Journal of Chromatography A, 660 (1994) 17–23
Elsevier Science B.V., Amsterdam

CHROMSYMP. 2952

Explanation of the selectivity differences between reversed-phase ion-pair chromatographic systems containing trifluoroacetate or heptafluorobutyrate as pairing ion

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ABSTRACT

The well documented selectivity differences found between reversed-phase ion-pair chromatographic systems containing trifluoroacetate or heptafluorobutyrate as pairing ion were explained after determining sorption isotherms for trifluoroacetate and heptafluorobutyrate on Nucleosil 100-5 C_{18} from a solution similar to the eluents used for the separation of transmitteramines and peptides. Based on the isotherms and retention data obtained with reversed-phase, ion-exchange and reversed-phase ion-pair chromatographic systems, it is proposed that the selectivity differences between the systems studied are caused by the fact that trifluoroacetate and heptafluorobutyrate are not interchangeable in terms of their surface concentrations at the practical eluent concentrations of the pairing ions concerned.

INTRODUCTION

It was found in earlier studies that, in reversed-phase ion-pair chromatography (RP-IPC), C_4–C_{12}-alkanesulphonate pairing ions are interchangeable in terms of their surface concentrations [1], suggesting that no selectivity difference can be expected in such systems from changing the chain length of pairing ions belonging to the same homologous series.

In view of the well documented selectivity differences between RP-IPC systems with trifluoroacetate (TFA) and heptafluorobutyrate (HFBA) as pairing ion [2–5], the above finding needs some adjustment or addition to explain the observed differences. The need for an explanation is justified also by the fact that both TFA and HFBA have been widely used in the RP-IPC separation and isolation of peptides of natural or synthetic origin [6–10].

To elucidate the basis of the selectivity differences concerned, this work draws on the double-layer sorption (DLS) model of RP-IPC [11], after measuring sorption isotherms for TFA and HFBA on Nucleosil 100-5 C_{18} chromatographic packing material. Selectivity differences are exemplified by the separation of transmitteramines and some acidic metabolites and the separation of some synthetic peptides.

EXPERIMENTAL

The chromatographic system for the separation of monoamine transmitters and some prominent metabolites and also the chemicals used were described in an earlier paper [4]. Of the further compounds separated in this work, 3,4-dihydroxyphenylalanine (DOPA), 6-hydroxydopamine (HODA) hydrochloride and 3-methoxytyramine (MTA) hydrochloride were obtained from Sigma (St. Louis, MO, USA) and the peptides Ac–Ala–Tyr (II), Asp–Phe–Gly–amide (III), Ala–Pro–Val–Arg–Ser–amide (IV), Ala–Phe–Pro (V) and Ala–Phe–Ile–Gly

0021-9673/94/$07.00
SSDI 0021-9673(93)E1046-3

(VI) were synthesized in this institute. The strong cation exchanger Mono S HR 5/5 was purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden).

The stationary phase surface concentrations of TFA and HFBA formed from an acetonitrile–aqueous buffer (10:90, v/v) eluent were determined as previously described [12]. The aqueous part of the eluent consisted of TFA or HFBA at various concentrations and 15 m*M* phosphate (with the concentration of each component calculated for the whole of the eluent). The pH of the aqueous part was adjusted to 2.2 with a 2 *M* solution of sodium hydroxide. The eluent concentrations of TFA and HFBA chosen for the determination of the isotherms are given in Table I and Fig. 6. The chromatographic columns and the compositions of the mobile phases are specified in the figure captions.

