n_C) for these sorbates was established, while hydrophobic interactions were found to be dominant for sorbates with $n_C > 3$.

The development of a successful ion-exchanger is a state-of-art process, which can be time-consuming or include multi-step syntheses that may affect manufacturing repeatability and add to the overall product cost [31, 32, 33]. As a rule, to protect their intellectual properties, the IC columns' manufacturers keep any information about the exact surface chemistry confidential but also provide only limited data on characteristics of columns' packing such as matrix type, ion-exchange capacity, surface area, and porous structure of adsorbents. Some characteristics of commercial ion-exchangers developed for IC can be found in reviews [15, 34, 35, 36, 37, 38] and classic research papers of Fritz et al. [39, 40, 41], Pohl et al. [42, 43, 44] and Lee [45] on design of organopolymer based ion-exchangers. However, there are very few systematic investigations on the role of hydrophobicity of ion-exchange resins in resulting IC separation selectivity [46, 47, 48, 49, 50], but it could be particularly important for low capacity anion-exchangers normally used in IC.

With a growing interest in the use of IC for the separation of ionogenic pharmaceuticals, their by-products and counterions, it becomes important to know the hydrophobicity of the ion-exchange resins used [17, 18, 19, 51]. The aim of this investigation was to develop a universal approach for the characterisation of hydrophobic properties of ion-exchange resins with an emphasis on the evaluation of their capability to retain hydrophobic organic ions. The retention regularities of alkanoic and alkanesulfonic acids were studied for a set of 37 commercially available anion-exchange columns. The cooperative effects of hydrophobic and electrostatic interactions in the retention of organic anions are considered.

2. Theory

2.1. Ion-exchange selectivity

The pure ion-exchange process of two singly charged anions A and B taking place on solid anion-exchanger phase R can be described as:

$$X_R^+ A^- + B^- = X_R^+ B^- + A^- \tag{1}$$

At equilibrium (1) with thermodynamic activity coefficients being omitted the following concentration constant or selectivity coefficient can be expressed for the "pure" ion-exchange process as follows:

$$K_{B/A}^c = \frac{[B^-]_R [A^-]_e}{[A^-]_R [B^-]_e} \tag{2}$$

The retention factor k for anion B is defined (3) by distribution coefficient ($K_D^{B,ie}$) and phase ratio ($\omega = V_R / V_0$, where V_0 is the dead volume of the column and V_R is the volume of the resin in the column) which is constant for a selected chromatographic column and separation conditions such as pressure, temperature, pH and ionic strength of the eluent and some other factors [52]. While it is accepted that a large fraction of the resin particle in IC material is non-functional, thus all comprehensive IC columns will possess a surface-modified or surface-layered structure where all charged groups responsible for ion-exchange are located in a relatively narrow zone on the outer surface. Taking into account the variation in structure, the concentration of surface groups, the fact that every single ion-exchange group has its own solvent and hydrated ion environment with an unknown number of coordinated solvent molecules, counterions, etc., the calculation of the true ion-exchange phase volume becomes practically impossible. To date no single method for calculation of true volume of the stationary phase has been reported, so a possible solution is to accept the volume of the stationary phase as a volume of all insoluble pasts in the chromatographic column. Thus, the retention of ions is proportional to the amount of charged groups in the chromatographic column and these groups belong to the insoluble stationary phase.

$$k_{ie} = K_D^{B,ie} \omega = \frac{[B^-]_R V_R}{[B^-]_e V_0} = K_{B/A}^c \frac{[A^-]_R V_R}{[A^-]_e V_0} \tag{3}$$

If anion B^- is present in a significantly lower concentration than the eluent concentration $[A^-]_e$ (mmole/L) and ion-exchange resin is saturated with A^- ion, then $[A^-]_R$ is naturally equal to the ion-exchange resin capacity Q (meq/g), which is constant under selected separation conditions. In this case, combining Eqs. (2) and (3) gives the following equation in logarithmic form (4):

$$\begin{aligned}
\log k_{ie} &= \log K_{B/A}^c + \log \omega + \log Q - \log[A^-]_e \\
&= \mathrm{const}(K_{B/A}^c,\ \omega,\ Q) - \log[A^-]_e
\end{aligned} \tag{4}$$

For the molecules of organic acids selected as sorbates in this study the negatively charged carboxylic and sulfonic acid groups are responsible for electrostatic interactions with the functional groups of anion-exchange resins. It should be noted that the effective charge or acidity of carboxylic and sulphonic acid groups in the molecules of first homologues ($n_C = 0–1$) is higher than for the rest of homologues with $n_C \geq 2$ due to $+I$ inductive effect of alkyl groups linked to the acidic group. This effect is well illustrated by pK_a changes for alkanoic acids with the increase of alkyl chain length as shown in Table 1.

It should be noted that some of the common anion-exchangers can be considered as zwitterionic ion-exchangers [53]. For example, a popular class of, so called, agglomerated anion-exchangers possesses a core-shell structure with sulfonated PS-DVB microspherical particles representing a core coated with an electrostatically retained layer of positively charged latex nanoparticles. The diameter of latex particles is varied from 60 nm (OmniPac PAX-500) to 530 nm (IonPac AS7) as presented in Table 2, so the space between these particles is easily accessible for ions that can influence anion-exchange selectivity. For example, IonPac AS7 column has the cation-exchange capacity of 43 µequiv/col, which is about a half of its anion-exchange capacity of 100 µequiv/col and such a zwitterionic nature of this column was used for simultaneous separation of anions and cations [37, 54].

Electrostatic repulsion of anions from alkanoldialkylammonium functional groups (Type II anion-exchangers) is also possible, when a zwitterionic structure is formed due to dissociation of hydroxyls in the concentrated alkaline eluent. The effect of negatively charged groups' presence in anion-exchange on selectivity is quite strong, especially for bulky anions [55]. This, however, is applicable to only rather short linkers (e.g., one methylene group) and an internal salt cannot be formed.

Table 1. Properties (acid dissociation constants and partition coefficients) of alkanoic and alkanesulfonic acids used as analytes.

Alkanoic acids	pK_a[a]	$\log P_{exp}$[b]	$\log P_{cal}$[b]	n_C	Alkanesulfonic acids	pK_a[a]	$\log P_{cal}$[b]
Formic	3.75	-0.54	-0.46	0			
Acetic	4.76	-0.17	0.09	1	Methanesulfonic		-2.38
Propionic	4.86	0.33	0.58	2	Ethanesulfonic		-1.89
Butyric	4.83	0.79	1.07	3	Propanesulfonic	1.53	-1.40
Valeric	4.84	1.39	1.56	4	Butanesulfonic		-0.91
Caproic	4.85	1.92	2.05	5	Pentanesulfonic		-0.42
Heptanoic acid	4.89	2.42	2.54	6	Hexanesulfonic		0.07
Octanoic acid	4.89	3.05	3.03	7	Heptanesulfonic		0.56
				8	Octanesulfonic		1.06

[a] Data adapted from NIST database.
[b] both experimental ($\log P_{exp}$) and calculated ($\log P_{cal}$) values are obtained from EPA KOWWIN database and software.

