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Factors affecting retention of basic solutes in ion-exclusion chromatography using an anion-exchange column

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Abstract

Retention volumes have been measured for a variety of inorganic and organic (both aliphatic and aromatic) bases on a quaternary ammonium functionalized styrene–divinylbenzene stationary phase using dilute sodium hydroxide as eluent. The retention behaviour of the inorganic bases and some of the aliphatic bases could be explained on the basis of ion-exclusion effects alone, with strong bases (which are cationic at the eluent pH) being co-eluted at the column void volume and very weak bases (which are neutral at the eluent pH) being co-eluted at the sum of the column void and inner volumes. Solutes intermediate between these extremes were eluted in order of increasing pK_{b1} and their retention could be varied by changing the eluent pH. A mixed retention mechanism involving hydrophobic adsorption and steric effects was observed for other aliphatic amines. Aromatic amines were found to be retained almost solely by a reversed-phase mechanism involving interaction of the solute with the unfunctionalized regions of the stationary phase. For such solutes, retention could be manipulated most easily by addition of acetonitrile to the eluent.

1. Introduction

Ion-exclusion chromatography, first introduced by Wheaton and Bauman in 1953 [1], has been used predominantly for the separation of organic acids and some inorganic weak acid anions using a sulphonate-type cation-exchange stationary phase (usually in the hydrogen form) with an eluent comprising a dilute solution of a mineral acid. Several studies [2–7] have been devoted to the elucidation of the mechanism of ion-exclusion chromatography under these conditions. Tanaka *et al.* [3] found that the retention volume of an acidic solute was dependent primarily on the first acid dissociation constant (pK_{a1}) of the solute. They showed that the dependence between the sample retention volume and its pK_{a1} value could be explained, at least to a first approximation, by the magnitude of the charge on the solute. That is, all solutes which were fully ionized at the eluent pH were unretained by virtue of their repulsion by the anionic functional groups of the stationary phase and were eluted at the column void volume. On the other hand, solutes which were neutral were all co-eluted at a retention volume equal to the sum of the void and inner volumes of the column since they are able to partition freely between the eluent and the inner volume (that is, the occluded liquid trapped within the pores of the stationary phase). Solutes having intermediate charge exhibited retention volumes which fell between the above extremes.

This behaviour considers only the effects of

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solute charge and has been described quantitatively by Glod and co-workers [4,5]. Further studies [2,4,6,7] have identified other factors which can influence the solute retention, including hydrophobic adsorption of the solute on the underivatized regions of the stationary phase resin and a size-exclusion effect which mediates the ability of the solute to penetrate the pores of the stationary phase. The magnitude of the effects of these factors depends on such solute parameters as the length of the molecular chain, molecular mass, solubility in water, etc. A comprehensive review of these effects may be found elsewhere [2]. The existence of these factors results in significant departures from the retention behaviour predicted on the basis of solute charge alone. For example, some neutral solutes show retention volumes which are much greater than expected due to the additional retention caused by hydrophobic adsorption.

Ion-exclusion chromatography may also be used for the separation of basic compounds using a quaternary ammonium anion-exchange resin and an alkaline eluent [8]. In this case, a dependence between the retention volume and the pK_{b1} value of the solute would be anticipated, along similar lines to those observed for acidic solutes. To our knowledge, no detailed study of the retention behaviour of basic solutes has been reported and the aim of the present paper has been to examine the factors contributing to the retention of such solutes. These factors included solute characteristics such as the pK_{b1} value, length of aliphatic chain, presence of aromatic groups, and degree of substitution, together with eluent characteristics such as concentration, pH, and presence of organic modifiers.

2. Experimental

2.1. Instrumentation

The chromatographic instrumentation comprised a Millipore–Waters (Milford, MA, USA) Model 510 chromatographic pump, Model U6K universal injection valve, Model 430 conductivity detector and Model TCM temperature-control module. A Shimadzu (Kyoto, Japan) model SPD-6AV UV–Vis photoabsorbance detector was also used in tandem with the conductivity detector and was operated at either 214 nm (for aliphatic amines) or 254 nm (for aromatic amines). The ion-exclusion column used was a Bio-Rad (Richmond, CA, USA) Model HPX-72-O, 300 × 7.8 mm I.D., packed with 11-μm particles of polystyrene–divinylbenzene co-polymer (8% cross-linking) derivatized with quaternary ammonium groups. Chromatograms were recorded using a Goerz–Metrawatt (Vienna, Austria) SE-120 dual-pen chart recorder.