RESULTS AND DISCUSSION

To demonstrate the selectivity differences between the RP-IPC systems with TFA or HFBA as pairing ion, we plotted the retentions (k' values determined in the usual way) of the transmitteramines, noradrenaline (NA), dopamine (DA) and serotonin (5-HT), the internal standard, α-methyldopamine (MDA), the acidic metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA) and homovanillic acid (HVA), and the peptides II, III, IV, V and VI as a function of the TFA and HFBA concentrations in the eluent (Figs. 1–3). The elution orders of the compounds examined, obtained at the most frequently used TFA or HFBA eluent concentrations (10–20 m*M*), were as follows:

in Fig. 1 (TFA system): NA, DA, MDA, DOPAC, 5-HT, 5-HIAA, HVA

in Fig. 2 (HFBA system): NA, DOPAC, DA, 5-HIAA, HVA, MDA, 5-HT

in Fig. 3 (TFA system): IV, III, II, V, VI

in Fig. 3 (HFBA system): II, III, IV, V, VI

The chromatograms of the amines and acidic metabolites in the TFA and HFBA systems are shown in Figs. 4 and 5, respectively.

To explain the selectivity differences demonstrated in Figs. 1–5, we first determined the

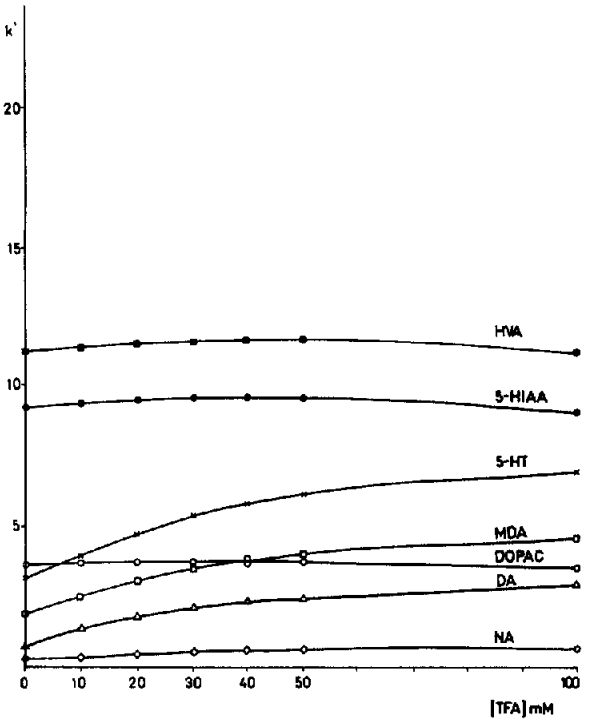

Fig. 1. Dependence of k' values of some amines and anionic metabolites on the concentration of TFA in the eluent. Column, Nucleosil C_{18} (5 μm), 100 × 4.0 mm I.D.; eluent, aqueous buffer (pH 4.15)–acetonitrile (94:6); the aqueous buffer consisted of 0.05 *M* K_2HPO_4, 0.1 m*M* EDTA and 0–100 m*M* TFA (TFA concentration 0, 10, 20, 30, 40, 50 or 100 m*M*). The pH of the aqueous buffer was adjusted with 42.5% (w/v) orthophosphoric acid or 5 *M* potassium hydroxide solution. Flow-rate, 0.83 ml/min. Detection, amperometric. From ref. 4.

surface concentrations of TFA and HFBA on the stationary phase (Nucleosil 100-5 C_{18}, 5 μm, with a BET surface area of 310 m^2/g and a carbon content of 14%) obtained after a 60-min equilibration with the respective eluent (see Experimental), using the elution method and gas chromatography, and also ion-pair extraction (for HFBA), as described in ref. 12. The surface concentrations of the pairing ions (in units of 10^{-8} mol/m^2) and the respective eluent concentrations are given in Table I. The isotherms drawn from the data in Table I are presented in Fig. 6.