2.2. Hydrophobic selectivity

Certainly, an n-alkyl chain of the sorbate molecule is also contributing to its retention due to hydrophobic interactions with the ion-exchanger. Hydrophobic properties of anion-exchange resins depend on multiple factors including the polarity of a polymer matrix, the configuration of ion-exchange layer (classic neutral carrier with bonded functional groups, a grafted polymer layer, *etc.*) or shell (agglomerated ion-exchangers), porosity, and related surface area, the surface density of ion-exchange groups, structures of functional groups and linkers and others. Therefore, the hydrophobicity of anion-exchangers only can be evaluated by using empirical methods. One of the methods that is widely used in reversed phase HPLC is the calculation of methylene selectivity or methylene increment $\alpha(CH_2)$ through the measurement of chromatographic retention for a series of homologues with a linear alkyl chain $-(CH_2)_nH$ with n_C methylene groups [56]. The retention of homologues can be expressed in the form of Eq. (5):

$$\log k = \alpha(CH_2)n_C + const \tag{5}$$

where $\alpha(CH_2)$ is the retention increment for one methylene group, and the constant reflects the ion-exchange contribution of electrostatic interactions between charged functional group of the sorbate and the ion-exchanger surface. The slope of the dependence (eqn. 5) $\alpha(CH_2)$ can be regarded as a measure of the hydrophobic selectivity or affinity of anion-exchange resins towards alkyl chains in homologues. The linearity of the $\log k$ vs. n_C plot has been validated for various polymer based adsorbents [50, 57] including PS-DVB based anion-exchange resin Dowex 1 × 8. The $\alpha(CH_2)$ values measured for adsorption of alkanols from water on Dowex 1 × 8 in sulphate and chloride forms were 0.470 [28] and 0.375 [29], respectively. The $\alpha(CH_2)$ of 0.167 for alkanols adsorbed from water on Dowex 1 × 2 in chloride form was reported by Small *et al.* [11]. As discussed earlier, data points for ionogenic homologues with $n_C \leq 3$ are normally outside the linear range of $\log k - n_C$ plots for ion-exchange resins due to difference in the charge of the terminal functional group following changes in pK_a values (Table 1).

According to Eq. (5), $\alpha(CH_2)$ value reflects the separation selectivity ($\alpha_{n+1/n}$) of two homologues with n and $n+1$ carbon atoms in alkyl chain according to the following expression:

$$\log \alpha_{n+1/n} = \log (k_{n+1}/k_n) = \log k_{n+1} - \log k_n = \alpha(CH_2) \tag{6}$$

This Eq. (6) is correct only for neutral or ionogenic homologues with equal contribution of electrostatic interactions into retention. This requirement is met for alkanoic acid homologues having practically equal pK_a values of carboxylic groups at $n_C > 3$ (see Table 1).

2.3. Mixed-mode retention mechanism

In a real IC separation of anions, it is practically impossible to observe a pure single mode retention mechanism, especially for complex anions like alkanoates and alkanesulfonates. Apart from the most dominant electrostatic and hydrophobic interactions, a certain contribution in the retention can be provided by hydrogen bonding between anions and hydroxyls- in functional groups, linkers, and residual polar groups at the surface of the ion-exchanger matrix. Various steric hindrance effects related to the different structures of complex multi- element anions can influence retention and separation selectivity. Thus, the carboxylate group with the trigonal planar structure and the sulfonate group with trigonal pyramidal structure are expected to interact differently with an ionic polymer-grafted type of ion-exchangers or microporous anion-exchangers, where pore structure can influence the accessibility of functional groups.

Since its formal introduction by Cox and Stout in 1987 [58] the combination of reversed phase and ion-exchange interaction became the most popular variant of mixed-mode HPLC. The authors suggested that the resulting retention factor is determined not only by *"the sum of individual distribution coefficients but also by the addition of the products of interacting distribution coefficients and phase ratios"* [58]. So, in the case of mixed ion-exchange and hydrophobic interactions we have the following dependence:

$$k_{total} = K_D^{B,ie}\varphi_1 + K_D^{B,hyd}\varphi_2 + K_D^{B,ie}K_D^{B,hyd}\varphi_1\varphi_3 \tag{7}$$

If $K_D^{B,ie}$ is related to the retention of the first homologue in a series, which is a hydrophilic inorganic anion (formate for alkanoic acids or bisulfate for alkanesulfonic acids), and $K_D^{B,hyd}$ is related to the retention of neutral hydrophobic alkane, the phase ratios for these two separate interactions can be accepted as equal ($\varphi_1 = \varphi_2$) for the same stationary phase. In the case of cooperative interaction the phase ratio φ_3 in (7) is different as alkyl radical from organic acid anion coordinated around the functional group has a limited possibility for interaction with hydrophobic sites. Then the following Eq. (8) is true:

$$k_{total} = k_{ie} + k_{hyd} + pk_{ie}k_{hyd} \tag{8}$$

where p is a probability of cooperative ionic and hydrophobic interactions for a selected sorbate. If ion-exchange is a primary and dominant interaction for alkanoic and alkanesulfonic acids, the cooperative interaction coefficient p in (8) reflects the accessibility of hydrophobic sites around anion-exchange groups for interaction with alkyl radicals, as shown in Figure 1. In this case, factor p is proportional to the surface area, free of charged groups, or reciprocal to the surface density of charged groups. It also depends on the hydrophobicity of the polymeric core and the linker connecting the surface and the functional group, the length and flexibility of the linker, as well as the alkyl chain length in sorbates.

The ion-exchange capacity of commercially available IC columns varies in a broad range from 13 (IonPac AS4) to 700 μeq/col (Metrohm A Supp 8) and so can their hydrophobicity as shown by calculated methylene selectivities which range from 0.050 (IonPac AS21) to 0.646 (ICSep

Table 2. Properties of anion-exchange columns and pre-column as specified by producers [15, 53, 65, 69, 70, 71].

Columns	Structure of ion-exchange group	Column properties				Capacity	
		Size, mm	D_p, µm	Matrix and morphology		µeq/col	µeq/g
PRP-X100	$-CH_2N^+(CH_3)_3$	150 × 4.1	5	PS-DVB, 415 m²/g, d_{pore} 10 nm, V_{pore} 0.79 cm³/g		75	190
ICSep AN1[c]	$-CH_2N^+(CH_3)_2CH_2OH$	250 × 4.6	9	PS-DVB, 415 m²/g, d_{pore} 8 nm		35	50
Metrosep A Supp 8	$-N^+R_3$	150 × 4.0	5	PS-DVB		700[d]	
Metrosep A Supp 5	$-N^+R_3$	100 × 4.0	5	Polyvinylalcohol		34[d]	94–107
TSK-Gel IC-Anion-PW	$-N^+(C_2H_5)_2CH_3$	150 × 3.0	10	PMMA		>32[a]	30 ± 3 µeq/ml
Separon HEMA-S 1000 Q-L[b]	$-CH(OH)CH_2N^+(CH_3)_3$	80 × 8.0	10	HEMA-EDMA, 200 m²/g, d_{pore} 100 nm		32	100–150
IonPac AS4A	$-CH_2NR_2R'OH$	250 × 4.0	15	sulphonated PS - DVB (4%, nonporous) with layer of 180 nm PVBC-DVB (0.5%) beads		13–20/~10*	10–50
IonPac AS4A SC	$-CH_2NR_2R'OH$	250 × 4.0	13	sulphonated PEVB - DVB (55%, microporous) with layer of 160 nm PVBC-DVB (0.5%) beads		20–24/~10*	
IonPac AS5	$-CH_2NR_2R'OH$	250 × 4.0	15	sulphonated PS - DVB (2%, nonporous) with layer of 120 nm PVBC-DVB (1.0%) beads		20/~10*	
IonPac AS5A-5u	$-CH_2NR(R'OH)_2$	150 × 4.0	5	sulphonated PS-DVB (2%, nonporus) with layer of 60 nm PVBC-DVB (4.0%) beads		35/~10*	
IonPac AG7 (same as AS7)	$-CH_2N^+R_3$	50 × 4.0	10	sulphonated PS-DVB (2%, nonporous) with layer of 530 nm PVBC-DVB (5%) beads		20	50/8.6*
IonPac AS9	$-CH(OH)CH_2N^+R_3$	250 × 4.0	15	sulfonated PS-DVB (2%, nonporous) with layer of 140 nm PGDMA(20%) beads		20–26	
IonPac AS9-SC	$-CH(OH)CH_2N^+R_3$	250 × 4.0	13	sulphonated PEVB-DVB (55%, microporous) with layer of 110 nm aminated PGDMA (20%) beads		30–35/residual*	
IonPac AS9-HC	$-CH(OH)CH_2N^+R_3$	250 × 4.0	9	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) with layer of 90 nm aminated PGDMA (18%) beads		190	
IonPac AG10 (same as AS10)	$-N^+R_2R'OH$	50 × 4.0	8.5	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) with layer of 65 nm microporous PVBA-DVB beads with 5% cross-linking		34/~10*	
IonPac AS11	$-CH_2N^+CH_3(CH_2OH)_2$	250 × 4.0	13	sulphonated PEVB-DVB (55%, microporous) with layer of 85 nm aminated PVBA-DVB (6%) beads		45/~10*	
IonPac AS11 HC	$-CH(OH)CH_2N^+CH_3(CH_2CH_2OH)_2$	250 × 4.0	9	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) with layer of 70 nm aminated PVBGE-DVB (6%) beads		290	
IonPac AS12A	$-CH_2N^+(C_2H_5)_3$	200 × 4.0	9	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) with layer of 140 nm microporous PVBC-DVB (0.15%) beads		52	
IonPac AS14	$-N^+R_3$	250 × 4.0	9	PEVB-DVB (55%, d_{pore} 10 nm, 450 m²/g) grafted layer		65	
IonPac AS14A	$-N^+R_3$	250 × 4.0	7	PEVB-DVB (55%, d_{pore} 10 nm, 450 m²/g), grafted layer		120	
IonPac AS15–5 um	$-CH_2N^+(CH_3)(CH_2CH_2OH)_2$	150 × 3.0	5	PEVB-DVB (55%, d_{pore} 10 nm, 450 m²/g) modified with PVBC and methyldiethanolamine		70	
IonPac AS16	$CH(OH)CH_2N^+(CH_3)(CH_2CH_2OH)_2$	250 × 4.0	9	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) with layer of 80 nm PVBGE-DVB (1%) beads functionalized with methyldiethanolamine		170	
IonPac AS17	$-N^+R_2R'OH$	250 × 4.0	10.5	sulphonated PEVB-DVB (55%, microporous), layer of 75 nm PVBA-DVB (6%) beads		30	
IonPac AS-18 Fast	$-N^+R_2R'OH$	150 × 2.0	7.5	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) with layer of 65 nm PVBA-DVB (8%) beads		45	
IonPac AS19	$N^+(CH_3)(CH(CH_2OH)O-)_3$	250 × 4.0	7.5	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g), layer of hyperbranched BDDE-methylamine polymer		240	160 µeq/ml
IonPac AS20	$-N^+R_2R'OH$	250 × 4.0	7.5	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g), layer of hyperbranched BDDE-amine polymer		310	