2.2. Reagents

The mobile phase comprised water with varying concentrations of analytical reagent-grade sodium hydroxide (BDH, Port Fairy, UK) and HPLC-grade acetonitrile (Millipore–Waters). The aliphatic amines were obtained from Sigma (St. Louis, MO, USA) and aromatic amines, pyridines and inorganic bases were from Fluka (Buchs, Switzerland) or from Ega-Chemie (Steinheim, Germany). All reagents were of analytical-reagent grade and were used without any further purification.

2.3. Procedures

Water was triply distilled and was passed through a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus. Eluents were filtered through a 0.45-μm membrane filter and were degassed in an ultrasonic bath, boiled and purged with nitrogen prior to use. Eluent reservoirs were fitted with a sodium hydroxide trap to exclude carbon dioxide from the air. All experiments were performed using an eluent flow-rate of 1 ml/min. The column was conditioned with the mobile phase for 30 min prior to the recording of chromatographic data and the column temperature was maintained at 25°C.

Stock solutions of solute bases were prepared as 10 mM solutions in Milli-Q water and diluted to the required concentrations before use. Injections (20 μl) of sample solutions were made using a 100-μl syringe (Hamilton, Reno, NV,

USA), and chromatograms were recorded simultaneously on the conductivity and UV-absorbance detectors.

The void and the inner column volumes for the Bio-Rad column were determined by the method described [3] and were found to be 3.8 and 6.5 ml, respectively.

3. Results and discussion

3.1. Ion-exclusion effect

Retention data for a wide variety of organic (both aliphatic and aromatic) and inorganic bases using 10 m*M* sodium hydroxide as eluent are listed in Table 1, together with the pK_{b1} value for each solute. It can be seen that the strong inorganic bases were eluted at or close to the column void volume (3.8 ml) since they are excluded from the resin by electrostatic repulsion from the positively charged quaternary ammonium functional groups. Very weak aliphatic bases which are neutral at the eluent pH, together with methanol which was used as a neutral marker compound, can partition freely into the inner volume of the resin and were eluted at or close to a retention volume equal to the sum of the dead and the inner column volumes (10.3 ml). Most of the other aliphatic amines were eluted between these boundaries, with the exception of higher alkylamines, di-

Table 1
Retention data for basic compounds

Solute	pK_{b1}	V_R (ml)	K_d	Solute	pK_{b1}	V_R (ml)	K_d
Inorganic bases				*Aromatic amines*			
KOH	−10.00	3.90	0.02	Pyridine	8.79	22.40	2.86
NaOH	−5.00	3.90	0.02	2-Picoline	8.08	33.10	4.51
$Ca(OH)_2$	2.43	4.00	0.03	3-Picoline	8.48	41.78	5.84
$Zn(OH)_2$	3.02	3.80	0.01	4-Picoline	7.92	39.36	5.47
$Pb(OH)_2$	3.02	4.00	0.03	2,3-Lutidine	7.43	66.92	9.71
AgOH	3.96	3.80	0.01	2,4-Lutidine	7.01	66.40	9.63
$As(OH)_3$	3.96	3.95	0.02	2,6-Lutidine	7.28	51.36	7.32
NH_4OH	4.75	6.96	0.49	3,4-Lutidine	7.51	81.76	11.99
				3,5-Lutidine	7.85	90.40	13.32
Organic bases				3-Aminopyridine	7.97	27.40	3.63
Hydrazine	5.77	6.20	0.37	4-Aminopyridine	4.89	35.16	4.82
Hydroxylamine	7.97	10.40	1.02	Aniline	9.39	114.00	16.95
Urea	13.82	10.32	1.00	2-Methylaniline	9.56	206.70	31.22
Thiourea	14.26	10.40	1.02	3-Methylaniline	9.30	223.20	33.75
$(CH_3)_4NOH$	−15.00	3.80	0.00	4-Methylaniline	8.89	200.88	30.32
Methylamine	3.34	6.60	0.43	2,4-Dimethylaniline	9.11	394.30	60.08
Ethylamine	3.30	7.15	0.52	3,5-Dimethylaniline	9.09	456.20	69.60
Propylamine	3.40	8.00	0.65	2-Aminoaniline	9.51	70.80	10.31
Butylamine	3.37	14.56	1.66	4-Aminoaniline	7.84	19.56	2.42
Pentylamine	3.37	28.00	3.72	Benzylamine	4.67	49.06	6.96
Hexylamine	3.36	62.70	9.06	4-Methylbenzylamine	4.64	104.92	15.56
Trimethylamine	4.19	7.36	0.55	2-Phenylbenzylamine	4.16	84.56	12.42
Diethylamine	2.96	8.60	0.74	2-Methylbenzylamine	4.81	87.12	12.82
Triethylamine	3.00	16.70	1.98				
Dibutylamine	2.99	81.42	11.94				
Triethanolamine	6.24	7.00	0.49				
Ethylenediamine	4.07	5.30	0.23				
Methanol	15.00	10.30	1.00				

