*Journal of Chromatography*, 243 (1982) 253–261
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,862

# GEL CHROMATOGRAPHIC BEHAVIOUR OF ALIPHATIC AMINES AND AMMONIUM IONS ON SEPHADEX G-10

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(Received March 1st, 1982)

## SUMMARY

The gel chromatographic behaviour of aliphatic amines and ammonium ions on Sephadex G-10 was investigated in aqueous systems. A good linear relationship was found between the distribution coefficients ($K_d$) of monoalkylamines or ammonium ions and those of primary *n*-alkanols, the behaviour of which was governed predominantly by a hydrophobic interaction in aqueous dextran gel systems. A salting-out effect was observed even with ammonium ions. The results showed that a hydrophobic interaction contributed primarily to the separation mechanisms of both amines and ammonium ions. An attempt was made to separate the solute–gel interaction into two parts: the interaction between the gel matrix and the hydrophobic site of a solute, and that between the gel and the hydrophilic site of the solute.

## INTRODUCTION

A hydrophobic interaction, one of the important side-effects in gel chromatography, has often been observed for organic, biochemical and biological solutes in aqueous systems[1]. Among those solutes the mechanism of separation of aliphatic *n*-alkanols on tightly cross-linked dextran gels has been elucidated primarily in terms of the hydrophobic interaction between the solute and the gel matrix by using thermodynamic and extra-thermodynamic approaches[2–4].

As aliphatic amines resemble closely aliphatic alcohols in molecular structure, their gel chromatographic behaviour in aqueous systems is assumed to be governed chiefly by hydrophobic interactions. In fact, we recently found that the mechanism of separation of tetraalkylammonium ions on Sephadex G-10 could be interpreted in terms of the hydrophobic interaction and steric exclusion effects[5]. The availability of a wide variety of aliphatic amines and their ready conversion into the corresponding ammonium ions are advantageous for the systematic investigation of hydrophobic interactions in gel chromatography.

However, no study on the behaviour of aliphatic amines and ammonium ions in aqueous dextran gel systems has been reported, other than our earlier work[5], although several papers on their separation by cation-exchange[6,7] and thin-layer

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chromatography[8,9] have been published. The aim of the present work, therefore, was to investigate the mechanisms of the separation of mono-, di- and trialkylamines and ammonium ions on Sephadex G-10. The empirical equations representing the logarithm of the distribution coefficients of the solutes (ln $K_d$) as a function of number of carbon atoms in the solute molecule or ion were obtained for individual series of amines and ammonium ions. A theoretical treatment with a slight approximation was made in order to separate the $K_d$ values into two components, which were correlated with the interactions between the hydrophobic and hydrophilic sites of the solute and the gel matrix. The difference in the separation mechanism between amine molecules and ammonium ions is also discussed.

## EXPERIMENTAL

All reagents used were of guaranteed reagent grade from Wako (Osaka, Japan), except methyl-, dimethyl-, trimethyl- and ethylamines, which were 40, 50, 30 and 70 % aqueous solutions, respectively. Fifteen sample solutions of amines (see Fig. 2 or Table II) were prepared by dissolving them in the eluent at pH 11.8. Nineteen sample solutions of ammonium ions at pH 2.3 were also prepared in the same way. The sample concentration was $1 \cdot 10^{-2}$ $M$, except for 1-hexyl- and 1-heptylamine at pH 11.8 and 1-heptylammonium ion at pH 2.3, for which saturated solutions were employed because of their poor solubilities.

Blue Dextran 2000 or Dextran T 2000 (Pharmacia, Uppsala, Sweden; 0.36 or 0.08 %) and tritiated water (New England Nuclear, Boston, MA, U.S.A.) were used as standard materials with $K_d = 0$ and 1, respectively. Tritiated water was not utilized for examining the effect of salting-out.

The eluent used was 0.1 $M$ sodium chloride solution at pH 2.3 or 11.8 adjusted with hydrochloric acid or sodium hydroxide solution. A gel column (Pharmacia, K16/100) packed with Sephadex G-10 (Pharmacia, dry particle size 40–120 $\mu$m) was prepared according to the procedure described previously[10]. The dimensions of the gel bed were 70 × 1.6 cm.

