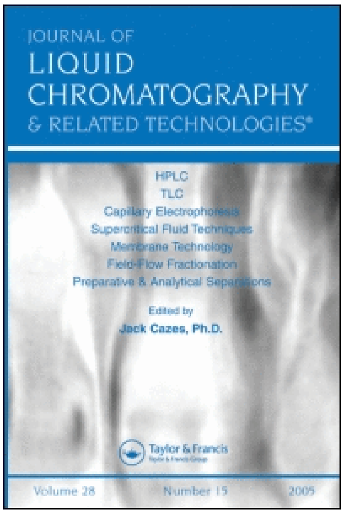

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RETENTION OF ACIDIC AROMATIC COMPOUNDS IN ION EXCLUSION CHROMATOGRAPHIC SEPARATIONS

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ABSTRACT

Aromatic acidic compounds have been separated by ion exclusion chromatography. The theoretical equation, which relates the retention volume of an eluted acid to its dissociation constant, failed for aromatics, which show higher retention volumes than predicted. An important role is played by hydrophobic adsorption, which can be increased by addition of ion interaction reagents to the mobile phase .

INTRODUCTION

Ion exclusion chromatography (IEC) is a widely applied technique to separate ionic compounds, since the retention of a single compound depends on the ratio of concentrations of its ionised and neutral forms.[1] The characteristic feature of IEC technique is the electric charge of dissociated ion-exchange resin functional groups which has the same sign of the electric charge of the analysed ionic compound. It follows that samples containing negatively charged ions, as dissociated acidic compounds, are separated on cation exchange resins with anionic functional groups. For this purpose a large ion exchange capacity column is preferred, which can be obtained maximising the column dimensions and the functional group concentration in the support, and using strong ion-exchange resins.

When such a column is filled up with water, which is pumped through it as a mobile phase, the water molecules build up hydration spheres around the dissociated functional groups of the support. Water contained in the pores of the support and in the hydration spheres is immobilised and it forms the stationary phase, in which the resin functional group ions and the hydrogen ions are dissolved. The retention mechanism of ion exclusion[2] is based on the following phenomenon. Neutral, uncharged molecules are allowed to penetrate the resin, while similarly charged coions are repulsed owing to the presence of dissociated functional groups immobilised in the stationary phase. The hydrated resin network behaves like a semipermeable membrane between the stationary phase and the mobile phase. With the exception of the covalently bound functional groups and of the counterions because of the electroneutrality requirement, all other species are freely exchanged through such a hypothetical membrane, which is permeable to neutral molecules. As a consequence, strong acids, which are completely dissociated and, therefore, electrostatically repulsed are eluted, not separated, in the column dead volume (V_M), which corresponds to the volume of the mobile phase in the column. On the other hand, undissociated compounds can completely penetrate the resin and are also not separated being eluted in a volume correspondent to the sum of the inner and the dead volumes of the column, where the inner column volume is just the volume of its stationary phase. This behaviour makes the determination of the inner and dead column volumes straightforward as it has been confirmed by Tanaka et al.[3] Only the acids of intermediate strength, with their dissociation constants falling in the range 10^{-7} - 10^{-2}, can be separated by this technique.

Ion exclusion can seldom be considered as the sole retention mechanism even on an ion-exclusion resin. Like, in other chromatographic techniques, it is classified according to the primary mechanism of solute retention. This primary mechanism in IEC is coulombic repulsion between solute and dissociated groups of the resin. Besides ion exclusion, let us recall hydrophobic adsorption on the resin network as in reversed phase chromatography, size exclusion,[4,5] effect of functional group screening in the analysed sample, normal phase retention, van der Waals, and polar interactions of the sample compound with the support.[1]

The aim of the presented paper is to investigate how hydrophobic adsorption influences the retention of aromatic acidic compounds in ion exclusion chromatographic separations. To this purpose, the retention volumes of aliphatic and aromatic acids are compared with a theoretical model. Furthermore, the addition of ion interaction reagents is studied in order to investigate the possibility to govern hydrophobic adsorption.

EXPERIMENTAL

Materials

Analytical reagent-grade sulphuric acid (Carlo Erba, Milan, Italy) dissolved in triply distilled water at the selected concentration was used as mobile phase. Tetrabutylamonium bromides used as ion-interaction reagents were Aldrich-Chemie (Steinheim, Germany) products. All other chemicals were Research Grade products and were used without further purification.

Apparatus

The chromatographic analyses were carried out by Beckman equipment consisting of a 110B Solvent Delivery Module, a 340 Organizer, and a 160 Absorbance Detector, and equipped with a HPX-72-O Bio-Rad (Richmond, USA) column (300 x 7.8 mm I.D., hydroxide form strong ion exclusion column packed with 11 μm particles of polystyrene-divinylbenzene copolymer, crosslinking 8%). The retention times of the eluted compounds were measured by a Hewlett Packard HP 3394A Integrator.