(continued on next page)

Table 2 (*continued*)

Columns	Structure of ion-exchange group	Column properties					
		Size, mm	D_p, µm	Matrix and morphology	Capacity		
						µeq/col	µeq/g
IonPac AS21	$-N^+R_2R'OH$	250 × 2.0	7	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g), layer of hyperbranched BDDE-methylamine polymer	45	
IonPac AS22	$-CH(OH)CH_2N^+R_2R'OH$	250 × 4.0	6.5	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g), layer of hyperbranched BDDE-amine polymer	210	
IonPac AG22	$-N^+R_2R'OH$	50 × 4.0	11	sulphonated PEVB-DVB (55%, nonporous), layer of hyperbranched BDDE-amine polymer	6	
IonPac AS23	$-CH(OH)CH_2N^+R_2R'OH$	250 × 4.0	6	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) layer of hyperbranched BDDE-amine polymer	320	
IonPac AS25	$-CH(OH)CH_2N^+R_2R'OH$	250 × 4.0	7.5	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) layer of hyperbranched polymer	350	
IonPac AG25	$-N^+R_2R'OH$	50 × 4.0	13	sulphonated PEVB-DVB (55%, d_{pore} <0.1 nm) layer of hyperbranched polymer	3.5	
IonPac AS26	$-CH(OH)CH_2N^+R_2R'OH$	250 × 4.0	7.5	sulphonated PEVB-DVB (55%, d_{pore} 200 nm, 20 m²/g) layer of hyperbranched polymer	250	
OmniPac PAX 100	$-CH_2N^+R_2R'OH$	250 × 4.0	8.5	sulphonated PEVB-DVB (55%, microporous, < 1 m²/g) with layer of 60 nm PVBC-DVB (4%) beads functionalized with tertiary amine	40	25
OmniPac PAX500	$-CH_2N^+R_3$	250 × 4.0	8.5	sulphonated PEVB-DVB (55%, d_{pore} 6 nm, 300 m²/g) with layer of 60 nm PVBC-DVB (4%) beads functionalized with tertiary amine	40/<1*	16
ProSwift SAX 1S	$-N^+R_3$	50 × 4.6	n/a	PS-DVB based monolith, transport pore size 1600–2700 nm	no data	

BDDE – 1,4-Butanediol diglycidyl ether.
  [a] Calculated by using ion-exchange capacity (30 ± 3 µeq/mL) and empty column volume.
  [b] 150 × 3.0 mm ID column is also known as Metrosep Dual 1 column.
  [c] This is analogue of IonPac AS9-HC according to the producer.
  [d] Measured for Cl⁻.
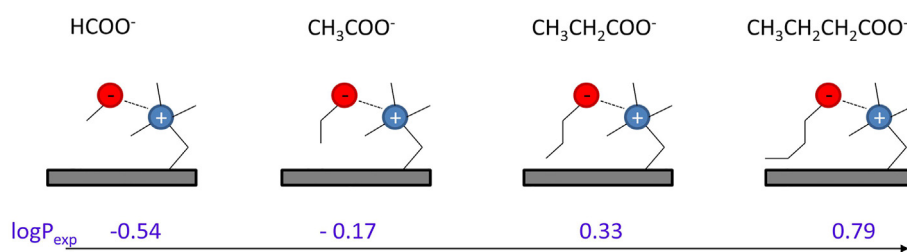 [*] Residual cation-exchange capacity from sulphonated core of the particle.

Figure 1. Mixed-mode interactions of alkanoic acids with hydrophobic anion-exchanger.

AN1). As such, for practical use, it is important to know the ratio of electrostatic and hydrophobic interactions to understand the separation selectivity of organic anions.

Relative hydrophobicity of ion-exchangers and its contribution to the retention of organic ions under defined separation conditions (constant temperature, eluent composition, etc.) can be expressed by using the ratio of methylene selectivity $\alpha(CH_2)$ and distribution coefficient ($K_D^{B,ie}$, see eqn. 3) for a small spherical and singly charged anion such as chloride, which is retained exclusively due to electrostatic interactions. If phase ratio ω is assumed as an insignificant parameter for the comparison of the ion-exchange columns' selectivity, the retention factor k_{Cl} can be used instead of $K_D^{Cl,ie}$ and the following factor F can be introduced:

$$F = \frac{\text{ion exchange selectivity}}{\text{methylene selectivity}} = \frac{K_D^{Cl,ie}}{\alpha(CH_2)} = \frac{k_{Cl}}{\alpha(CH_2)} \quad (9)$$

An alternative approach was suggested by Carr *et al.* [59] who used the slope of a plot k_{ie} *vs.* $1/[A^-]_e$ (eqn. 3) as a measure of the ion-exchange interaction strength for the characterisation of mixed-mode stationary phases instead of using k_{Cl} in Eq. (9). However, this slope is proportional to the ion-exchange equilibrium constant $K_{B/A}^c$.only under the condition of constant ion-exchange capacity Q at a varied concentration of the eluent $[A^-]_e$ used for plotting k_{ie} vs. $1/[A^-]_e$. Notably, this condition is not valid for Type II resins in sodium hydroxide eluent of varied concentration, as hydroxyl group in alkanolalkylammonium ion-exchange groups can dissociate with the formation of zwitterionic sites that changes the effective ion-exchange capacity of the resins. It should be noted that the possibility of dissociation of hydroxyl groups in choline type substances mimicking the structure of ion-exchange groups in Type II resin is well known from the literature. For example, pKa values of the hydroxyl group in formocholine $(CH_3)_3N^+CH_2OH$ and in choline $(CH_3)_3N^+(CH_2)OH$ are

equal to 10.1 ± 0.02 and 12.10 ± 0.02, respectively [60]. The pK_a value reported for choline hydroxide is 11.2, which is significantly less than that of chloride form [61]. Undoubtedly, the possibility of formation bipolar ion-exchange cites is high for Type II anion-exchange resins.