A 1-cm$^3$ volume of the sample solution was introduced on to the column with a hypodermic syringe through a line sample injector of the septum type. The elution was allowed to proceed at a constant flow-rate of 1 cm$^3$/min at 20°C. The effluent from the column was monitored continuously with a differential refractometer (Waters Assoc., Milford, MA, U.S.A.; Model R-403). The activities of tritiated water of the effluent collected in each fraction (*ca.* 1 cm$^3$) were measured with a liquid scintillation spectrometer (Packard, Downers Grove, IL, U.S.A.; Model 2660). Other experimental details were as reported previously[4].

The volumetric distribution coefficients used, $K_d$ and $K_{av}$, can be represented by the following equations:

$$K_d = (V_e - V_0)/V_t - V_0) \quad (1)$$

$$K_{av} = (V_e - V_0)/(V_T - V_0) \quad (2)$$

where $V_e$ is the elution volume of the sample and $V_t$, $V_T$ and $V_0$ are the total liquid volume, the total bed volume and the void volume of the gel column, respectively.

The elution volume of tritiated water was used as the $V_t$ value and that of Blue Dextran 2000 or Dextran T 2000 as $V_0$. The dead volume from the cell of the refractometer to the fraction collector was corrected in the calculation of the $K_d$ and $K_{av}$ values. The $K_d$ values were uncorrected for that of tritiated water, which possibly varies depending on both the amounts and the dissociative states of hydroxyl and carboxyl groups of the gel matrix. However, the $K_d$ value of $1.09_1$ for tritiated water on Sephadex G-10[11] was used in evaluating the accurate $V_g/V_t$ value mentioned later.

## RESULTS AND DISCUSSION

As the p$K_a$ values of the alkylammonium ions used are in the range 9.8–11.0, all of the compounds are converted completely into the corresponding ammonium ions in the eluent at pH 2.3 and the samples exist almost entirely as amine molecules at pH 11.8. Accordingly, the chromatographic behaviour of amines and ammonium ions on Sephadex G-10 could be observed with the eluents at pH 11.8 and 2.3, respectively. In addition, the carboxyl groups fixed to the gel matrix do not dissociate at pH 2.3. With the eluent at pH 2.3 the electrostatic interaction between the solute ions and the gel matrix, pointed out by Neddermeyer and Rogers[12], should be suppressed because the eluent concentration was greater than 0.01 *M*. In fact, the $K_d$ values of sodium and chloride ions as the eluting agent are approximately equal at this pH[13].

Fig. 1 shows the correlation of the $K_d$ values of monoalkylamines or ammonium ions with those of corresponding primary *n*-alkanols, resulting in an excellent linear relationship in each instance. The straight line with a slope of 1.05 and an

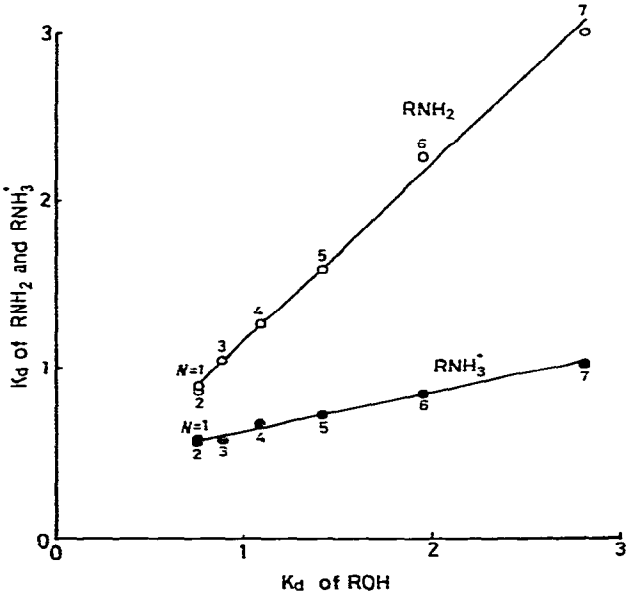


Fig. 1. Correlations of $K_d$ values of monoalkylamines ($RNH_2$) and ammonium ions ($RNH_3^+$) with those of primary *n*-alkanols (ROH). Gel: Sephadex G-10. Temperature: 20°C. Eluent: 0.1 *M* NaCl at pH 2.3 and 11.8. *N* is the number of carbon atoms in the molecule and the ion. Data for ROH taken from ref. 4.

