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Multifunctional ion-exchange stationary phases for high-performance liquid chromatography

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Abstract

The preparation and properties of multifunctional ion-exchangers, including one cation and two zwitterion types, for high-performance liquid chromatography (HPLC) are described. These ion-exchange stationary phases (IXSPs) were synthesized through two major steps; first by bonding the corresponding organic moieties, either undecenoic acid, aniline or ethyl *p*-fluorobenzoate, onto silica surfaces with appropriate silane coupling agents. The bonded silica was subsequently derivatized to the corresponding ion exchanger by free-radical partial addition, sulfonation or hydrolysis, respectively, and worked up by end-capping. The ion exchangers thus prepared were characterized by elemental analysis and FTIR spectroscopy. These IXSPs produce effective separations of aromatic amines, aromatic acids, amino acids, aminobenzoic acids and nitroanilines by HPLC. Effects of pH, concentration of the electrolyte and the polarity of the mobile phase on the capacity (retention) factor were investigated. The chromatographic results for these IXSPs as separators of the positional isomers of aminobenzoic acids and nitroanilines were compared with those for commercial phases. On the basis of the chromatographic behaviour observed in this study, it is concluded that the effective selectivity of these IXSPs is due to the sulfo-(SO_3H) group or the carboxylic group for the cation-exchange ability, the amino group for the anion-exchange ability, the long-chain alkenyl moiety for the hydrophobic character, and the double bond of the long-chain alkenyl moiety as well as the aromatic rings for the $\pi-\pi$ charge-transfer interactions. These multifunctional interaction mechanisms render these readily preparable phases more efficient in the separation of organic weak bases and organic weak acids than a simple ion exchanger.

1. Introduction

Silica-based ion-exchange stationary phases (IXSPs) for high-performance liquid chromatography (HPLC) have proved useful in separating compounds which are ionic in acidic solutions [1]. The ion-exchange functional groups are typically aliphatic or aromatic sulfonic acids for strong-cation exchangers and quaternary amine for strong-anion exchangers. Obviously, the ion-exchange interactions provided by the IXSPs play an important role in the separation of ionic compounds. However, for separations of ionic organocompounds, contributions from forms of interaction other than ion exchange, such as H-bonding, $\pi-\pi$ interaction or hydrophobic interaction, may be involved if the stationary phase provides the corresponding functional moiety [2–4]. Wilson and co-workers [5] also confirmed that columns prepared by mixing together ion-exchange and reversed-phase packing materials

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could simultaneously separate ionized/ionizable and non-ionic compounds. It seems that an ion exchanger bearing sites of $\pi-\pi$ charge transfer interaction, hydrophobicity and cation/anion exchange could effectively separate both organic acids and bases. However, the synthesis of silica-based cation exchangers is quite difficult, and some problems exist with the synthesis of silica-based multifunctional phases. Recent research has led to a proposed solution to this problem [6]. For the purposes of the present paper, three multifunctional ion exchangers bearing sites of $\pi-\pi$ charge-transfer interaction, hydrophobicity and cation/anion exchange were prepared for the separation of both organic acids and organic bases. Two synthetic approaches were examined. One was bonding the organic moiety to the silica surface and subsequently sulfonating under mild condition; the other was bonding the pre-sulfonated organic moiety to the silica surface. In order for us to understand the possible interactions made available by these IXSPs, various organic acids and bases, as well as positional isomers of disubstituted benzene derivatives, were studied as analytes. The effects of pH, electrolyte concentration and mobile-phase composition on the factors determining the capacity to separate these analytes were also studied.

2. Experimental

2.1. Chemicals

Silica gel (Nucleosil; pore size 100 Å, particle size 10 μm, surface area 350 m^2/g), was obtained from Macherey-Nagel and dried at 180°C for 10 h. The chemicals used in the synthetic processes and analytes used in the chromatographic experiments were all of reagent grade: 3-aminopropyltriethoxysilane (APS, Chisso); 3-chloropropyltrimethoxysilane (CPS) and ethyl *p*-fluorobenzoate (Aldrich); dicyclohexylcarbodiimide (DCC), *n*-butyl lithium–*n*-hexane solution (1.6 *M*) and copper (II) acetate (Merck); α,α'-azobisisobutyronitrile (AIBN, Janssen) and L-amino acids (Sigma). The solvents used for HPLC were LC-grade THF, methanol, acetonitrile and deionized water.

