

CHROM. 13,668

GEL CHROMATOGRAPHIC BEHAVIOUR OF TETRAALKYLAMMONIUM IONS ON SEPHADEX G-10

KIKUJIRO UJIMOTO*, KUMIKO SUZUKI and HIRONDO KURIHARA

Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma 11, Nishi-ku, Fukuoka 814-01 (Japan)

(First received July 28th, 1980; revised manuscript received January 20th, 1981)

SUMMARY

The gel chromatographic behaviour of tetraalkylammonium (TAA) ions on Sephadex G-10 was studied chiefly by a thermodynamic and an extra-thermodynamic approach. The pH dependence of the distribution coefficients of TAA ions was observed, and compared with those of alkali metal and halide ions. The extra-thermodynamic approach showed that no enthalpy-entropy compensation was operative in the partitioning processes of TAA ions between the mobile and the gel phase. Examination of the effects of eluent concentration, organic eluent and temperature suggested that hydrophobic interaction and sieving effects contribute to various extents to the separation mechanism of individual TAA ions in the temperature range studied.

INTRODUCTION

In gel chromatography it is well known that chromatograms of relatively small molecules and ions on tightly cross-linked gels in aqueous systems are often adversely affected by several kinds of side effects. One of the important side effects is a hydrophobic interaction, which sometimes plays a dominant rôle in the gel chromatographic behaviour of organic and biological solutes¹.

Several workers²⁻⁹ have used a thermodynamic approach to describe the hydrophobic interaction. We have recently studied the separation mechanism of aliphatic *n*-alcohols in aqueous dextran gel systems by a thermodynamic and an extra-thermodynamic approach, where the hydrophobic interaction between the solute and the gel matrix was interpreted principally by correlating ΔS_{GC}° values of the alcohols with their ΔS_{HY}° values¹⁰; ΔS_{GC}° denotes the standard entropy change upon transfer of the solute from the mobile phase outside the gel beads to the gel phase at infinite dilution, and ΔS_{HY}° , usually called the entropy of hydration, is the standard entropy change upon dissolution of the gaseous solute in water at infinite dilution.

However, no study on the behaviour of tetraalkylammonium (TAA) ions in aqueous dextran gel systems has been reported, although such ions are regarded as a

homologous series of hydrophobic solutes^{11,12}. The aim of the present work, therefore, was to study the behaviour of TAA ions on Sephadex G-10.

The pH dependence of the distribution coefficients, K_d , of TAA ions was investigated with 0.1 *M* sodium chloride solutions in order to find the pH range in which the electrostatic interaction can almost be suppressed. The effects of eluent concentration, organic eluent and temperature upon the K_d values of TAA ions were examined. Enthalpy-entropy compensation tests were applied to the partitioning processes of TAA ions to differentiate the separation mechanisms.

EXPERIMENTAL

Sample solutions

Tetramethylammonium (Me_4N^+), tetraethylammonium (Et_4N^+), tetra-*n*-propylammonium ($n\text{-Pr}_4\text{N}^+$) and tetra-*n*-butylammonium ($n\text{-Bu}_4\text{N}^+$) chlorides were of guaranteed reagent grade from Nakarai Chemicals (Kyoto, Japan). Tetra-*n*-pentylammonium ($n\text{-Pe}_4\text{N}^+$) bromide was purchased from Fluka (Buchs, Switzerland). Sample solutions were prepared by dissolving each TAA salt in the eluents at a concentration of 0.01 *M*, except for *n*- Pe_4NBr which because of its poor solubility was used as a saturated solution.

Standard materials with $K_d = 1$ and 0 were used as in a previous study¹⁰.

Columns and eluents

Gel columns packed with Sephadex G-10 (dry particle size 40–120 μm ; Pharmacia, Uppsala, Sweden) were prepared according to the procedure described previously¹⁰.

The eluents used were 0.1 *M* sodium chloride solutions at various pH values, 95% (v/v) methanol and 0.1 *M* lithium chloride in 95% methanol. Sodium chloride solutions at concentrations of 0.01, 0.1, 0.3, 0.5 and 1.0 *M* at pH 2.3 were also employed to investigate the effect of eluent concentration on the K_d values of TAA ions.

Other experimental details and the procedures for calculating K_d values and thermodynamic functions were as reported previously¹⁰.