A Bio-Rad HPX-72-O column (300 × 7.8 mm I.D.) was used with 0.01 *M* NaOH as mobile phase. K_d = Distribution coefficient.

butylamine and triethylamine which, together with the aromatic bases, were eluted at retention volumes greater than 10.4 ml.

Values of the distribution coefficient for each solute were calculated from the retention volumes and are presented in Table 1. The strong bases are characterised by a distribution coefficient close to zero, whilst the very weak bases show a distribution coefficient close to unity. Since we are considering the distribution of the solutes between two phases with the same chemical composition, the largest theoretical value that the distribution coefficient can attain is unity; that is, when an equal solute concentration exists in both phases. When the distribution coefficient exceeds unity (as is the case, for example, for the aromatic bases), this indicates that a retention mechanism other than ion exclusion is in operation.

Fig. 1 shows a plot of pK_{b1} *vs.* retention volume for the inorganic and aliphatic solutes shown in the left-hand column of Table 1. Many of the data points (those represented as open circles in Fig. 1) can be joined by three straight lines characteristic of ion-exclusion behaviour in which the retention volume can be predicted from the pK_{b1} of the solute. This part of Fig. 1 is identical to a plot of pK_a *vs.* retention volume obtained previously for aliphatic carboxylic acids [3]. The remaining data points do not follow the ion-exclusion model. Linear alkylamines (△) and the secondary and tertiary amines (□) show greater retention than expected, whilst triethanolamine and hydrazine (●) show less retention than expected. The retention behaviour of these species will be rationalised below.

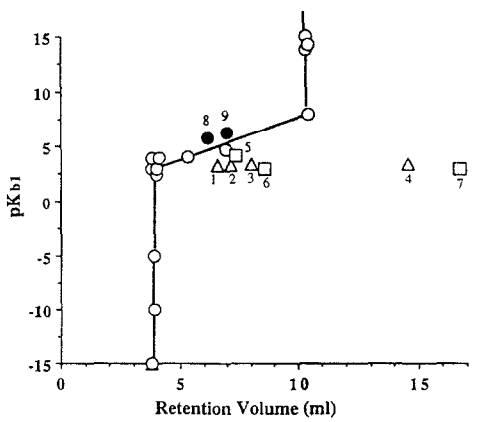

Fig. 1. Plot of pK_{b1} *versus* retention volume for the inorganic and aliphatic organic bases listed in Table 1. A 10 m*M* sodium hydroxide eluent was used with a Bio-Rad HPX-72O ion-exclusion column. Solutes: 1 = methylamine; 2 = ethylamine; 3 = propylamine; 4 = butylamine; 5 = trimethylamine; 6 = diethylamine; 7 = triethylamine; 8 = hydrazine; 9 = triethanolamine.