As shown by the different elution orders found with the RP-IPC eluents containing TFA or HFBA (see Figs. 1–5), changing the chain length

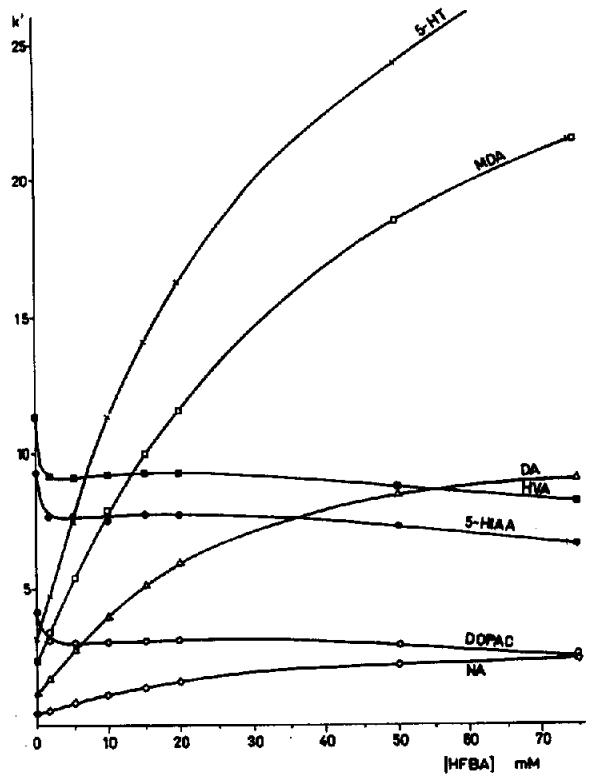

Fig. 2. Dependence of k' values of some amines and anionic metabolites on the concentration of HFBA in the eluent. HFBA concentration in the aqueous buffer: 0, 2, 5, 10, 15, 20, 50 or 75 m*M*. Other details, without TFA, were the same as in Fig. 1. From ref. 4.

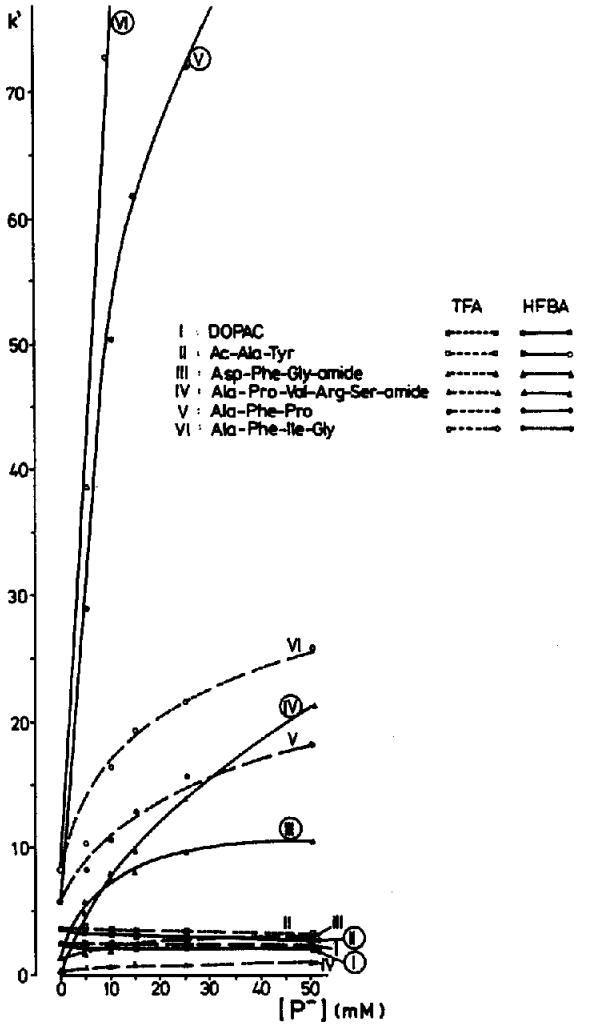

Fig. 3. Dependence of k' values of some synthetic peptides and DOPAC (for comparison) on the concentration of TFA (dotted lines) and HFBA (continuous lines) in the eluent. Column, Nucleosil C_{18} (5 μm), 170 × 4.0 mm I.D.; eluent, aqueous buffer (pH 2.20)–acetonitrile (90:10); the aqueous buffer consisted of 15 m*M* orthophosphoric acid and 0–50 m*M* TFA or HFBA (with the concentration of each component calculated for the whole of the eluent). TFA and HFBA concentrations: 0, 5, 10, 15, 25 and 50 m*M*. The pH of the aqueous buffer was adjusted with 2 *M* sodium hydroxide solution. Flow-rate, 1.0 ml/min. Detection, UV at 214 nm.