3. Results

3.1. Eluent selection

According to IC theory, the retention of anions is proportional to the column ion-exchange capacity. For the columns selected in this study, it varies from 3.5 to 700 μeq/column as it can be seen in Table 2. Such a broad range of column capacities makes difficult the choice of eluent concentration, which can provide reasonable retention ($0.5 < k < 100$), suitable for reliable detection of chromatographic peaks. The two most common IC eluents namely hydrocarbonate/carbonate and hydroxide at varying concentrations were initially tested, and 10 mM sodium hydroxide was found to be a good compromise for the majority of columns used.

As the application of suppressors for conductimetric detection can contribute to the increase in retention of hydrophobic solutes, suppressed conductivity detection could not be used in this study. The use of 10 mM NaOH as eluent allowed sufficient sensitivity of indirect (non-suppressed) conductimetric detection of analytes due to a significantly higher value of equivalent conductivity of hydroxide. Additionally, as this eluent is UV transparent, selective photometric detection of alkanoic acids could be carried out at 210 nm. Finally, the use of singly charged counter-ion based eluent simplifies modeling of ion-exchange equilibrium occurring in anion-exchange columns as all interactions take place between singly charged ionic species with a minimal possibility of their coordination around two charged functional groups located nearby on the surface.

The important condition for the use of anion-exchange columns in this work is connected with their hydrolytic stability in the eluent. The data on hydrolytic stability of studied anion-exchange columns are summarised in Table 3. The stability can be defined by the stability of the matrix itself and grafted polymer layer or latex. According to manufacturers' data, all studied anion-exchangers, except IonPac AS5, AS9, and AS9-SC, are compatible with 10 mM sodium hydroxide as the eluent. In fact, the manufacturer's manuals (ThermoFisher Scientific, USA) recommended "not to use hydroxide eluents" only with the IonPac AS9-SC column used in this work. Other columns with limited pH stability (pH up to 12) from this manufacturer described as designed for the application with carbonate/bicarbonate eluents. For an IonPac AS9-SC column (probably, also for AS9 column) the hydrolytic stability range is pH 2–11, which is below pH 12 of 10 mM NaOH eluent. This column represents agglomerated ion-exchanger composed of sulfonated nonporous PEVB-DVB core and a layer of poly(glycidylmethacrylate) (PGMA) based anion-exchange latex nanoparticles. Obviously, PEVB-DVB core matrix is hydrolytically stable, so the upper pH limit of 11 can be related only to the stability of PGMA based anion-exchange latexes. However, the pH range of hydrolytic stability documented for other methacrylate-based anion-exchangers used in this study, namely TSK-Gel IC-Anion-PW and Separon HEMA-S 1000 Q-L is between 2 and 12. An additional information on hydrolytic stability of methacrylate based anion-exchangers solutions with concentration up to 1M NaOH can be found in the literature [62]. So, one can expect stable work of IonPac AS5, AS9, and AS9-SC column for short time with 10 mM hydroxide eluent. No ion-exchange capacity loss was observed for these columns during relatively short experiments with this eluent.

3.2. Column selection

Two main criteria were applied to the selection of anion-exchange columns for the study of hydrophobic properties of ion-exchange resins. The first criterium is related to the matrix properties with a key focus on the possible hydrophobicity of bare materials. The first group of the studied anion-exchangers has a hydrophilic base such as poly-vinylalcohol (PVA, Metrosep A5 Supp 5), polymethylmethacrylate, or PMMA (TSK-Gel IC-Anion-PW), co-polymer of 2-hydroxyethyl methacrylate (HEMA), and ethylene dimethacrylate (EDMA) (Separon HEMA-S 1000 Q-L).

However, hydrophobic matrices including PS-DVB and poly(ethylvinylbenzene-divinylbenzene) or PEVB-DVB are most popular for the preparation of anion-exchangers including well known PRP-X100, Metrosep A Supp 8, IonPac AS, and AG family resins. It should be noted

Table 3. The hydrolytic stability of studied anion-exchangers according to manufacturer and literature data.

Matrix	Structure of anion-exchange group	Anion-exchangers	pH range
PS-DVB	Covalently bonded groups	PRP-X100,	1–13
		Metrosep A Supp 8	1–14
	Immobilised or grafted layer	IonPac AS14A; AS15–5 um; AS19; AS20; AS21; AG22; AS22; AS23; AG25; AS25; AS26;	0–14
		IonPac AS14	2–12
	Adsorbed polymer layer	ICSep AN1[c]	0–14
	Monolayer of electrostatically retained latex nanoparticles	PVBC-DVB beads:	
		IonPac AS5[a]	2–11
		IonPac AS4A; AS4A SC; AS5A-5u; AS7; AS12A; OmniPac PAX 100 and PAX 500	0–14
		PGDMA beads:	
		IonPac AS9; IonPac AS9-SC[a];	2–11
		IonPac AS9-HC	0–12
		PVBA-DVB:	
		IonPac AG10; AS11; AS17; AS-18 Fast;	0–14
		PVBGE-DVB beads:	
		IonPac AS11 HC; AS16;	0–14
	Porous monolith	ProSwift SAX 1S	2–12
Polyvinylalcohol	Covalently bonded groups	Metrosep A Supp 5	3–12
Polymethacrylate	Covalently bonded groups	TSK-Gel IC-Anion-PW	2–12
		Separon HEMA-S 1000 Q-L	1–12

[a] Manufacturer does not recommend to use hydroxide eluents.

that IonPac type agglomerated anion-exchangers have a core-shell structure with microparticles of sulfonated PS-DVB or PEVB-DVB as a central core and a layer of electrostatically retained nanosized anion-exchange latex. The latex matrix bearing anion-exchange functional groups is usually different from that in the central core. Ion-exchange latex matrix can be either hydrophobic such as poly(vinylbenzyl chloride – divinylbenzene) (PVBC-DVB), poly(vinylbenzylammonium – divinylbenzene) (PVBA-DVB), poly(vinylbenzyl glycidyl ether – divinylbenzene) (PVBGE-DVB), poly(glycidoxystyrene) styrene epoxide-divinylbenzene (PGS) or hydrophilic including poly(glycidylmethacrylate) or PGDMA, polymethacrylate or PMA beads. Additionally, the surface chemistry can be further modified to tune ion-exchange selectivity [63]. Obviously, it would be extremely difficult to identify the resulting hydrophobicity of these composite anion-exchangers without special experiments considered later in this work.

Another factor, which affects the hydrophobic properties of anion-exchangers is related to the nature of anion-exchange functional groups themselves as well as linkers connecting functional groups with the surface of ion-exchanger. Traditionally, two types of anion-exchange functional groups have been used in IC. Type I anion-exchangers have bonded trialkylammonium- functional groups, which are more hydrophobic than dialkyl(hydroxyalkyl)ammonium functional group with the same number of carbons used for the preparation of Type II anion-exchanger. Historically, Type I anion-exchangers are considered preferable for the use in carbonate eluent, while Type II are commonly used with hydroxide eluents.

The surface concentration of functional groups or bonding density can also influence the balance of the hydrophobic/electrostatic interactions balance in anion-exchange resins and through this affect ion-exchange selectivity, especially for complex and multivalent anions due to possible multipoint interactions [64]. Obviously, ion-exchange capacity is proportional to the surface concentration for the same type of bare resin used for the preparation of ion-exchangers. The upper limit of ion-exchange capacity (μequiv/g) for a studied resin is related to the ability to elute strongly retained analytes in a reasonable time. Therefore, the size of the corresponding anion-exchange column should also be considered along with an ion-exchange capacity of anion-exchanger, as chromatographic retention of ions is proportional to the total number of oppositely charged functional groups in the column. The values of column capacities are presented in Table 2 and were within the range from 20 to 350 mequiv/column except for Metrosep A Supp 8 with column capacity 700 μequiv/col.

Thus, a set of the studied IC stationary phases included 36 columns packed with anion-exchangers having different properties such as type of organopolymer matrix, its micro- and macroporous morphology, particle size, structure of bonded layer and grafted functional groups, and ion-exchange capacity. All of these parameters can influence the hydrophobic interactions and separation selectivity of ions.