2.2. Preparation of PB-1

APS (10 ml) was added under stirring at 0°C to a solution of 8.65 ml 11-undecenoic acid and 8.86 g DCC in 100 ml toluene. The reaction mixture was stirred for 24 h at room temperature and 24 h at the reflux temperature (110°C). After removal of the suspended solid dicyclohexylurea, 10 g of pre-dried silica gel was added and refluxed for 17 h. The bonded phase was collected by filtration and washed with toluene, methanol, water, methanol and ether, and then dried under vacuum in the presence of the drying agent P_2O_5. Characterization was done by FTIR spectroscopy (ν_{CO}: 1638 cm^{-1} for the amide group) and elemental analysis (Table 1).

2.3. Preparation of IXSP-1

AIBN (1.24 g) and 8.65 g sodium bisulfite were added at 70°C to a suspension of 5 g PB-1 in a mixture of 50 ml toluene and 50 ml water,

Table 1
Characteristics of the prepared stationary phases[a]

	PB-1	IXSP-1	PB-2	IXSP-2	PB-3
Elemental analysis (%)	N 2.70	S 1.36	N 0.32	S 0.34	N 1.51
Loading capacity[b]					
(mmol/g)	1.93	0.43	0.23	0.11	1.08
(μmol/m^2)	5.51	1.21	0.65	0.30	3.09

[a] Before end capping.
[b] Based on elemental analysis of the corresponding functional group.

and the mixture was stirred for 17 h. The sulfonated phase was filtered, washed with methanol and water and dried under vacuum in the presence of P_2O_5. The elemental analysis of the resulting product is shown in Table 1.

2.4. Preparation of PB-2

A solution of 2.5 ml aniline and 13.1 ml *n*-butyl lithium–*n*-hexane in 10 ml toluene was refluxed for 1 h. Subsequently, 5 ml CPS and 100 ml toluene were added to the above solution and the whole refluxed for 28 h. After removal of the suspended solid lithium chloride, 5 g of pre-dried silica gel was added and the mixture was stirred for 17 h at 110°C. After filtration, the product was washed with toluene, methanol, water, methanol, ether and dried under vacuum in the presence of P_2O_5. Characterization was by FTIR spectroscopy ($\nu_{C=C}$: 1505, 1605 cm^{-1} for the phenyl group) and elemental analysis (Table 1).

2.5. Preparation of IXSP-2

A solution of 1.81 ml chlorosulfonic acid in 200 ml carbon tetrachloride was cooled in an ice bath, 5 g PB-2 was added in stages and the mixture was stirred for 20 min. After neutralizing with 100 ml 0.1 *M* sodium bicarbonate, the product was obtained by filtration and washed with toluene, methanol, water and acetone and dried under vacuum in the presence of P_2O_5. The elemental analysis of the product is shown in Table 1.

2.6. Preparation of PB-3

A solution of 2.64 ml ethyl *p*-fluorobenzoate, 5 ml APS and 3.06 ml N,N-diisopropylethylamine in 100 ml N,N-dimethylformamide (DMF) was refluxed for 28 h. Subsequently, 5 g pre-dried silica gel was added to this solution, which was then reacted at 110°C for 17 h. After cooling, the reaction mixture was filtered and the product was washed and dried as described for the preparation of PB-1. Characterization was by elemental analysis (Table 1).

2.7. Preparation of IXSP-3

PB-3 (5 g) was added to 200 ml of a 0.05 *M* copper(II) acetate aqueous solution, which was then stirred at room temperature for 40 h. The product was collected, washed with water and acetone and dried under vacuum in the presence of P_2O_5. Complete hydrolysis of PB-3 to IXSP-3 was confirmed by FTIR analysis (ν_{CO}: 1673 cm^{-1} and ν_{OH}: 3000–3600 cm^{-1} for the carboxylic group).

2.8. End capping of the prepared IXSPs

The prepared IXSP (3 g) was suspended in 100 ml toluene. After 3 ml of dichlorodimethylsilane had been added, the reaction mixture was stirred at 0°C for 1 h and 40°C for 4 h. The end-capped IXSP was collected and washed with toluene, methanol and ether and dried.

2.9. Chromatographic studies

The chromatographic studies were carried out with a Kratos liquid chromatographic system which consisted of a Spectroflow 400 solvent-delivery system, a Rheodyne 7125 injector and a Spectroflow 757 variable-wavelength UV detector. The recorder used was a Model 11 SIC chromatocorder. Stainless-steel columns (300 mm × 4 mm I.D.) were packed by the balanced-density slurry method. Reversed-phase ODS (nucleosil 10 C_{18}) and cation exchanger (LiChrosorb 10 SO_3H) were obtained commercially. The mobile-phase flow-rate was 1.0 ml min^{-1} and the detector was operated at 254 nm.