of the pairing ion resulted in a remarkable change in the system selectivity, which is in apparent contrast with the suggestion based on the results obtained for C_4–C_{12}-alkanesulphonate pairing ions [1]. The surface concentration data for the adsorbed pairing ions (which we denote as $[LP^-]$, with reference to the pairing ion, P^-, on the lipophilic adsorbent surface) and the isotherms drawn from them reveal that the $[LP^-]$ range within which TFA and HFBA are interchangeable and, therefore, can be regarded as identical from the point of view of their effect on system selectivity is very limited and too low for HFBA (see Fig. 6).

It should be pointed out that the results in ref. 1 were obtained with salt-controlled RP-IPC systems. However, as our primary aim was to explain the observed selectivity differences between TFA and HFBA systems under chromatographic conditions as close as possible to those applied in practice [typical elution conditions with TFA or HFBA systems used, *e.g.*, for the isolation of peptides include low ionic strength

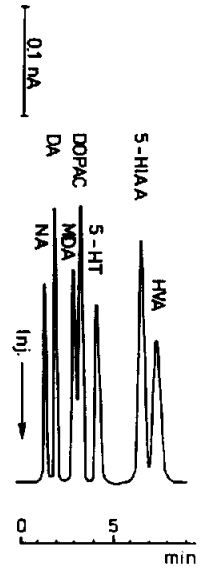

Fig. 4. Separation of some amines and acidic metabolites in a TFA system. Column, Nucleosil C_{18} (5 μm), 80 × 4.0 mm I.D. The TFA concentration in the eluent was 20 m*M*. Other conditions as in Fig. 1.

(<0.025), low pH (1.9–2.2) and an acetonitrile concentration of 0–40% (v/v)], the isotherms for TFA and HFBA were measured from eluents without salt control.

Salt control in the [P^-] range studied would

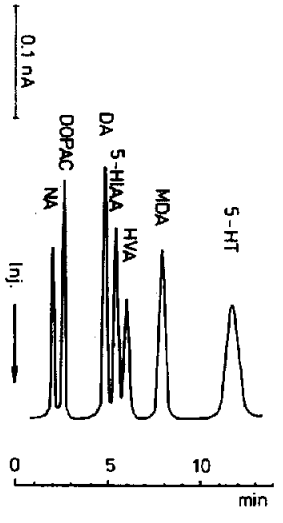

Fig. 5. Separation of some amines and acidic metabolites in an HFBA system. The HFBA concentration in the eluent was 20 m*M*. Column as in Fig. 4. Other conditions, without TFA, as in Fig. 1.

have required a counter ion concentration of at least 200 m*M* at each of the [P^-] levels chosen for the isotherms, and although the results thus obtained might have been more tractable for a rigorous theoretical analysis, they might also have been less meaningful for the explanation of the elution orders found in practice. In addition, a comparison of isotherms for two different pairing ions should lead, in our case, to valid practical conclusions even when the ionic strength of the eluent is not controlled, because the difference between the isotherms measured under the same conditions is usually less affected (especially at lower [P^-] levels) by the counter ion concentration level of the eluent than the position and shape of the individual isotherms themselves (compare the isotherms of two different pairing ions in Fig. 1 in ref. 1 at changing and constant counter ion concentrations).