intercept of 0.11 for amines strongly suggests that the separation mechanism of monoalkylamines on Sephadex G-10 is very similar to that of primary *n*-alkanols, and that the difference between the hydrophilic sites of the solutes such as amino and hydroxyl groups has hardly any effect on the difference in the gel chromatographic behaviour of the solutes. In contrast, the straight line with a smaller slope for monoalkylammonium ions (slope 0.23, intercept 0.40) implies that the introduction of an electric charge into the solute molecule reduces its $K_d$ value considerably. This phenomenon is in good agreement with those observed in gel chromatography[10,14–17] and in reversed-phase chromatography[18–21], which have been interpreted in terms of the acid dissociation of solutes depending on the pH of eluent. Fig. 2 shows plots of ln $K_d$ against the number of carbon atoms contained in a solute molecule or ion ($N$) for alkylamines or ammonium ions. The ln $K_d$ value increases substantially with increasing $N$ in each series of mono-, di- and trialkylamines or ammonium ions. This tendency is the opposite of that expected from the steric exclusion effect. Murakami[7] also reported a similar phenomenon on a sulphonated polystyrene cation exchanger, where the logarithm of the selectivity coefficients of aliphatic ammonium ions with borate buffer as eluent at pH 8 increased with increasing carbon number in a solute ion.

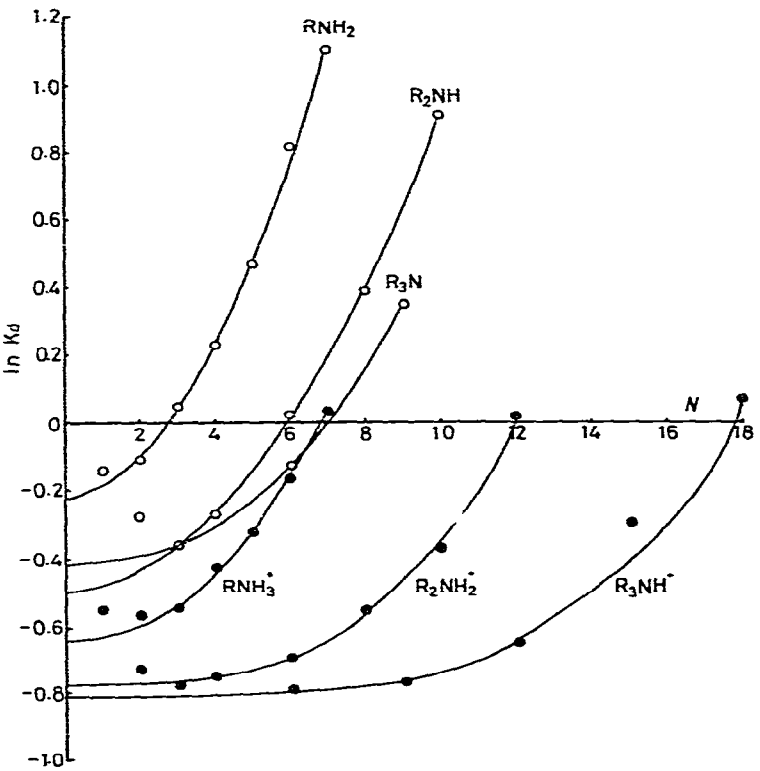


Fig. 2. Plots of ln $K_d$ against the number of carbon atoms in the molecule and the ion ($N$). Gel: Sephadex G-10. Temperature: 20°C. Eluent: 0.1 $M$ NaCl at pH 2.3 and 11.8.