3. Results and discussion

Amino derivations were evaluated as possible agents for introducing the desired unsaturated organic moiety effectively. For convenience, APS and CPS were used as coupling agents. As shown in Fig. 1, a long-chain alkenyl-derived silica phase (PB-1) was prepared by APS through amide formation with undecenoic acid and condensation with dry silica. Sulfonation of PB-1 through AIBN-initiated free-radical sulfo-addi-

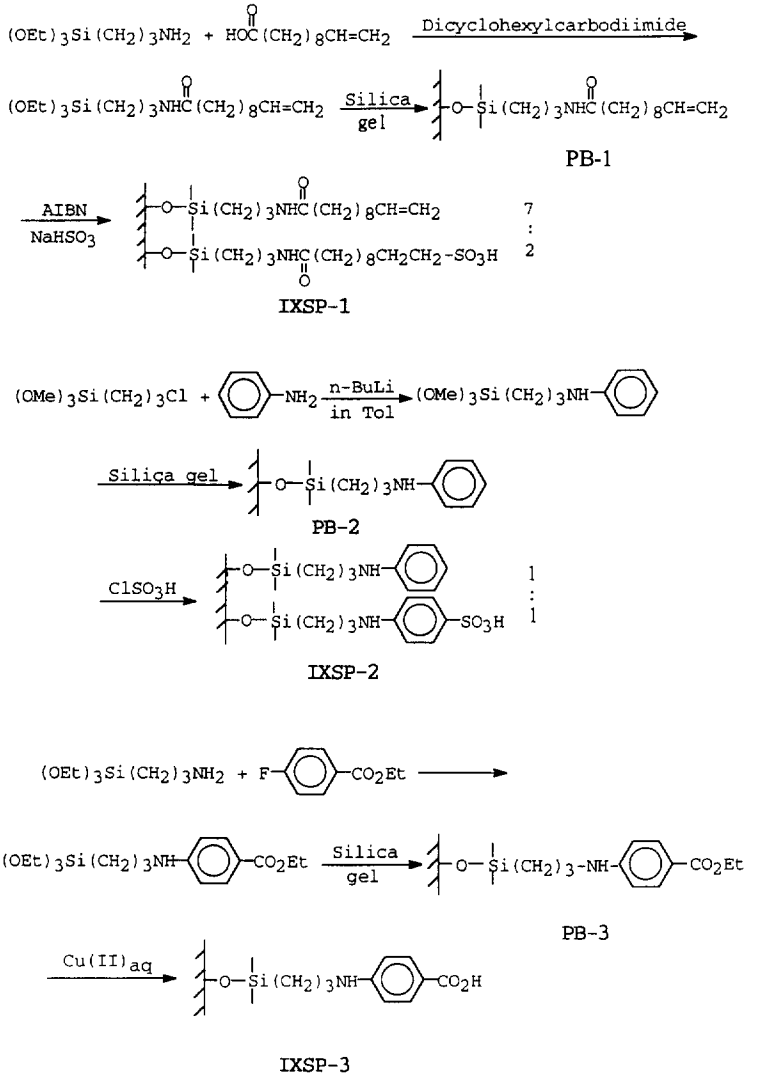

Fig. 1. Preparation of ion-exchange stationary phases (IXSPs).

tion [7] affords a mixed phase (IXSP-1) with 23% sulfonated moiety (according to the results of elemental analysis: see Table 1). As an alternative, the phenyl moiety was first bonded to silica by CPS through amination with aniline and condensation with dry silica. The PB-2 thus obtained was subsequently sulfonated by chlorosulfonic acid [8] with 50% conversion to yield a 1:1 mixed phase of IXSP-2. The amination of benzoate with CPS and *p*-aminobenzoate failed. However, that of the ethyl *p*-fluorobenzoate with APS was achieved in the presence of diisopropylethylamine: PB-3 is the result. After hydrolysis of the ester moiety of PB-3 in a copper(II) aqueous solution [9], IXSP-3 was obtained as expected. All these IXSPs were of high purity. The structures of these prepared phases are fully corroborated by elemental analyses and FTIR spectra. The characteristics of these IXSPs are shown in Table 1. According to FTIR analysis, the hy-

drolysis of the ester moiety of PB-3 is complete; thus the surface coverage of the function moiety of IXSP-3 should be the same as that of PB-3.