3.3. Hydrophobicity of anion-exchangers

The effect of anion-exchanger's hydrophobicity on retention of organic acids homologues was studied for anion-exchange columns listed in Table 2. For this purpose, the retention factors for alkanoates and alkansulfonates were recorded under the optimum flow rate for each column and the average value of triplicate measurements was used to build $\log k - n_C$ plots. Then for linear parts of plots the slopes $\alpha(CH_2)$ are calculated. The obtained values of $\alpha(CH_2)_{CA}$ for alkanoic and $\alpha(CH_2)_{SA}$ for alkanesulfonic acids are presented in Table 4. For convenience, the data are divided into groups according to the structure of anion-exchangers.

3.3.1. Effect of polymer matrix

As discussed earlier, apart from electrostatic attraction to positively charged functional groups the most significant effect on the retention of

organic anions is due to matrix hydrophobicity of ion-exchangers. To evaluate this effect, the retention of model compounds was measured and slopes for $\log k - nC$ plots were calculated for anion-exchange columns with different polymer matrices including PS-DVB (ICSep AN1), PVA (Metrosep A5, PVA), PMMA (TSK Gel IC-Anion-PW, PMMA), HEMA-EDMA co-polymer (Separon HEMA-S 1000 Q-L) and PEVB-DVB coated with a layer of hyperbranched ionic polymer (IonPac AS21). For the latter anion-exchanger, based on results obtained for the retention of homologues under the same experimental conditions, the layer formed by the surface reaction of BDDE with methylamine (MA) was considered to be thick enough to provide good shielding of PEVB-DVB core microparticles, so in this case, the experiments measured hydrophobicity of the outer hyperbranched layer. The obtained $\log k - n_C$ plots (Figure 2) look similar both for alkanoates and alkanesulfonates, but for all anion-exchangers sulfonates are retained stronger than carboxylates. This is due to the higher affinity of anion-exchangers towards the bulkier and more polarisable sulfonic acid group in analytes. The $\alpha(CH_2)_{CA}$ values calculated from retention of valeric and hexanoic acids are increased in the following row: BDDE-MA (0.050) < PVA (0.130) < PMMA (0.175) < HEMA (0.209) < PS-DVB (0.646), with ICSep AN1 column having demonstrated the highest hydrophobicity among studied anion-exchangers. Corresponding $\alpha(CH_2)_{SA}$ values for alkanesulfonic acid homologues were also higher as compared with $\alpha(CH_2)_{CA}$ and changed in the same order (see Table 4).

3.3.2. Effect of ion-exchange capacity

The matrix hydrophobicity measurement results need some correction by taking into consideration differences in charge densities and surface areas of studied adsorbents. Obviously, for the surface area of ion exchangers (S, m^2/g) the density of charged groups is proportional to ion-exchange capacity (Q/S, μequiv/g), so the surface area (S) variation may increase or decrease apparent hydrophobicity of anion-exchanger depending on properties of the polymer matrix. The accurate evaluation of this effect is complicated due to the absence of accurate data on the porous structure of studied anion-exchangers and on the exact chemistry of substitutes in $-N^+R_3$ functional groups. However, it was possible to compare hydrophobicity for anion-exchangers PRP-X100 and Metrosep A8 which have a similar PS-DVB matrix, but the different ion-exchange capacities of 71 and 700 μeq/col, respectively as normalized for 150 × 4.0 mm ID columns. The methylene increment value $\alpha(CH_2)_{CA}$ = 0.387 obtained for high capacity Metrosep A8 anion-exchanger was found to be less than $\alpha(CH_2)_{CA}$ = 0.489 for PRP-X100. The lower surface density of charged groups for PRP-X100 contributes to its higher hydrophobicity as compared with Metrosep A8. Interestingly, according to Lee [45], the functionalisation degree of aromatic rings located on the surface of PRP X-100 is only about 0.15, so one can expect either functionalization of the matrix in internal volumes or higher surface area for Metrosep A8. Nevertheless, the value $\alpha(CH_2)_{CA}$ = 0.387 obtained for Metrosep A matches well methylene selectivity value of 0.375 obtained for adsorption of alkanols homologues on Dowex 1X8 anion-exchange resin in chloride form with the ion-exchange capacity of 3500 μeq/g [29]. The obtained results keep demonstrating the possible effects of charge density and ion capacity of anion-exchangers on hydrophobic selectivity.

It should be also underlined that hydrophilicity of $-PhCH_2N^+(CH_3)_2CH_2CH_2OH$ functional group (logP = -3.94) typical for Type II resins is higher than that of $-PhCH_2N^+(CH_3)_3$ functional group (logP = -2.17) for Type I resins.

3.3.3. Effect of central core surface area

The matrix surface area of adsorbents is responsible for the interaction with alkyl radicals of organic acids, so an increase in the surface area should result in a proportional increase of hydrophobicity increments. This is a well established regularity in reversed phase HPLC. In IC and related mixed-mode HPLC the ideal experiment would require the preparation of a set of ion-exchangers with equal ion-exchange capacities

Table 4. Methylene selectivity values obtained for alkanoates and alkansufonates homologues for anion-exchange columns, retention factors of chloride and mixed-mode factors F_{CA}.

Column	Hydrophobicity as defined by producer	$\alpha(CH_2)_{CA}$	$\alpha(CH_2)_{SA}$	k_{Cl^-}	F_{CA}
Sulphonated PS-DVB or PEVB-DVB core particles with electrostatically retained positively charged latex particles					
IonPac AS4A	Medium-low	0.123	0.191	0.93	7.56
IonPac AS4A SC	Medium-low	0.150	0.211	2.49	16.6
IonPac AS5	Low	0.108	0.130	2.04	18.89
IonPac AS5A-5u	Low	0.147	0.196	3.23	21.97
IonPac AS7	Medium-high	0.137	0.136	-	-
IonPac AS9	Non specified	0.109	0.180	1.29	11.83
IonPac AS9-SC	Medium-low	0.093	0.196	2.45	26.34
IonPac AS9-HC	Medium-low	0.144	0.180	11.49	79.79
IonPac AS11	Very low	0.170	0.228	-	-
IonPac AS11-HC	Medium-low	0.256	0.383	1.97	7.70
IonPac AS12A	Medium	0.078	0.136	1.19	93.59
IonPac AS16	Ultra-low	0.203	0.302	1.77	8.72
IonPac AS17	Low	0.202	0.258	1.41	6.98
IonPac AS18	Low	0.323	0.344	1.68	5.20
OmniPac PAX 100	Medium	0.147	0.172	2.27	15.44
OmniPac PAX500	Hydrophobic	0.377	0.467	6.19	16.42
PEVB-DVB based resins with grafted layer of cationic polymer					
IonPac AS14	Medium-high	0.569	0.557	1.93	3.39
IonPac AS14A	Medium	0.502	0.474	6.30	12.55
IonPac AS15–5 um	Medium-high	0.605	0.438	6.56	10.84
PEVB-DVB based resins with hyperbranched layer of cationic polymer					
IonPac AS19	Low	0.076	0.099	3.65	48.02
IonPac AS20	Ultra-low	0.080	0.140	2.22	27.75
IonPac AS21	Ultra-low	0.050	0.109	1.10	22.00
IonPac AS22	Ultra-low	0.072	0.066	1.79	24.86
IonPac AS23	Ultra-low	0.077	0.082	2.71	35.19
IonPac AS25	Ultra-low	0.058	0.164	6.03	103.97
IonPac AS26	Ultra-low	0.181	0.184	4.57	25.25
PMMA based monolithic column					
ProSwift SAX 1S	Low	0.320	0.288	6.55	20.46
Miscellaneous					
Hamilton PRP-X100	High	0.332 0.489[a]	0.350	12.27	36.96
ICSep AN1	High	0.646	0.704	0.55	0.85
Metrosep A5		0.130	0.141	1.89	14.54
Metrosep A8		0.387[a]	no data	-	-
Separon HEMA-S 1000 Q-L	Low	0.209	0.217	5.49	26.27
TSK-Gel IC-Anion-PW	Low	0.175	0.192	2.61	14.91

[a] Calculated for pair propanoic/acetic acids.

but different surface areas, which is not a trivial synthetic task. However, some ion-exchange materials considered in this work matched this requirement, so their hydrophobicities were compared.