Apart from the general shape of Fig. 1, the existence of an ion-exclusion mechanism for those data points falling on the lines can be confirmed by the influence of other factors which affect the degree of ionization of the solute. When water alone was used as the eluent, the retention volume was found to decrease when the amount of injected solute was decreased. This behaviour can be attributed to increased ionization of the solute at low concentration, in accordance with theoretical prediction [5], and results in the appearance of fronted peaks. The dependence of retention volume on the amount of solute injected is eliminated when sodium hydroxide is used as eluent since the degree of solute dissociation is maintained at a constant value regardless of solute concentration. Symmetrical peaks are also obtained with this eluent. Solute dissociation can also be manipulated by changing the concentration (and hence the pH) of the sodium hydroxide eluent, with an increase in retention volume being observed with an increase of eluent concentration. Other alkaline buffers such as carbonate buffers might be used as well but have not been investigated during this study.

3.2. Hydrophobic interaction between solute and stationary phase

The retention volumes for the linear alkylamines and the secondary and tertiary amines in Fig. 1 are larger than those predicted on the basis of their pK_{b1} values alone. Retention volumes increase steadily for the homologous series of alkylamines as the alkyl chain length increases, despite the fact that all have very similar pK_{b1} values. Similarly, triethylamine has a much greater retention volume

than diethylamine, without any significant change in pK_{b1}. As mentioned previously, hydrophobic interaction between the solute and the stationary phase has been observed in ion-exclusion chromatography of carboxylic acids [4,6,7] and is clearly also a factor in ion-exclusion chromatography of aliphatic bases.

This effect is even more pronounced for aromatic amines, as seen from their anomalously large distribution coefficients listed in Table 1 and can be attributed to strong π-electron interaction with the aromatic rings of the solute and the resin. In order to determine whether such hydrophobic adsorption effects were the predominant cause of solute retention, two further experiments were performed. First, the dependence between the logarithm of the solute capacity factor and the number of the carbon atoms in the solute molecule was determined and is shown in Fig. 2. A linear dependence was observed for higher (propyl to hexyl) aliphatic amines and is indicative of hydrophobic adsorption, but was not observed for the lower aliphatic amines (methyl and ethyl), suggesting that the retention of these latter species occurs through a mixed retention mechanism combining ion-exclusion and hydrophobic adsorption.

The second experiment involved measurement of retention volumes after addition of an organic solvent to the eluent. Organic solvents are usually characterised by a smaller dielectric constant than water so that, if one considers only the ion-exclusion mechanism, an increase in the retention volume might be anticipated as the percentage of organic modifier in the eluent increases. The observed relationship between the logarithm of capacity factor of some aromatic amines and the concentration of acetonitrile in the eluent is given in Fig. 3, from which it can be seen that the addition of acetonitrile caused solute retention to decrease. This fact, together with the linearity of the plots, is again indicative of reversed-phase behaviour wherein the hydrophobic interaction of the solute with the stationary phase is diminished as the percentage of acetonitrile is increased. Separation of aromatic amines in ion-exclusion chromatography can therefore be manipulated most conveniently by adjusting the percentage of organic modifier in the eluent and the magnitude of this effect is illustrated in Fig. 4 which shows chromatograms obtained with 1 m*M* sodium hydroxide made up in water and in 30% acetonitrile.

3.3. Other factors influencing retention

Fig. 1 reveals that some solutes (*e.g.* triethanolamine) show retention volumes which are somewhat smaller than those predicted from

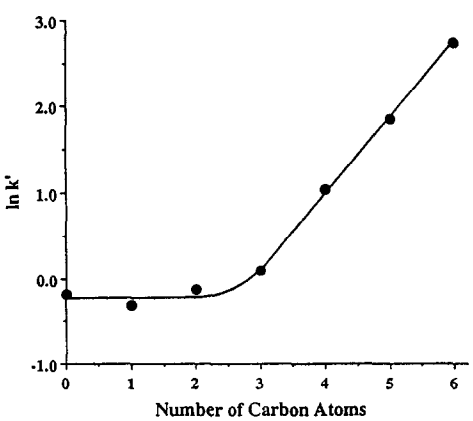

Fig. 2. Effect of the number of carbon atoms on retention volume for aliphatic amines. Chromatographic conditions as in Fig. 1.