Procedure

Triply distilled water was passed through a Millipore (Bedford, USA) Milli-Q water purification system, filtered through a Millipore 0.45 μm membrane filter, and degassed in an ultrasonic bath, before the addition of sulphuric acid. The column was equilibrated for at least 1 hour prior to being used.

All of the analyses were carried out at ambient temperature. Each sample was injected six times and the average value of its retention time was taken.

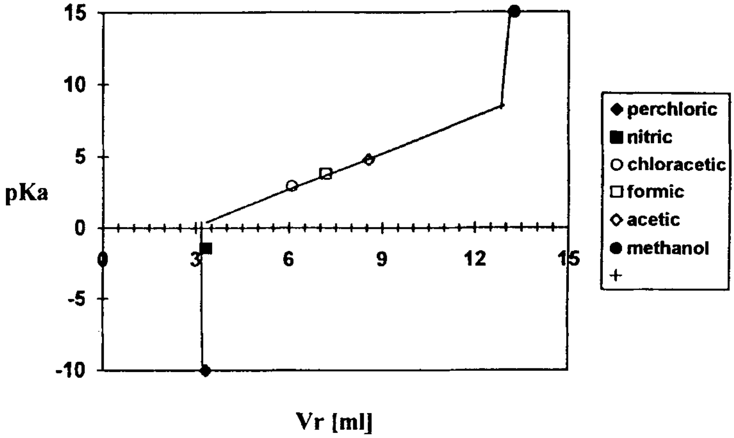

Figure 1. Effect of pK_a values on retention volumes, V_R. Bio-Rad Aminex ion-exclusion HPX-87H organic acid analysis column, 300 x 7.8 mm I.D., hydrogen form 8% crosslinked cation exchanger, 9 μm particle diameter. Mobile phase: 1 mN H_2SO_4. Solute acids separated by pure ion-exclusion effect.

RESULTS AND DISCUSSION

Many models have been used to describe the IEC mechanism. The simplest one, which assumes pure water as a mobile phase, equal inner and dead volumes, and the support functional groups completely dissociated, leads to simple equations.[6] The distribution coefficient, K_d, can be obtained from the chromatographic data:

$$K_d = (V_R - V_M)/V_S \qquad (1)$$

where V_R and V_S are the retention and the stationary phase volumes, respectively.

The influence of the dissociation constant of an acid on its retention volume is shown in Figure 1. The characteristic for the ion-exclusion s-shape dependence between the pK_a and retention volume can be observed.

Strong acids, like perchloric or nitric, are eluted at the dead column volume ($K_d = 0$). Very weak acidic compounds are eluted at a distribution coefficient equal one. Other acids (chloroacetic, formic, acetic) are eluted between these boundaries, as confirmation of the theoretical predictions.[1,3]

Table 1

Observed Retention Volumes (mL) Against Expected Retention Volumes from pKa Values

Acid	pKa	Vr obs.	Vr exp.
Benzoic	4.20	72.00	7.4 - 8.2
Salicylic	3.00	44.87	6.0 - 6.8
Anisic	4.47	41.09	7.6-8.4
Tannic	4.32	20.03	7.5 - 8.3

From the s-shape of Fig. 1. the retention of other acids can be calculated. In Table 1 the found retention volumes of aromatic acids are compared with the calculated values. It can be observed that these compounds are characterised by much higher retention than expected on the basis of a pure ion-exclusion. It means that their retention is governed by the mixed ion-exclusion - hydrophobic adsorption mechanism.[5]

A theoretical model can be proposed.

The distribution coefficient (K_d) in the case of acidic solutes can be written as:

$$K_d = \frac{[HR]_S + [R^-]_S}{[HR]_M + [R^-]_M} \qquad (2)$$

where the subscripts M and S refer to the mobile and the stationary phase, respectively.

Under the conditions that a strong acid is used as a buffer and that its concentration is much higher than that one of solute, Eq. (2) can be written as:

$$K_d = \frac{[HR]_S + [HR]_S \, {}^{K_a}/_{c_f}}{[HR]_M + [HR]_M \, {}^{K_a}/_{c_b}} \qquad (3)$$

where c_f and c_h are the functional groups and the buffer concentrations, respectively, and K_a is the dissociation constant of the acid.

Table 2

Comparision of Retention Volumes (mL) and pK_a Values of Some Isomers of Substituted Benzoic Acids. Mobile Phase: 1mN H_2SO_4 Column: Aminex HPX-87H

Acid	Ortho-		Para-		Meta-	
	pK_a	V_R	pK_a	V_R	pK_a	V_R
Toluic	3.91	59	4.36	61.6	4.27	103.
Nitrobenzoic	2.17	9.6	3.44	55.0	3.49	88.0
Phthalic	2.98	11.8	3.51	45.3	3.54	4.5

In an analytical chromatographic process we assume linear adsorption:

$$K_d = \frac{K_H\left(1+\frac{K_a}{c_f}\right)}{1+\frac{K_a}{c_b}} \tag{4}$$

where K_H consists of the two effects: the ion-exclusion and hydrophobic adsorption of the neutral molecule.