In eluents used for the separation of peptides, both pairing ions are mostly used at a 0.1% (v/v) concentration, which corresponds to an eluent concentration of 12.9 m*M* with TFA and 7.7 m*M* with HFBA. As shown by the isotherms, these eluent concentrations generate [LP^-] levels of *ca.* $1.5 \cdot 10^{-8}$ mol/m^2 for TFA and *ca.* $10 \cdot 10^{-8}$ mol/m^2 for HFBA. It is fairly obvious from its isotherm that the latter [LP^-] level is virtually unattainable with TFA.

The surface concentration of the pairing ion and the ionic strength in the eluent play a major role in determining the "character" of an RP-IPC system. As discussed by Liu and Cantwell [11], in RP-IPC an electrical double layer is formed on the hydrophobic surface of the stationary phase owing to the selective adsorption of the pairing ion used. It was pointed out that retention in such systems is governed both by dynamic ion exchange in the diffuse part of the double layer and adsorption on the electrically charged surface, and that it is the experimental conditions that determine which of the two is the dominant process.

It can be concluded from the discussion that at low ionic strength (<0.1) in the eluent, and at sufficiently high [LP^+] or [LP^-] levels (*e.g.*, at [LP^+] levels between $35 \cdot 10^{-8}$ and $65 \cdot 10^{-8}$ mol/m^2, as tested by the authors), the dominant process governing the sorption of a sample ion is

TABLE I

ADSORPTION OF TFA AND HFBA ON THE STATIONARY PHASE[a] FROM AN ELUENT[b] CONTAINING THE RESPECTIVE PAIRING ION AT VARIOUS CONCENTRATIONS[c]

TFA			HFBA		
$[P^-]$ (m*M*)	$[LP^-]$		$[P^-]$ (m*M*)	$[LP^-]$	
	10^{-8} mol/m^2	R.S.D. (%)		10^{-8} mol/m^2	R.S.D. (%)
–	–	–	5	6.6	14.2
10	1.4	21.1	10	11.3	10.4
20	3.4	20.2	20	25.7	9.7
50	3.8	20.8	50	41.7	8.5
100	6.1	16.8	100	54.7	8.6
200	5.7	17.1	200	62.7	8.1

[a] Nucleosil 100-5 C_{18}, 100 Å, 5 μm.
[b] Acetonitrile–aqueous buffer (10:90, v/v); see Experimental.
[c] $[P^-]$ = eluent concentration of the pairing ion (m*M*); $[LP^-]$ = surface concentration of the adsorbed pairing ion (10^{-8} mol/m^2); R.S.D. = relative standard deviation; for the calculation of *n* for TFA and HFBA see ref. 12, p. 147.

dynamic ion exchange. The contribution of surface adsorption to the overall chromatographic distribution coefficient is well below 20% in such systems [11].

The ionic strength in both the TFA- and HFBA-containing eluents used for the separation of peptides or other compounds is low (usually less than 0.1). There is, however, a substantial difference of up to an order of magnitude between the $[LP^-]$ levels formed from the eluents, resulting in the consequence that, owing to the very low $[LP^-]$ levels, the conditions in a TFA system favour surface adsorption, whereas the higher $[LP^-]$ levels in HFBA systems increase the chances of dynamic ion exchange.

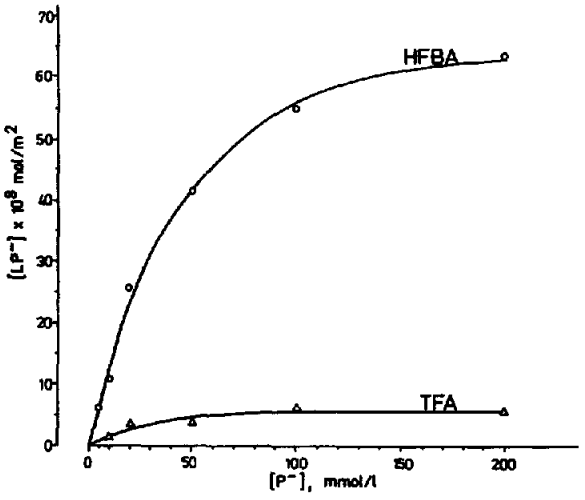

Fig. 6. Adsorption isotherms for TFA and HFBA on Nucleosil 100-5 C_{18}, 5 μm (170 × 4.0 mm I.D.), determined as described in Experimental, and constructed from the data in Table I.