Excellent selectivity towards various organic acids and bases was observed on these IXSPs. Representative chromatograms are shown in Figs. 2–4. The results with respect to the effects of pH (Fig. 5, Table 2), the concentration of electrolyte (KH_2PO_4) (Fig. 6) and the polarity (Fig. 7) of the mobile phase on the capacity factor (k') demonstrate clearly the possibly available contributions by these IXSPs to the separation process. An increase in the concentration of electrolyte, KH_2PO_4, in the mobile phase, resulting in an obvious decrease in k', explains the role of the cation exchanger of IXSP-1 (Fig. 6). A decrease in methanol content leads to increasing polarity of the mobile phase; thus the increase in k' with decreasing methanol content of the mobile phase observed for IXSP-1 indicates the hydrophobic nature of the prepared phase (Fig. 7). In general, IXSP-1 and IXSP-3 were found to be more effective than IXSP-2 for the separation of the analytes chosen in this study. This result may be ascribed to the relatively lower surface coverage of IXSP-2.

3.1. The multifunctional nature of IXSP-1

In ion-exchange chromatography (IEC), the retention of an analyte on the organic stationary phase bearing an ionic functional group can be predicted as the result of two processes: (a) the distribution of an analyte between an aqueous mobile phase and the organic stationary phase and (b) the reaction of an analyte with ionic sites of the stationary phase (i.e. ion exchange). The long-chain alkenyl structure of IXSP-1 (Fig. 1) suggests that IXSP-1 would have a hydrophobic nature, which was confirmed by the effect of mobile-phase polarity on the retention of the analytes (Fig. 7). Moreover, comparing the chromatographic behaviour of the positional isomers of nitroanilines and aminobenzoic acids on commercial ODS phase and PB-1, one sees both stronger retention and better selectivity towards these isomers on PB-1 (of which Fig. 8 provides an example), indicating that an additional

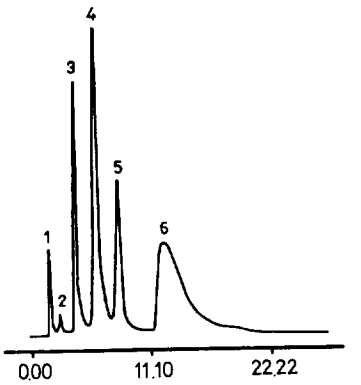

Fig. 2. Chromatogram of the separation of aniline derivatives on IXSP-1. Mobile phase: CH_3OH–H_2O (1:1), [KH_2PO_4]: 5 mM, pH 6.0, flow-rate: 1 ml min^{-1}, analytes: (1) p-anilinesulfonic acid, (2) p-aminobenzoic acid, (3) ethyl p-aminobenzoate, (4) aniline, (5) p-nitroaniline, (6) p-toluidine.

charge-transfer interaction existed between PB-1 and the analytes. These two additional interactions, charge-transfer and hydrophobic interactions, provided by the long-chain alkenyl moiety

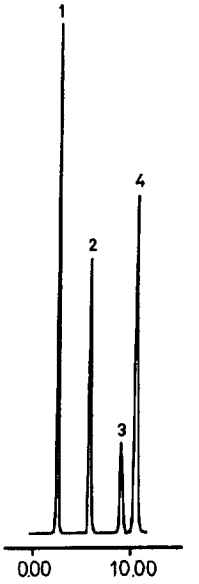

Fig. 3. Chromatogram of the separation of weak organic acids on IXSP-3. Mobile phase: CH_3CN–H_2O (2:1), [KH_2PO_4]: 1 mM, pH 6.0, flow-rate: 1 ml min^{-1}, analytes: (1) 1-naphthylacetic acid, (2) p-aminobenzoic acid, (3) benzoic acid, (4) p-nitrobenzoic acid.

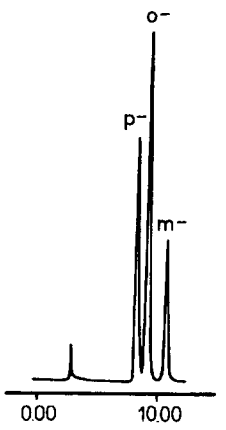

Fig. 4. Chromatogram of the separation of positional isomers of aminobenzoic acids on IXSP-3. Mobile phase: CH_3CN–H_2O (2:1), [KH_2PO_4]: 1 m*M*, pH 6.0, flow-rate: 1 ml min^{-1}.

of IXSP-1, also significantly influenced the retention of analytes (Fig. 5b). This behaviour means that not only organic bases but also aromatic acids can be separated on IXSP-1.