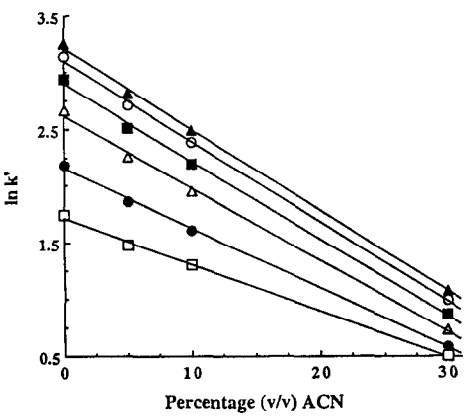

Fig. 3. Dependence of the logarithm of capacity factor on percentage of acetonitrile (ACN) in the eluent for some aromatic amines. Chromatographic conditions as in Fig. 1, but with the indicated percentages of acetonitrile added to the eluent. ▲ = 3,5-Lutidine; ○ = 3,4-lutidine; ■ = 2,3-lutidine; △ = 2,6-lutidine; ● = 2-picoline; □ = pyridine.

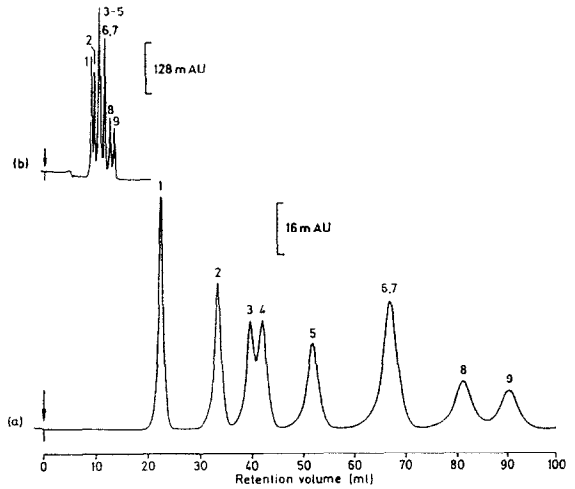

Fig. 4. Chromatograms showing the effect of acetonitrile added to the eluent on the separation of some pyridine derivatives. Eluent: (a) 1 m*M* sodium hydroxide, (b) 1 m*M* sodium hydroxide containing 30% (v/v) acetonitrile. Detection was by UV absorbance at 254 nm. Solutes: 1 = pyridine; 2 = 2-picoline; 3 = 4-picoline; 4 = 3-picoline; 5 = 2,6-lutidine; 6 = 2,4-lutidine; 7 = 2,3-lutidine; 8 = 3,4-lutidine; 9 = 3,5-lutidine. Other conditions as in Fig. 1.

consideration of the ion-exclusion mechanism alone. In the case of organic acids, this behaviour has been attributed to size-exclusion effects [3] and this appears to also be a factor in ion-exclusion chromatography of bases. Triethanolamine is a relatively large molecule in comparison to other solutes in Fig. 1 and can be partially excluded from the pores of the stationary phase through size-exclusion effects. This effect occurs in competition with enhanced hydrophobic adsorption anticipated as the size of the solute molecule is increased. However, a decrease in retention is apparent for triethanolamine since it is quite hydrophilic and would show little reversed-phase adsorption.

A second factor which could be considered in the prediction of retention volume is the effective charge of the solute. Only the first ionization constant has been plotted in Fig. 1 and where the solute has more than one amine functionality it might be necessary to consider further ionization steps if these are significant at the eluent pH. In the case of ethylenediamine ($pK_{b1} = 4.3$, $pK_{b2} = 6.8$, $V_R = 5.3$ ml) the retention volume is predictable from consideration of pK_{b1} alone since the second ionization does not occur under the conditions used.