Fig. 7 shows the chromatogram of some amines and acidic metabolites obtained with a simple reversed-phase system, without a pairing ion, but otherwise under the same conditions as those in Fig. 4. A comparison of the elution orders shown in Figs. 4 and 7 reveals essential similarities. Both systems are characterized by a relatively high retention for the acids and lower retention for the amines. It appears that TFA can effectively function as a pairing ion, *i.e.*, can satisfactorily increase the retention of cations, only if the cation concerned is sufficiently hydrophobic and exhibits a k' value of 2 or larger in the corresponding simple reversed-phase system (see the retention behaviour of MDA and 5-HT in Figs. 1 and 4 and the peptides III, V and VI in Fig. 3).

Fig. 8 shows the chromatogram of the same compounds as in Fig. 7 but obtained with a simple ion-exchange system, using the same eluent as in Fig. 7. When the elution orders in

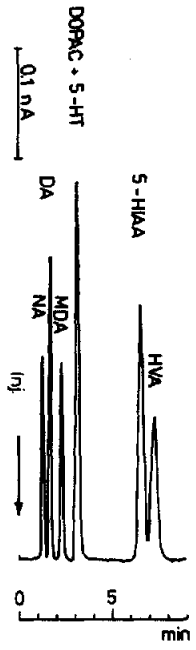

Fig. 7. Separation of some amines and acidic metabolites in a reversed-phase system. Column as in Fig. 4. The eluent contained 20 m*M* nitric acid to replace the pairing ion concentration. Other conditions, without TFA, as in Fig. 1.

Figs. 5 and 8 are compared, similar retention tendencies can be discovered, with an increased retention for most of the amines (NA is an exception) and a decreased retention for the acids, as compared with Figs. 4 and 7. These retention characteristics are stronger with the ion-exchange system because of the higher concentration of the negatively charged groups on the surface of the stationary phase.

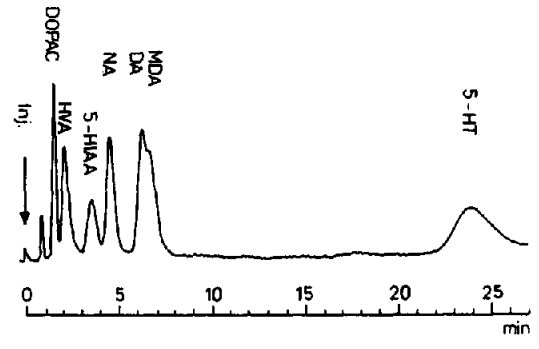

Fig. 8. Separation of some amines and acidic metabolites in an ion-exchange system. Column, Mono S HR 5/5 (10 μm), 50 × 5.0 mm I.D., with an ionic capacity of 0.14–0.18 m*M* per column. Eluent and flow-rate as in Fig. 7. Detection, UV at 274 nm.