The sample retention in IEC can be controlled via optimization of the pH of the mobile phase. The effect of the pH of the mobile phase on the retention of some aniline derivatives on the IXSP-1 is shown in Fig. 5a. In the range pH 3 to 7, the amphoteric *p*-anilinesulfonic acid behaves almost as an anion and is thus retained only slightly, whereas other bases are retained selectively in a manner parallel to their basicity. The fact that analyte retention consistently decreases with increasing pH in this pH range is evidence of the strong cation-exchange behaviour of IXSP-1. Further evidence of this property of IXSP-1 is provided by the relation between the retention of analyte and the concentration of electrolyte in the mobile phase. In Fig. 6, as the concentration of KH_2PO_4 increases in this ion-exchange system, sample retention decreases consistently. Clearly, an increase in the concentration of the potassium ion leads to stronger competition of these ions for a place within the ion exchanger. Thus, IXSP-1 could act as a strong cation exchanger, with a sulfonate group serving as the cation-exchange site.

The various aspects of chromatographic behaviour reported above show that IXSP-1 is a

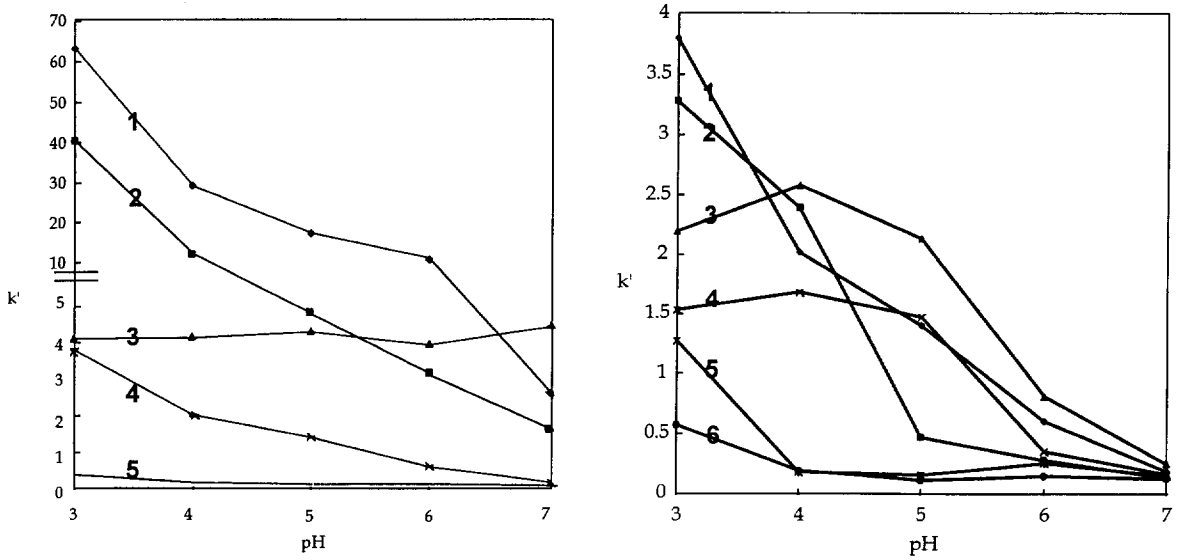

Fig. 5. Effect of the pH of the mobile phase on the values of *k'* for (a) aniline derivatives: (1) *p*-toluidine, (2) aniline, (3) *p*-nitroaniline, (4) *p*-aminobenzoic acid, (5) *p*-anilinesulfonic acid; and (b) aromatic acids: (1) *p*-aminobenzoic acid, (2) *p*-nitrobenzoic acid, (3) 1-naphthylacetic acid, (4) benzoic acid, (5) *p*-toluenesulfonic acid, (6) *p*-anilinesulfonic acid on IXSP-1. Mobile phase: CH_3OH–H_2O (1:1), [KH_2PO_4]: 5 m*M*, flow-rate: 1 ml min^{-1}.