The effect of eluent concentration on the $K_{av}$ values of monoalkylammonium ions is shown in Fig. 3. A salting-out effect from the bulk to the gel phase was apparently observed with increase in concentration of sodium chloride from 0.1 to 1.0 *M*, although the effect for methyl- and ethylammonium ions was relatively small. The more carbon atoms there are in an ammonium ion, the greater is the effect. Moreover, preliminary experiments showed that the transfer processes for all of the monoalkylammonium ions from the bulk to the gel phase were endothermic.

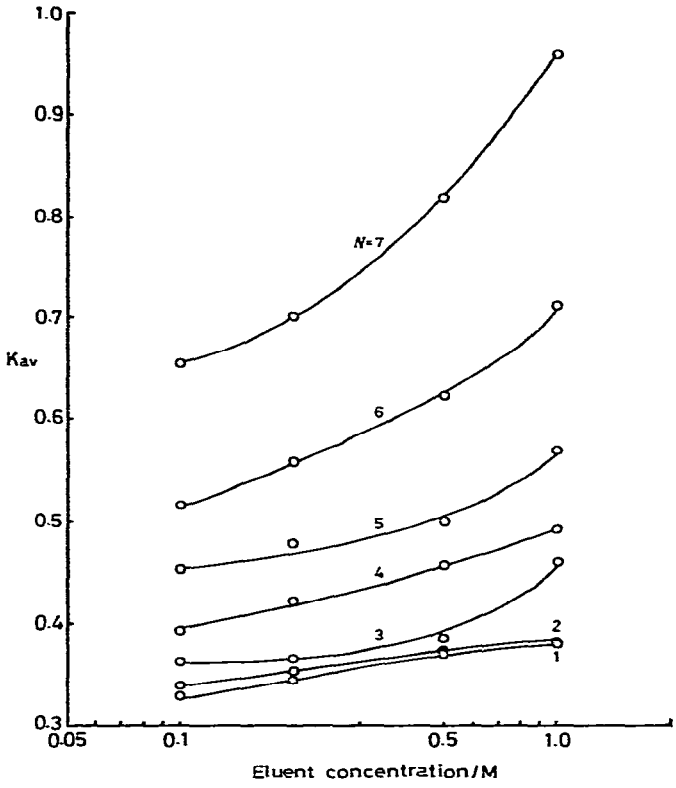


Fig. 3. Effect of eluent concentration on $K_{av}$ values of $RNH_3^+$ ions. Gel: Sephadex G-10. Temperature: 20°C. Eluent: NaCl solutions of various concentrations at pH 2.3. Key as in Fig. 1.

All of the facts mentioned above strongly suggest that the hydrophobic interaction plays a dominant role in the gel chromatographic behaviour of both aliphatic amines and ammonium ions on Sephadex G-10 in aqueous systems, as in the case of aliphatic *n*-alkanols.

In order to elucidate the mechanism of the hydrophobic interaction mentioned above, the relationships between ln $K_d$ and $N$ for individual series of amines and ammonium ions in Fig. 2 were approximated by the following equation:

$$\ln K_d = pN^q + r \tag{3}$$

where $p$, $q$ and $r$ are the parameters for each series of mono, di- and trialkylamines or

ammonium ions. The sets of parameters obtained by the best-fit method are given in Table I, together with those for primary *n*-alkanols. The $K_d$ values, both observed ($K_{d(obs)}$) and calculated by using the parameters in Table I ($K_{d(calc)}$), are summarized in Table II, where the $K_{d(calc)}$ values are in good agreement with the corresponding $K_{d(obs)}$ values except for methyl derivatives and tripentylammonium ion (relative standard deviations: 1.4% for amines and ammonium ions).