The first pair of compared adsorbents comprised two agglomerated ion-exchangers OmniPac PAX-100 and OmniPac PAX-500. Both adsorbents have the equal anion-exchange capacity of 40 μeq/column due to the same amount of electrostatically retained 60 nm PVBC-DVB latex particles, functionalized with tertiary amines (see Table 2). PEVB-DVB (55% crosslinking degree) particles with diameter 8.5 μm were used for the preparation of both adsorbents, but the central core of OmniPac PAX-100 has a microporous structure with very low specific surface area ($S < 1$ m²/g) while OmniPac PAX-500 has a well developed mesoporous structure with a pore diameter of 6 nm and surface area of 300 m²/g [64]. As a result, the interior surface of OmniPac PAX-500 cannot be coated with significantly bigger, b but more hydrophilic latex particles of diameter 60 nm. The results on retention of homologues of organic acids show remarkably higher methylene increment values for PAX-500

($\alpha(CH_2)_{CA} = 0.377$, $\alpha(CH_2)_{SA} = 0.467$) compared to PAX-100 ($\alpha(CH_2)_{CA} = 0.147$, $\alpha(CH_2)_{SA} = 0.172$). Probably, the difference in hydrophobicity of the exterior surface of PAX-100 and PAX-500 is not too big, but the latter anion-exchanger has a structure of restricted access media (RAM) adsorbent when the more hydrophobic interior porous structure of PEVB-DVB matrix is accessible for interaction with low molecular weight solutes. To an extent, a big difference in obtained $\alpha(CH_2)$ values can be attributed to stronger adsorption of sorbates within developed mesopores structure of OmniPac PAX-500 comparing to adsorption onto the surface of OmniPac PAX-100. It should be also noted that the matrix used for the preparation of OmniPac resins contains anionic dispersant used for decoration with latex particles.

Another pair of compared adsorbents comprised an analytical IonPac AS25 column and a corresponding precolumn AG25. These ion-exchangers have similar layers of hyperbranched functional ionic polymer on the surface of sulphonated PEVB-DVB core microparticles, but a macroporous matrix ($d_{pore} = 200$ nm, $S = 20$ m²/g) was used for the
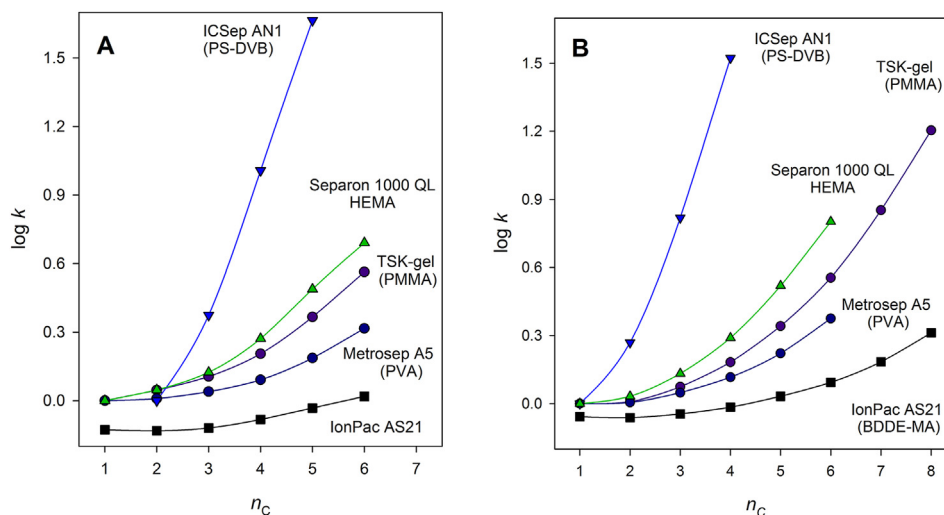
Figure 2. The effect of polymer matrix type on the retention of homologues of alkanoic (a) and alkanesulfonic acids (b).

preparation AS25, while for AG25 a practically nonporous matrix with d_{pore} <0.1 nm was used. The observed difference between hydrophobicities of precolumn and analytical column was insignificant. The methylene increment values of $\alpha(CH_2)_{CA} = 0.058$, $\alpha(CH_2)_{SA} = 0.074$ and $\alpha(CH_2)_{CA} = 0.082$, $\alpha(CH_2)_{SA} = 0.085$ were obtained for AS25 and AG25, respectively. The substantial difference in surface area of compared ion-exchangers is not reflected by the obtained $\alpha(CH_2)$ values, which is, probably, connected with high hydrophilicity of hyperbranched ionic polymer and a rather effective coating of hydrophobic PEVB-DVB matrix by this polymer layer. The ratio of column capacities as calculated for 250 × 4.0 mm ID packed with AS25 and AG25 is equal to 20, that is in good agreement with the ratio of specific surface areas for these ion-exchangers. This confirms the similar characteristics for the layers of hyperbracnhed ionic polymer in compared ion-exchangers.

3.3.4. Effect of functional group structure

Due to the lack of information on the exact structure of functional groups in commercially available anion-exchangers and the presence of several types of ion-exchange groups on the surface, it is difficult to accurately measure the hydrophobicity related effect of a functional group on retention of model compounds. However, an excellent investigation was carried out by Barron and Fritz, who studied ion-exchange selectivity of anion-exchangers with equal ion-exchange capacity, same polymer matrix, and bonding chemistry, but with different lengths of alkyl substitutes in trialkylammonium functional groups [39]. The retention time of hydrophobic anion nicotinate (logP = 0.36) increased from trimethylammonium- (2.57 min) to trihexylammonium- (3.29 min) functionalised resin which could potentially indicate an increase in hydrophobicity of anion-exchangers with longer alkyl substitutes in quaternary ammonium functional groups. However, the use of hydrophobic benzoic acid (logP = 1.87) as an eluent results in an indefinite mechanism of nicotinate retention due to the strong adsorption of benzoic acid on the PS-DVB surface. No significant changes in retention of nicotinate were observed for the resins possessing trimethylammonium-, dimethylethanolammonium- and methyldiethanolammonium- functional groups with an equal ion-exchange capacity of 0.025–0.030 mequiv/g. Probably, the low surface density of charged groups does not allow to see a small change in hydrophobicity between compared Type I and Type II anion-exchange resins.

3.4. Selectivity map based on hydrophobic/electrostatic interactions ratio

Finally, all studied anion-exchangers were distributed in three groups based on their ability to retain organic anions *via* a combination of

electrostatic and hydrophobic interactions as shown by Eq. (8). The apparent electrostatic interactions were estimated as a logarithm of retention factor ($logk_{Cl^-}$) of chloride, which is a spherical anion with a small ionic radius (0.181 nm). Due to these properties, one can expect minimal hydrophobic interactions between this anion and the matrix, as well as high permeability or minimal steric hindrance in accessing charged ammonium groups. Methylene selectivity values $\alpha(CH_2)_{CA}$ obtained for alkanoates homologues were used as hydrophobicity measures of selected anion-exchangers. The resulting selectivity map is shown in Figure 3.

Based on hydrophobicity, these anion-exchangers can be divided into three selectivity groups: very hydrophobic with $\alpha(CH_2)_{CA} > 0.30$; hydrophobic with $0.30 > \alpha(CH_2)_{CA} > 0.10$ and hydrophilic ion-exchangers with $\alpha(CH_2)_{CA} < 0.10$. When using purely aqueous eluents, an almost pure ion-exchange retention mechanism is applies only to the separation of organic anions on hydrophilic anion-exchangers, however, for the rest of ion-exchangers, the possibility of mixed-mode separation should be considered. It should be noted that the data obtained in this work show some inconsistency with the hydrophobicity description given by the producer for the corresponding columns.