Table 2
Effect of the pH of the mobile phase on the values of k' on IXSP-2 and IXSP-3. Mobile phase: CH_3CN–H_2O (2:1), [KH_2PO_4]: 1 mM

Sample number	Compound	k' on IXSP-2					k' on IXSP-3				
		pH 3	pH 4	pH 5	pH 6	pH 7	pH 3	pH 4	pH 5	pH 6	pH 7
1	1-Amino-2-phenylethane	3.18	7.77	19.2	64.5	77.9	0.21	0.23	0.34	0.35	0.89
2	Benzylamine	3.07	7.39	19.0	61.4	71.0	0.27	0.27	0.37	0.58	0.91
3	Aniline	2.34	2.27	1.84	1.34	1.25	0.49	0.53	0.61	0.67	0.82
4	Ethyl *p*-aminobenzoate	1.43	1.32	1.31	1.28	1.28	0.50	0.50	0.56	0.61	0.83
5	*p*-Nitroaniline	1.33	1.33	1.33	1.33	1.29	0.51	0.51	0.56	0.63	0.85
6	*o*-Aminobenzoic acid	1.23	1.21	1.20	0.72	0.33	0.58	0.64	2.91	4.09	1.66
7	*m*-Aminobenzoic acid	1.36	1.21	1.10	0.52	0.24	0.59	0.78	4.16	4.97	1.57
8	*p*-Aminobenzoic acid	1.12	1.10	1.10	0.81	0.43	0.62	0.64	2.86	3.56	1.64
9	*p*-Toluenesulfonic acid	5.14	1.01	0.57	0.23	0.22	14.8	6.91	5.56	4.96	3.40
10	*p*-Anilinesulfonic acid	2.46	0.78	0.38	0.19	0.16	17.0	13.1	7.20	4.51	2.00
11	*p*-Nitrobenzoic acid	1.39	1.35	0.91	0.23	0.17	0.70	0.93	3.39	3.93	1.46
12	1-Naphthylacetic acid	1.38	1.34	1.34	1.32	1.31	0.44	0.52	0.54	0.60	0.85
13	Benzoic acid	1.25	1.24	1.20	0.50	0.25	0.57	0.68	3.14	4.16	1.58
14	Glycine	1.02	1.09	1.03	0.98	0.93	2.45	3.81	1.92	0.98	0.72
15	Leucine	1.48	1.32	1.27	1.13	1.10	0.64	1.82	1.24	0.87	0.71
16	Phenylglycine	1.34	1.17	0.97	0.96	0.92	0.67	2.15	1.47	0.92	0.79
17	Phenylalanine	1.59	1.35	1.07	1.00	0.99	0.57	1.82	1.26	0.87	0.79
18	Lysine	2.40	9.76	10.5	16.4	50.9	0.53	2.54	1.44	0.94	0.69
19	Aspartic acid	1.06	1.09	0.68	0.64	0.57	4.89	15.3	14.8	9.69	1.64
20	Glutamic acid	1.08	1.08	0.70	0.69	0.63	2.69	4.68	12.8	8.45	1.07

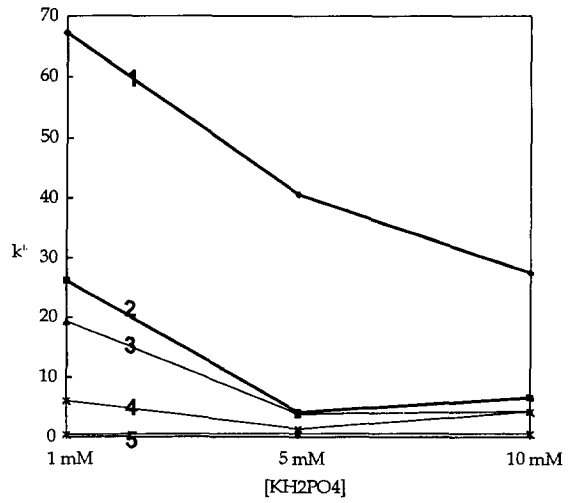

Fig. 6. Effect of the concentration of KH_2PO_4 electrolyte in the mobile phase on the values for k' for (1) aniline, (2) ethyl *p*-aminobenzoate, (3) *p*-aminobenzoic acid, (4) *p*-nitroaniline and (5) *p*-anilinesulfonic acid on IXSP-1. Mobile phase: CH_3OH–H_2O (1:1), pH 3.0, flow-rate: 1 ml min^{-1}.