TABLE I

PARAMETERS OBTAINED BY EQN. 3 FOR INDIVIDUAL SERIES OF MONO-, DI- AND TRIALKYAMINES AND AMMONIUM IONS

$K_d$ values of species with $N > 2$ were employed for the calculation of the parameters. The data used are shown in Table II.

| *Species* | *n* | *p* | *q* | *r* | $K_L$ |
|---|---|---|---|---|---|
| $R_nNH_{3-n}$ | 1 | $4.05 \cdot 10^{-2}$ | 1.81 | −0.257 | 1.3 |
| | 2 | $1.38 \cdot 10^{-2}$ | 2.00 | −0.490 | 1.0 |
| | 3 | $3.60 \cdot 10^{-3}$ | 2.44 | −0.416 | 1.1 |
| ROH | | $3.44 \cdot 10^{-2}$ | 1.92 | −0.404 | 1.1 |
| $R_nNH_{4-n}^+$ | 1 | $1.24 \cdot 10^{-2}$ | 2.06 | −0.657 | 0.86 |
| | 2 | $1.25 \cdot 10^{-4}$ | 3.51 | −0.759 | 0.78 |
| | 3 | $5.22 \cdot 10^{-6}$ | 4.16 | −0.808 | 0.74 |

Assuming that no solute–solute interaction is operative in both the bulk and the gel phases, the side-effects other than steric exclusion can be represented by the following two interactions: one is the interaction between the gel matrix and the hydrophobic site of the solute, and the other is that between the gel matrix and the hydrophilic site of the solute. Hence, the following expression describing the solute behaviour is obtained, similar to the case of affinity chromatography[22]:

$$V_e = V_0 + K_s V_i + K_B K_L V_g \tag{4}$$

where $K_s$, $K_B$ and $K_L$ denote the distribution coefficients for the steric exclusion effect and, the first and the second interactions, respectively, $V_i$ the pore volume of the gel beads and $V_g$ the skelton volume of the swollen gel. Eqn. 4 is correlated with $K_{d(obs)}$ as

$$K_{d(obs)} = (V_e - V_0)/V_i = K_S + K_B K_L (V_g/V_i) \tag{5}$$

The logarithmic form of eqn. 5 is

$$\ln K_{d(obs)} = \ln K_B + \ln [K_S/K_B + K_L(V_g/V_i)] \tag{6}$$

The $K_S$ value, limited to the region from zero to unity, should decrease with increasing molecular weight, whereas $K_B$ increases considerably in each series of amines and ammonium ions, as shown in Fig. 2. Accordingly, the term $K_S/K_B$ becomes negligibly

TABLE II

$K_{d(obs)}$ AND $K_{d(calc)}$ VALUES OF ALIPHATIC AMINES AND AMMONIUM IONS

Gel: Sephadex G-10. Temperature: 20°C. Eluent: 0.1 *M* NaCl at pH 2.3 and 11.8.

| *Species* | *R* | *n = 1* | | *n = 2* | | *n = 3* | | *ROH* | |
|---|---|---|---|---|---|---|---|---|---|
| | | $K_{d(obs)}$ | $K_{d(calc)}$ | $K_{d(obs)}$ | $K_{d(calc)}$ | $K_{d(obs)}$ | $K_{d(calc)}$ | $K^{*}_{d(obs)}$ | $K_{d(calc)}$ |
| $R_nNH_{3-n}$ and ROH | $CH_3$ | 0.805 | 0.827 | 0.757 | 0.621 | 0.699 | 0.696 | 0.755 | 0.693 |
| | $C_2H_5$ | 0.891 | 0.902 | 0.761 | 0.765 | 0.871 | 0.878 | 0.758 | 0.761 |
| | 1-$C_3H_7$ | 1.05 | 1.04 | 1.02 | 1.01 | 1.42 | 1.42 | 0.886 | 0.886 |
| | 1-$C_4H_9$ | 1.27 | 1.26 | 1.48 | 1.49 | | | 1.09 | 1.09 |
| | 1-$C_5H_{11}$ | 1.63 | 1.60 | 2.47 | 2.46 | | | 1.42 | 1.42 |
| | 1-$C_6H_{13}$ | 2.18 | 2.14 | | | | | 1.95 | 1.95 |
| | 1-$C_7H_{15}$ | 3.04 | 3.00 | | | | | 2.81 | 2.81 |
| $R_nNH^{+}_{4-n}$ | $CH_3$ | 0.525 | 0.532 | 0.484 | 0.468 | 0.461 | 0.446 | | |
| | $C_2H_5$ | 0.546 | 0.551 | 0.474 | 0.476 | 0.454 | 0.450 | | |
| | 1-$C_3H_7$ | 0.584 | 0.586 | 0.500 | 0.501 | 0.464 | 0.468 | | |
| | 1-$C_4H_9$ | 0.644 | 0.643 | 0.574 | 0.563 | 0.522 | 0.524 | | |
| | 1-$C_5H_{11}$ | 0.730 | 0.728 | 0.691 | 0.703 | 0.737 | 0.670 | | |
| | 1-$C_6H_{13}$ | 0.854 | 0.852 | 1.01 | 1.01 | 1.07 | 1.06 | | |
| | 1-$C_7H_{15}$ | 1.03 | 1.03 | | | | | | |