The mixed-mode performance of anion-exchangers depends on the ratio of hydrophobic and electrostatic interactions, so k_{Cl} retention factors were used to measure the ion-exchange ability of adsorbents under the same experimental conditions as used for hydrophobicity measurements. As shown in Figure 4, there is no clear correlation between the retention factor of chloride (k_{Cl^-}) and column capacities specified by manufacturers under the conditions used in this work. Nevertheless, anion-exchangers with $k_{Cl^-} > 5.0$ in 10 mM NaOH should be considered as high-capacity ion-exchangers. The group of anion-exchangers shown in inlay with retention factor $k_{Cl^-} < 5.0$ and declared column capacity less than 30 µeq/column can be identified as low-capacity ion-exchangers. The electrostatic/hydrophobic balance for anion-exchanger can be characterised by the ratio of chloride retention factor and methylene selectivity. The corresponding values of mixed-mode factors $k_{Cl}/\alpha(CH_2)_{CA}$ for studied anion-exchangers are presented in Table 4. The $k_{Cl}/\alpha(CH_2)_{CA}$ value varies in the range from 0.85 for the most hydrophobic ICSep AN1 column to 103.97 for the most hydrophilic IonPac AS25 column.

The ion-exchange retention mechanism appears to be strongly dominant for resins with $k_{Cl^-}/\alpha(CH_2)_{CA} > 20$. For the resins with $k_{Cl^-}/\alpha(CH_2)_{CA} < 10$, the probability of a mixed-mode mechanism for the separation of organic anions is high. The proposed mixed-mode factor $k_{Cl^-}/\alpha(CH_2)_{CA}$ can be used for the selection of anion-exchange columns suitable for certain practical applications connected with the separation
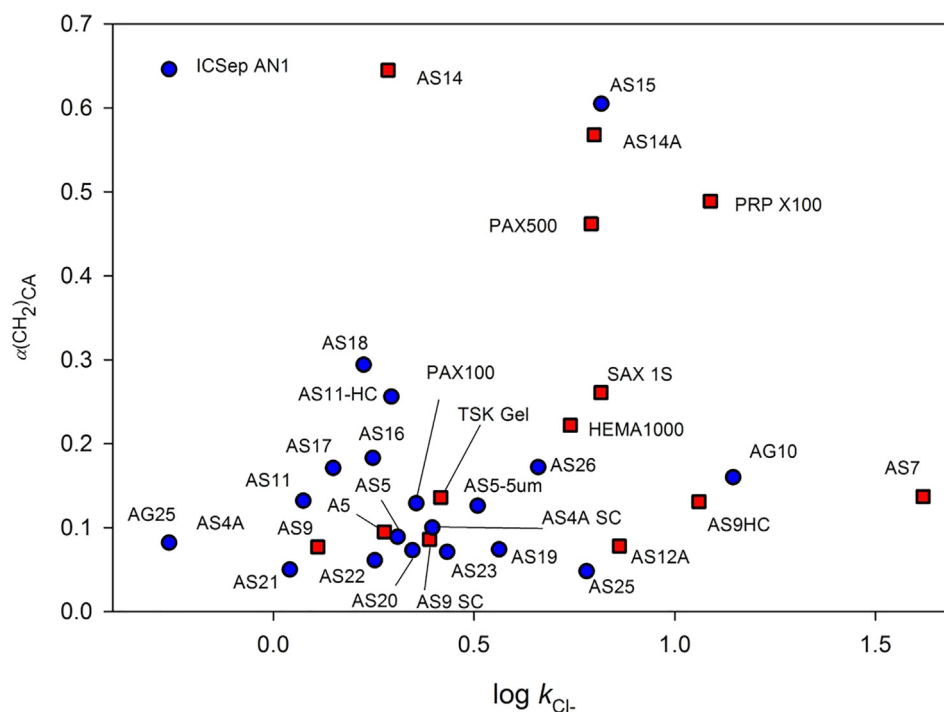
Figure 3. Selectivity map of anion-exchangers. The data points for Type I and Type II anion-exchangers are marked by red square and blue circles, respectively.
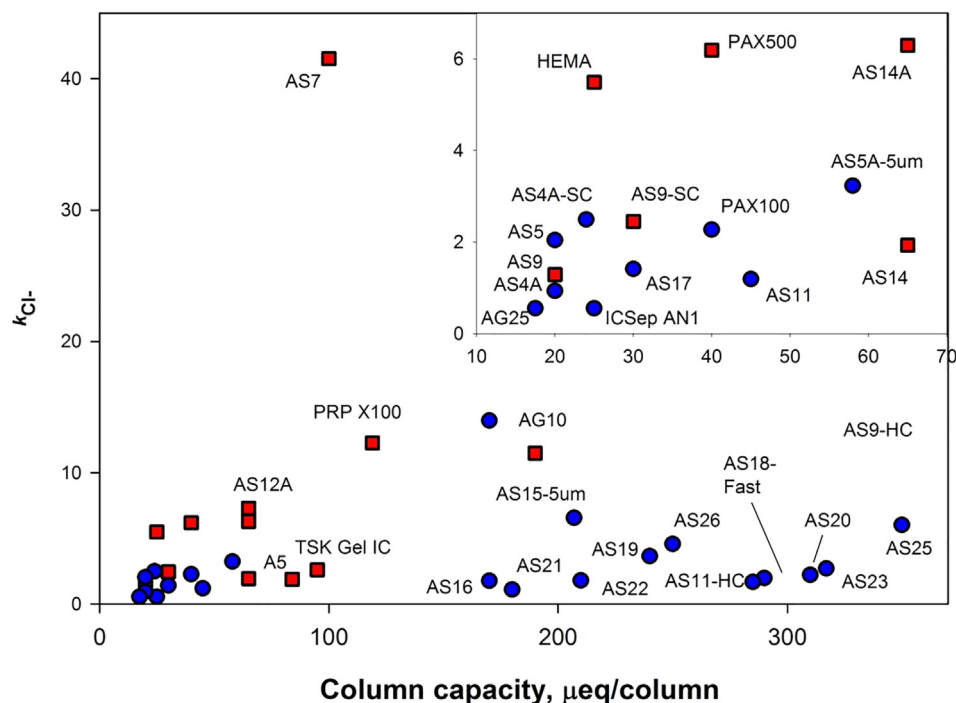
Figure 4. Correlation between experimentally obtained data on retention of chloride (k_{Cl^-}) in 10 mM NaOH eluent and column capacity values as specified by column manufacturers. All column capacities are presented for 250×4.0 mm ID dimension.

of organic anions. For example, three columns namely IonPac AS-4A-SC, AS11-HC, and AS15 are recommended for the separation of alkanoic acids according to the manufacturer column selection guide [66]. The corresponding mixed-mode factors values (7.56, 7.70, and 10.84, respectively) are close to each other and they show the presence of significant hydrophobic properties for these ion-exchange resins which are essential for the selective separation of organic acids. One can expect that the IonPac AS16 column with $k_{Cl^-}/\alpha(CH_2)_{CA} = 8.72$ may be selective for

the separation of organic acids, which has been confirmed in practice [67].

As presented in Table 4, there is a clear difference between methylene selectivity values $\alpha(CH_2)_{CA}$ and $\alpha(CH_2)_{SA}$ obtained for carboxylic and sulfonic acids, respectively. The $\alpha(CH_2)_{SA}$ values are higher for all of the studied anion-exchangers except for a group of resins with a grafted layer of cationic polymer (IonPac AS14, 14A, and 15–5 um) that is connected with a stronger synergetic effect as described by Eq. (7)

between hydrophobic and electrostatic interactions for alkanesulfonic acids.

Another reason for the use of the obtained methylene selectivity data in IC should also be considered. The $\alpha(CH_2)_{CA}$ and $\alpha(CH_2)_{SA}$ values listed in Table 4 are true values as they were obtained without the use of any suppressor device usually placed before the conductivity detector. It has been established that the suppressor can significantly increase the retention of hydrophobic ions as was shown by Karu *et al.* [68]. For example, $\alpha(CH_2)_{SA}$ value of 0.340 was obtained from the data on retention of alkanesulfonic acid homologues in 10 mM NaOH on IonPac AS-11 column using conductivity detection with a suppressor [49], while the value of $\alpha(CH_2)_{SA}$ measured in this work using direct conductivity detection was significantly lower (0.228).

3.5. Separation selectivity of the first homologues of alkanoic acids

Explanation of the non-trivial retention order and separation selectivity for the first homologues of alkanoic acids is one of the most difficult challenges in ion-exchange theory since the first serious attempt in this field made by Diamond and Whitney [72]. They used trimethyl-, dichloro-, and trichloro- substituted acetic acids and found some correlation between retention and pKa values of these acids which have approximately the same size as estimated through limiting equivalent conductivity.