The increased significance of dynamic ion exchange in HFBA systems is supported also by the fact that the retention of peptide IV, a compound with two positive charges, increased markedly with increase in the eluent concentration of HFBA, whereas the same compound was very poorly retained in the TFA systems studied (see Fig. 3). The poor retention of the

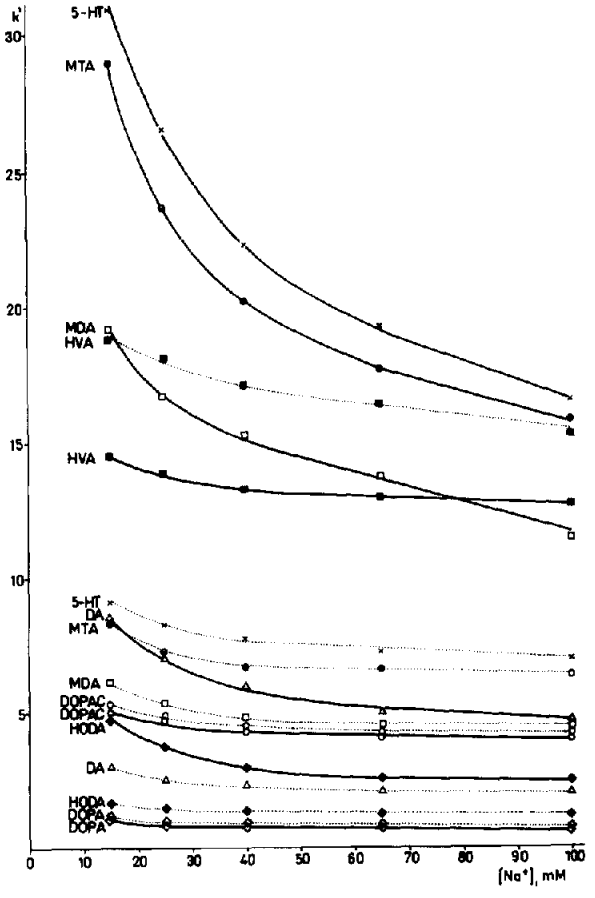

Fig. 9. Dependence of k' values of some amines, acidic metabolites and DOPA on the counter ion (Na^+) concentration in the eluent containing 10 m*M* TFA (dotted lines) or 10 m*M* HFBA (continuous lines). Column as in Fig. 1. The eluent consisted of 3% (v/v) acetonitrile and 97% aqueous component. Na^+ concentrations for the whole of the eluent were 15, 25, 40, 65 and 100 m*M*, adjusted with a calculated volume of 2 *M* sodium hydroxide solution. The pH of the aqueous component was adjusted to 4.25 using orthophosphoric acid (42.5%, w/v). Flow-rate and detection as in Fig. 1.

strongly hydrated, doubly charged cation in the TFA systems bears evidence of the dominance of non-specific surface adsorption with these systems.

Further support of the above distinction between TFA and HFBA systems is provided by Fig. 9. With HFBA systems (continuous lines), the retention of cations (HODA, DA, MDA, MTA and 5-HT) decreased "faster" with increase in the concentration of the eluent counter ion (Na^+) in the range 15–65 m*M* than the retention of the anions (DOPAC and HVA). With TFA systems (dotted lines), however, the difference between the retention behaviour of the cations and anions with increasing counter ion concentration of the eluent was negligible.

CONCLUSIONS

Selectivity differences between RP-IPC systems with TFA and HFBA as pairing ion can be explained by the fact that, as opposed to C_4–C_{12}-alkanesulphonates, the pairing ions concerned are not interchangeable in terms of their concentrations formed on the surface of the stationary phase at practical TFA and HFBA eluent concentrations. Most HFBA systems, depending on the [LP^-] level in the respective system, provide substantial evidence of an increased significance of dynamic ion exchange in governing the retention of a sample ion, whereas TFA systems, in general, are mainly characterized by non-specific surface adsorption.

In the light of the DLS theory, TFA and HFBA systems exhibit different selectivity effects, because the contributions of dynamic ion exchange and surface adsorption to the overall chromatographic distribution coefficient are very different with the two systems.

ACKNOWLEDGEMENTS

The author is grateful to Dr. É. Tomori for the GLC analyses, to Dr. A. Juhász for synthesizing the peptides, to Mrs. E. Blazsek for excellent technical assistance and to Mrs. J. Vámosi and Mrs. E. Tóth for their help in preparing the manuscript.

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