* Data taken from Ref. 4.

small with large $N$, compared with the term $K_L(V_g/V_i)$. In such a situation, eqn. 6 can be approximated by

$$\ln K_{d(\mathrm{obs})} \approx \ln K_B + \ln K_L(V_g/V_i) \tag{7}$$

The $V_g/V_i$ value was 0.60 under the experimental conditions used, regardless of the pH of eluent. The $K_L$ value is assumed to be invariant in each series because the hydrophilic site is the same in a series. These facts may lead to the assumption that ln $K_L(V_g/V_i)$ in eqn. 7 is constant in each series of solutes. Therefore, the following relationships were obtained by comparing eqn. 3 with eqn. 7:

$$\ln K_B = pN^q \tag{8}$$

$$\ln K_L(V_g/V_i) = r \tag{9}$$

The $K_L$ values, evaluated from $V_g/V_i$ and $r$ by using eqn. 9, are listed in the last column of Table I.

The $K_L$ value is a measure of the affinity that the hydrophilic site of the solute exhibits for the gel matrix. Whereas the $K_L$ values of amines and alcohols are larger than unity, those of ammonium ions are less than unity, indicating that the ionic groups, $-NH_3^+$, $=NH_2^+$ and $\equiv NH^+$, repulse the gel matrix. This is possibly attributable to an electrostatic interaction between the ionic solutes and the positive charge of the gel phase at low pH, which is brought about by the high $K_d$ value of hydrogen ions[13]. In addition, the $K_L$ values decrease slightly in the order mono- > di- > trialkyl series for both amines and ammonium ions. This phenomenon indicates a reduction of hydrogen bonding through the hydrogen atoms of the hydrophilic groups with the gel matrix.

The $K_B$ value is a measure of the interaction between the hydrophobic sites of the solute and the gel matrix. The $K_B$ values are larger than unity for all of the solutes ($pN^q > 0$), demonstrating that the hydrophobic sites of the solute attract the gel matrix. The relationship between ln $K_B$ and $N$ for each series is identical with the curve shifted by $r$ along the ordinate (ln $K_d$) in Fig. 2. The ln $K_B$ value does not show a linear increase in each instance. This means that there is no additivity of the free-energy change for transfer of the hydrophobic sites from the bulk to the gel phase with increase in $N$. One of the reasons is probably the steric exclusion effect, because its contribution may be included in ln $K_B$ by the approximation of eqn. 6 to eqn. 7. This effect may be serious, especially for the solutes with small $N$, and possibly cause differences between the $K_{d(\mathrm{obs})}$ and $K_{d(\mathrm{calc})}$ values in Table II.

It should be noted that Sephadex G-10, usually classified as a group of hydrophilic gels, possesses relatively high hydrophobic properties in aqueous systems.

ACKNOWLEDGEMENTS

The authors thank Professor S. Ohashi of Kyushu University for his many useful suggestions and encouragement, and Mr. Yuji Nishizaka for technical assistance.

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