There is a clear correlation between the dielectric constant and pKa values. A simple consideration of physico-chemical properties of aliphatic acids shows a significant drop in dielectric constants in the following order: formic (51.1) > acetic (6.6) > propionic (3.4) > butanoic (3.0) > rest of *n*-alkanoic acids having dielectric constant of 2.6–2.66 that corresponds to their pK_a values presented in Table 1. The case of alkanoic acids' homologues is relatively simple as the corresponding anions, formed due to dissociation, have the same geometric fragment COO- where the negative charge is concentrated. It is a well-established fact that for acids if looked at as a union of H^+ and anions with a similar structure, their strength (or pKa value) depends on the polarizability of anions or the ability of the alkyl group of alkanoic acid to compensate electric polarization effect induced by H^+. For example, the following change in acid strength for the same group elements/anions can be observed in order HF < HCl < HBr < HI and $H_2O < H_2S < H_2Se < H_2Te$. This is due to the ability of Hal^- or X^{2-} anions to compensate polarization caused by H^+ through their ability to be polarized or polarizability [73]. A weaker acid forms a less polarizable anion and for the molecules with two hydrogens the effect is synergetic. Correspondingly, the retention of anions is proportional to the strength of respective acids.

From the theoretical point of view, different polarizability of alkanoate homologues is related to a positive inductive effect $(+I)$ from alkyl moiety, which is reflected by changes in pK_a values and dielectric constants. The inductive effect works only through two adjacent carbon atoms in methylene chains and is fades out completely after three-four methylene chain bonds. Therefore this effect is responsible for the polarizability of alkanoates and it can be observed clearly only for the first three members: formate, acetate, and propionate, and to a lesser extent for butyrate. A further increase in the alkyl chain in alkanoates would not change the polarizability of the terminal carboxyl group.

Therefore, the retention of formate, acetate, and propionate is defined by a combination of the following interactions: columbic forces, which are the constant for all alkanoates having the same charged groups, the polarization of both trialkylammonium cite and alkanoates that includes both polarizing ability of anions and their ability to be polarized, and hydrophobic interactions. The polarizing ability of anions to shift electron density in the quaternary ammonium group of the resin increases in order propionate < acetate < formate and hydrophobic interactions increase in the opposite order formate < acetate < propionate. Combining this with constant ion-ion or columbic interactions

results in the following selectivity order: acetate < formate < propionate. Thus, the resulting U-shape dependence can be obtained as shown in Figure 2a.

4. Materials and methods

4.1. Instruments

A Waters (Milford, MA, USA) 2695 Alliance HPLC system equipped with a Waters 430 conductivity detector, Waters 2487 spectrophotometric detector connected via Waters Bus SAT/IN interface (Milford, USA) was used. Chromatographic data collection and processing were performed using the Waters Empower 3 software.

A set of analytical size anion-exchange columns including IonPac AS, (Thermo Fisher Scientific formerly Dionex, Sunnyvale, CA, USA), Metrosep (Metrohm AG, Herisau, Switzerland), PRP (Hamilton, Reno, Nevada, USA), Separon (Tessek Ltd., Prague, Czechoslovakia), Transgenomic (San Jose, Torrance, CA, USA, formerly Sarasep, Santa Clara, CA, USA) and TSK-Gel (Tosoh, Tokyo, Japan) was used in this study. Additionally, four IC pre-columns IonPac AG type and two mixed-mode OmniPac and Proswift columns were used (all from Thermo Fisher Scientific). Characteristics of these columns were collected from various literature sources and are summarized in Table 2.

4.2. Reagents

Sodium hydroxide 50% w/w (19.1 M) aqueous solution (Thermo Fisher Scientific, Sunnyvale, CA, USA) was used as a stock solution for the preparation of the eluent. Deionised water with 18.2 M cm^{-1} resistivity from a Millipore Milli-Q water purification system (Bedford, MA, USA) was used throughout this work. All prepared eluents were filtered through a membrane of 0.22 μm Nylon 66 filters (Millipore, USA) before use. A set of pure analytes used in this work included seven alkanoic acids (acetic, propionic, valeric, caproic, heptanoic, and octanoic) and eight sodium alkanesulfonates (methanesulfonate, ethanesulfonate, 1-propanesulfonate, 1-butanesulfonate, 1-pentanesulfonate, 1-hexanesulfonate, 1-heptanesulfonate, 1-octanesulfonate). All analytes were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Their basic characteristics are listed in Table 1.

Stock solutions of analytes in deionised water (100 mmol/L) were prepared. Working solutions (3 mmol/L) were prepared by further dilution with eluent before chromatographic experiments daily. The injection volume of analytes was 5 μL. All retention times were measured in triplicate for every analyte under optimized separation conditions and mean values were used for the evaluation of column properties. The detection of analytes was performed by sequentially connected non-suppressed conductivity and spectrophotometric (set up at 215 nm) detectors.

5. Conclusions

The hydrophobicity of a stationary phase, which can be also associated with its polarity [74], is one of the key parameters affecting separation selectivity in chromatography. The knowledge of the relative hydrophobicity of ion-exchangers is important for understanding the retention mechanisms of polar molecules and hydrophobic ions in various chromatographic modes. To date, the use of anion-exchangers in mixed-mode chromatography has been limited, in part due to the absence of data on their hydrophobic properties. In this work, accurate measurement of hydrophobic properties in a form of methylene selectivity increments of alkanoates and alkanesulfonates was performed for a broad range of anion-exchange resins under the same separation conditions, allowing analysis of various factors affecting hydrophobicity. The effects of a polymer matrix, specific surface area, functional group structure, ion-exchange capacity, and other parameters on column

hydrophobicity were considered and discussed in connection to the related literature data.

It was found that hydrophobicity of investigated phases varied significantly (obtained $\alpha(CH_2)$ values varied in a broad range from 0.05 to 0.605). It was shown that the matrix of ion-exchangers had the most significant contribution to phase hydrophobicity, which could be mitigated for phases with hydrophilic polymer coating. To some extent, the structure of functional groups and surface density of charged groups can further influence the hydrophobicity of anion-exchangers.

The obtained results indicate the presence of the synergetic effect of hydrophobic and electrostatic interactions for the retention of alkanoic and alkanesulfonic acids. A new mixed-mode factor $k_{Cl^-}/\alpha(CH_2)$ is suggested as a quantitative measure of dominant interactions for selected anion-exchange resins.

Special Notice. Thirty-seven different anion-exchange columns were used in this study to ensure a broad range of materials is investigated and compared. It should be noted that some (ca. 20%) of these columns, especially a first generation of the anion-exchange columns produced more than 20 years ago, were not bright new and their properties could have somewhat changed due to moderate exploitation for research projects and/or prolonged storage. This situation could be especially critical for short pre-columns packed with high-capacity hydrophobic anion-exchangers, which could be contaminated with irreversibly adsorbed hydrophobic counter ions, so it was ensured that all pre-columns are new or had minimal use. To minimize the probability of possible changes of the original properties and quality of anion-exchangers, all available columns were subjected to two checks. The first test comprised of assessing the column efficiency and peak shapes for obtained chromatographic peaks. Overall, no columns producing distorted chromatographic peaks (e.g., having shoulders, poor peak symmetry, or strong tailing) were used. The peak efficiencies under the test conditions were also compared with the column passports or with data from manuals and catalogues if available. The second test was specific to columns produced by ThermoFisher Scientific, formerly Dionex. For these, hydroxide type anion-exchange columns, the separation retention and selectivity data was compared with the data from the Virtual Column Separation Simulator 2 (Dionex, Sunnyvale, CA, USA)™.

While certain low degree of error is unavoidable for the data on retention of three first members of homologues and chloride. However, it should not influence the major pool of the data on the hydrophobicity of anion-exchangers which are related to homologues with $n_C > 3$ for alkanesulfonates and with $n_C > 4$ for alkanoates and on conclusions made from this investigation.

Declarations

Author contribution statement

Pavel N. Nesterenko: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ekaterina P. Nesterenko: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

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