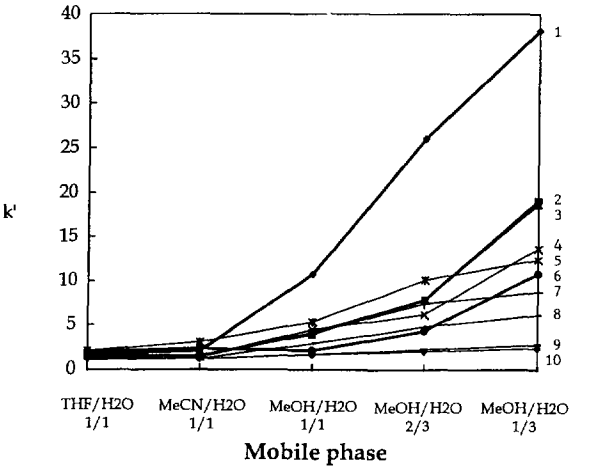

Fig. 7. Effect of the composition of the mobile phase on the values of k' for amine derivatives and some neutral compounds on IXSP-1. [KH_2PO_4]: 5 mM, pH 7.0, flow-rate: 1 ml min^{-1}, analytes: (1) N-nitroso-N-phenylbenzylamine, (2) *o*-nitrotoluene, (3) *p*-nitrotoluene, (4) *p*-nitroaniline, (5) 1-amino-2-phenylethane, (6) *p*-toluidine, (7) *m*-nitrotoluene, (8) ethyl *p*-aminobenzoate, (9) benzylamine, (10) aniline.

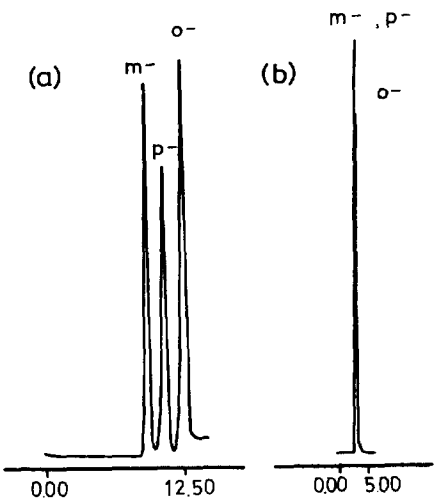

Fig. 8. Comparison of the chromatographic separation of nitroaniline isomers on (a) PB-1 and (b) ODS stationary phases. Mobile phase: CH_3OH–H_2O (1:1), [KH_2PO_4]: 5 m*M*, pH 4.0, flow-rate: 1 ml min^{-1}.

multifunctional ion exchanger. It provides strong cation-exchange, charge-transfer and hydrophobic interactions.

3.2. The amphoteric ion-exchange nature of IXSP-2 and IXSP-3

The effects of the pH of the mobile phase on analyte retention on IXSP-2 and IXSP-3 are given in Table 2. The mobile-phase component was chosen via optimization of the polarity of the phase and its electrolyte concentration.

For the stronger basic analytes, such as aliphatic amines and basic L-amino acids (i.e. lysine) on IXSP-2, an increase in the pH of the mobile phase resulted in long retention times and greatly increased k' (Table 2) suggesting that anion exchange dominates the retention process. With the weaker basic analytes, such as aniline derivatives, however, increased mobile-phase pH led to relatively shorter retention times and a reduction in k'. This suggests the existence of a weak cation-exchange process in the retention mechanism. From the structure of IXSP-2 (Fig. 1), it is observed that the unsulfonated group in this mixed phase serves as an anion-exchange site, while the sulfonated moiety in the same mixed phase serves as a cation-exchange site. The weaker contribution of the latter may be ascribed to the lower loading capacity of the sulfonate moiety. The similarity in chromatographic behaviour of organic acids on IXSP-2 to that on IXSP-1 can be ascribed to the hydrophobicity and $\pi-\pi$ charge-transfer site also provided by this stationary phase. Thus, IXSP-2 is a zwitterion exchanger that additionally provides hydrophobic-interaction and charge-transfer-interaction functions.