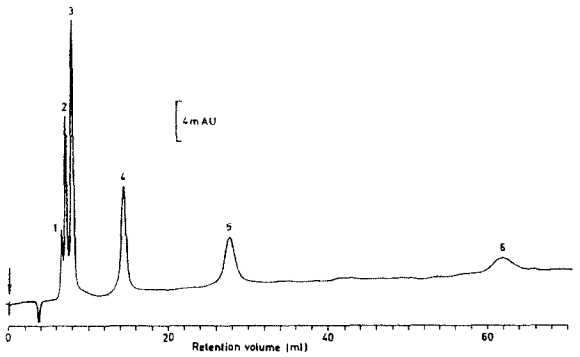

Fig. 5. Separation of aliphatic amines by ion-exclusion chromatography. Chromatographic conditions as in Fig. 1. Solutes: 1 = methylamine; 2 = ethylamine; 3 = propylamine; 4 = butylamine; 5 = pentylamine; 6 = hexylamine.

3.4. Separation of amines

The above-mentioned factors which have been shown to contribute to the retention of bases in ion-exclusion chromatography can be applied to their separation. Two examples of such separations are presented in Fig. 5 (aliphatic amines with different chain lengths) and Fig. 6

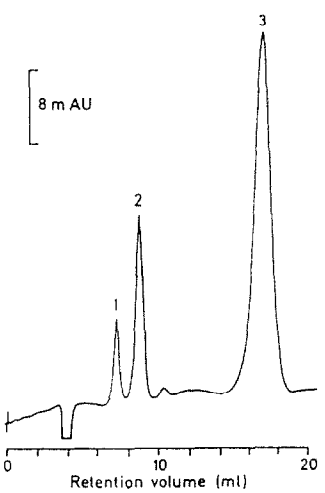

Fig. 6. Separation of ethylamines by ion-exclusion chromatography. Conditions as in Fig. 1. Solutes: 1 = ethylamine; 2 = diethylamine; 3 = triethylamine.

(ethylamines) and these chromatograms show that symmetrical peaks are observed.

4. Conclusions

The relationship between the retention volume and the pK_{b1} value of the solute using sodium hydroxide as eluent and a quaternary ammonium functionalized styrene–divinylbenzene stationary phase has been found for inorganic and some aliphatic amines to be analogous to the previously reported behaviour exhibited by carboxylic acids. This dependence made it possible to establish the dead volume and the inner column volumes and permitted prediction of retention volume for many amines on the basis of their pK_{b1} values. Solute retention increased with an increase in the concentration of sodium hydroxide in the eluent due to decreased ionization of the solute, enabling penetration into the resin network. Again, this effect was similar to that observed for acidic compounds separated using acidic buffers.

As the hydrophobicity of the solute was increased, retention volume also increased such that the retention behaviour no longer fitted the ion-exclusion model. This increased retention was due to hydrophobic interaction (adsorption) of the solute on the underivatized portions of the stationary phase resin. For the aliphatic amines, retention increased with the length of the alkyl chain and with the number of alkyl groups connected to the amine nitrogen. Very large retention volumes were observed for aromatic amines and were attributed to π-electron interactions between the solute and the stationary phase. The existence of reversed-phase behaviour for both longer chain aliphatic amines and aromatic amines has been confirmed. The magnitude of this hydrophobic adsorption effect for aromatic solutes was such that ion-exclusion played very little part in the retention process for these solutes. Some compounds were eluted earlier than predicted from their pK_{b1} value and this was attributed to size-exclusion (or steric) effects.

5. References

[1] R.M. Wheaton and W.C. Bauman, *Ind. Eng. Chem.*, 45 (1953) 228.
[2] P.R. Haddad and P.E. Jackson, *Ion Chromatography: Principles and Applications*, Elsevier, Amsterdam, 1990, pp. 195–220.
[3] K. Tanaka, T. Ishizuka and H. Sunahara, *J. Chromatogr.*, 174 (1979) 153.
[4] B.K. Glod and W. Kemula, *J. Chromatogr.*, 366 (1986) 39.
[5] B.K. Glod, A.K. Piasecki and J. Stafiej, *J. Chromatogr.*, 457 (1988) 43.
[6] K. Kihara, S. Rokushika and H. Hatano, *J. Chromatogr.*, 410 (1987) 103.
[7] B.K. Glod and R. Nowakowski, in preparation.
[8] *HPLC Columns, Methods and Applications*, Bio-Rad Labs., Richmond, CA, 1989.