In the absence of adsorption the concentrations of the neutral molecules in the both phases are equal. Since in ion-exclusion columns the concentration of the functional groups is generally much higher than that of the solute, the second term of the numerator can be generally omitted.

From Table 1 it can be read out that the aromatic compounds are characterised by a very high retention. This is probably due to the interaction between their π-electron and the resin network. This effect was found even stronger for aromatic bases[1,4] whose retention was a hundred times higher than that expected from the pure ion-exclusion effect.

As reported in Table 2, substituted aromatic acids are eluted mainly in the order of ortho-, para-, meta-. Even if they show different values of their dissociation constants, such differences seem to be too small to explain the big changes in their retention. An explanation can be found in their different molecular volumes, which are related to their surface area. It can be observed that their retention is inversely proportional to their density.

Table 3

Retention Volumes (mL) of Isomeric Nitrobenoic Acids in Different Mobile Phases (Bio-Rad Aminex Ion-Exclusion HPX-87H Organic Acid Analysis Column, 300 x 7.8 mm I.D., Hydrogen Form 8% Cross-Linked Cation Exchanger, 9 μm Particle Diameter)

			Mobile Phase		
Isomer	**pK_a**	**ρ [g/mL]**	**Water**	**1mN H_2SO_4**	**0.5mM TBABr**
Ortho	2.17	1.575	5.05	9.6	8.33
Para	3.44	1.55	5.14	55	49.05
Meta	3.49	1.494	9.3	88	74.49

As an example, m-nitrobenzoic acid is the last isomer eluted in the different experimental conditions, reported in Table 3. The meta- isomer is characterised by the smallest density value, 1.494 g/mL, in comparison with para- (1.55 g/mL) and ortho- (1.575 g/mL) isomers.

Since hydrophobic adsorption seems to play an important role in ion exclusion chromatographic separations, it seems useful to investigate how to govern it.

Hydrophobic adsorption can be decreased by addition of an organic modifier to the mobile phase. As a matter of fact,[7] it has been shown that cyclodextrine added to the mobile phase could also decrease retention.

On the other side, an increase of the hydrophobic adsorption can be obtained by addition of an ion-interaction reagent (IIR) to the mobile phase. In other chromatographic techniques,[8] it was found that the addition of small concentration of IIR to the mobile phase in the absence of buffer not only improved the retention, but led to more symmetrical peaks and avoided the dependence of the solute retention on its concentration. Also in IEC, IIR can be added to the mobile phase.

Since the electrostatic interactions between solutes and functional groups are very quick, there should not be time wasted waiting for column stabilisation. To confirm such a hypothesis, we changed the mobile phase from water to 0.5 mM tetrabutylamonium bromide (TBABr). The equilibrium was reached after few minutes. The same effect was found for the opposite variation.

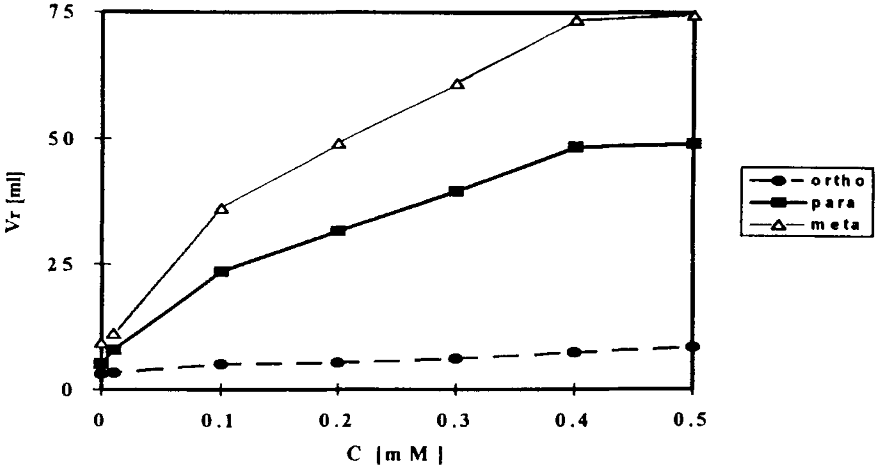

Figure 2. Influence of the tetrabutylammonium bromide concentration on the nitrobenzoic acids retention. Chromatographic conditions as in Fig. 1.

The influence of the IIR concentration on the retention for the isomeric nitrobenzoic acids is shown in Fig. 2. It can be seen that the addition of a small concentration of TBABr improves the separation of the isomers.

CONCLUSIONS

Aromatic acidic compounds are characterised by higher retention than predicted by a pure ion exclusion mechanism. We suggested that the reason can be found in a hydrophobic adsorption on the resin network, which is constituted mainly of styrene and divinylbenzene copolymerised resins. It means that, in this case, the retention is governed by the mixed ion-exclusion - hydrophobic adsorption mechanism.

This adsorption can be increased by the addition of an ionic interaction reagent to the mobile phase. Since, for this purpose, a small concentration (0.01 mM) of ionic interaction reagent can be used in the absence of buffer, the overall result is an improvement of solute retention without influencing the detection system.

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