IXSP-3 also exhibits multifunctional characteristics. A general increase in acid retention with an increase in mobile-phase pH reflects the anion-exchange capacity of the phase. An effective separation of a mixture of various aromatic acids can be carried out at pH 5, as can be seen from Table 2 (Nos. 6–13). However, the chromatographic results for L-amino acids such as glycine and lysine also suggest that cation exchange may dominate under certain conditions. Long retentions of L-amino acids with acidic side chains, as well as their changing retention with pH, suggests that these amphoteric compounds are dominantly retained by anion exchange at one pH and by cation exchange at another pH. Furthermore, L-amino acids with different side chains can be well distinguished on IXSP-3 at pH 4 with a mobile phase comprising CH_3CN–H_2O (2:1) and [KH_2PO_4] (1 m*M*). The retention mechanism for these amino acids on IXSP-3 may also include hydrophobicity and charge-transfer interaction and ion-exchange effects.

3.3. Evaluation of the IXSPs made in-house with respect to their ability to recognize the positional isomers of aniline derivatives

By virtue of the effective anion-exchange mechanism, positional isomers of aminobenzoic acids were well separated on IXSP-3 at pH 6 (Tables 2 and 3). In order to examine the characteristics of these IXSPs more closely, comparative studies of the chromatographic behaviour of the positional isomers of aminobenzoic acids and nitroanilines were conducted with the phases made in-house and commercial ODS and cation exchanger. Representative results are

Table 3
Relative retentions of nitroaniline isomers and aminobenzoic acid isomers on IXSPs made in-house

Compound	IXSP-1 k'	IXSP-2 k'	IXSP-2 k'	IXSP-3 k'
m-Nitroaniline	9.04[a]	2.29[a]		4.44[b]
p-Nitroaniline	4.20[a]	2.52[a]	1.33[c]	4.87[b]
o-Nitroaniline	4.06[a]	2.85[a]		5.17[b]
m-Aminobenzoic acid	24.06[a]	1.17[a]	0.52[c]	4.97[c]
p-Aminobenzoic acid	3.80[a]	1.50[a]	0.81[c]	3.56[c]
o-Aminobenzoic acid	2.93[a]	1.95[a]	0.72[c]	4.09[c]

[a] Mobile phase: CH_3OH–H_2O: 1:1, [KH_2PO_4]: 5 m*M*, pH: 3.
[b] Mobile phase: CH_3OH–H_2O: 1:3, [KH_2PO_4]: 5 m*M*, pH: 3.
[c] Mobile phase: CH_3CN–H_2O: 2:1, [KH_2PO_4]: 1 m*M*, pH: 6.

shown in Table 3 and Fig. 8. From the relation between the value of k' and the composition of the mobile phase, the order polarity of the IXSPs is: IXSP-3 > IXSP-2 > IXSP-1. For nitroanilines (from the comparison in Table 3 and Fig. 8), cation exchange and charge-transfer interaction dominate the retention mechanism. However, the contributions of these two factors are not parallel for all three isomers, and indicate a better contribution from simple charge transfer than a combination of the two interactions. As for aminobenzoic acids isomers, it was found that, on the commercial cation exchanger (sulfonated LiChrosorb silica gel 10 μm), all three positional isomers appeared around a k' value of 2.5 and were not well distinguishable, whereas on our multifunctional phases the three isomers can be separated. Therefore, the excellent selectivities of these IXSPs towards the positional isomers of aniline derivatives are achieved by a suitable combination of hydrophobicity and charge-transfer availability.

From all the results discussed above, the retention contributions of these phases have been summarized in Table 4.

4. Conclusions

The present results indicate that a multifunctional IXSP with either a long-chain alkenyl cation exchanger or zwitterion phase contained in an aromatic moiety can be successfully prepared and used to separate various weak organic acids and bases. The chromatographic behaviour of the phases as a function of differing phase composition within the sites shows that the effective selectivity of these IXSPs is due to the sulfo-(SO_3H) group or the carboxylic group (COOH) for the cation-exchange ability, the amino group for the anion-exchange ability, the long-chain alkenyl moiety for the hydrophobic character, and the double bond as well as the aromatic rings for the charge transfer.

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Table 4
Forces of interaction[a] with various analytes available on IXSPs prepared in-house

Analytes	IXSP-1	IXSP-2	IXSP-3
Aliphatic amines	CE	AE	AE
Aromatic amines	CE, CT	CE, CT	CE, CT
Aromatic acids	HB, CT	HB, CT	AE, CT
L-Amino acids	CE, HB	CE, AE, HB, CT	CE, AE, HB, CT
Aminobenzoic acids	CE, HB, CT	CE, HB, CT	AE, HB, CT
Nitroanilines	CE, CT	CE, CT	CE, CT

[a] CE: cation exchange, AE: anion exchange, HB: hydrophobicity, CT: π–π charge-transfer ability.

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