RESULTS AND DISCUSSION

pH dependence of K_d values

The pH dependence of the K_d values or capacity factors of aromatic acids, amines, ampholytes and monomeric oxo acids of phosphorus have been reported on Sephadex G-10¹³⁻¹⁵, LH-20¹⁶, Bio-Gel P-2¹⁷, polystyrene copolymers¹⁸⁻²⁴ and octadecylsilica²⁵. In these cases, the pH dependence was interpreted primarily in terms of acid or base dissociation of the solutes. We recently observed²⁶ that the K_d values of alkali metal and halide ions on Sephadex G-10 also vary significantly with the pH of the eluent over the range 2–12, although these ions are not transformed into any other form or species over this pH range. TAA ions also do not suffer any molecular change over a wide pH range. Therefore, their K_d values on Sephadex G-10 with 0.1 *M* sodium chloride as eluent were examined over the pH range 1.5–12.5. The results are shown in Fig. 1.

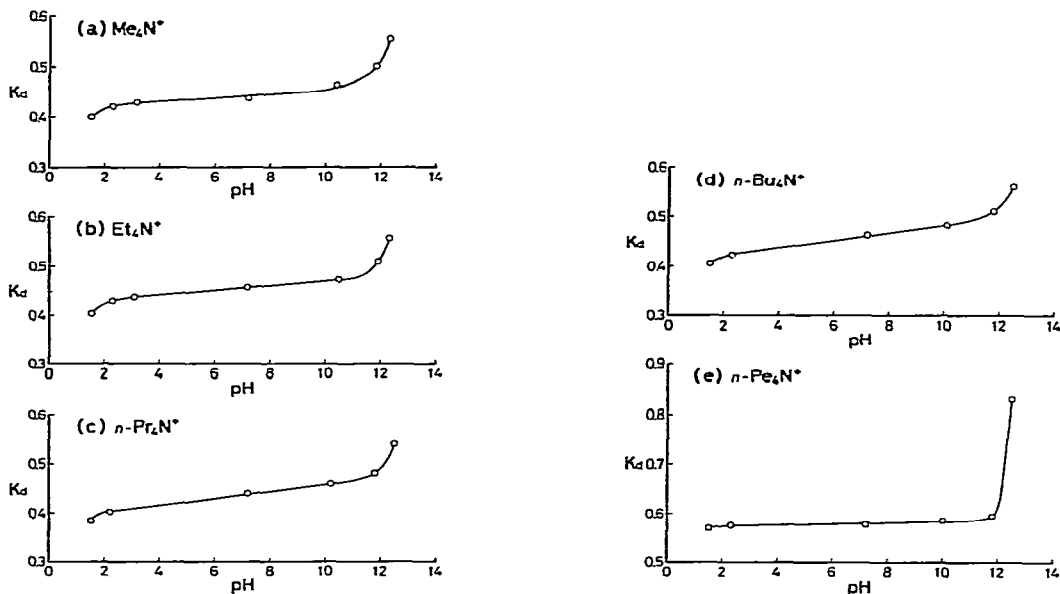


Fig. 1. pH dependence of K_d values of TAA ions on Sephadex G-10. Eluent: 0.1 M NaCl solutions at various pH values. Temperature: 20°C.

Each TAA ion showed a similar pH profile to those of the alkali metal ions. This behaviour may arise from the change in properties of the gel phase with the pH of the eluent. Namely, the slight increase in K_d value at pH < 3 may be ascribed to the change of hydrogen ion concentration in the gel phase, and the rapid increase at pH > 11.5 to the acid dissociation of alcoholic hydroxyl groups of the gel matrix.

In order to suppress the electrostatic interaction between the TAA ions and the charges of the gel matrix, the pH of the eluents used in subsequent experiments was adjusted to 2 or 2.3, except for 95% methanol systems.

Effect of eluent concentration

The salting-out of solutes from the mobile phase is a useful means of elucidating whether or not a hydrophobic interaction is operative in chromatographic processes¹.

Fig. 2 shows the effect of eluent concentration on the K_d values of TAA ions on Sephadex G-10 with sodium chloride eluents at pH 2.3. The K_d values of both sodium and chloride ion as the eluent are almost equal at pH 2.3²⁶.

As in the case of aliphatic *n*-alcohols¹⁰, the K_d values of TAA ions increase gradually with increasing eluent concentration, especially above 0.1 M. This effect is more significant for $n\text{-Pr}_4\text{N}^+$, $n\text{-Bu}_4\text{N}^+$ and $n\text{-Pe}_4\text{N}^+$ than for Me_4N^+ and Et_4N^+ . The relative increments of the K_d values with increasing eluent concentration from 0.01 to 1.0 M are 13% for Me_4N^+ , 14% for Et_4N^+ , 23% for $n\text{-Pr}_4\text{N}^+$, 37% for $n\text{-Bu}_4\text{N}^+$ and 68% for $n\text{-Pe}_4\text{N}^+$.

Deguchi²⁷ observed that differences between the K_{av} values of TAA ions on Sephadex G-15 are remarkable with sodium chloride, potassium sulphate and lithium sulphate as eluent, although those with water are negligibly small.

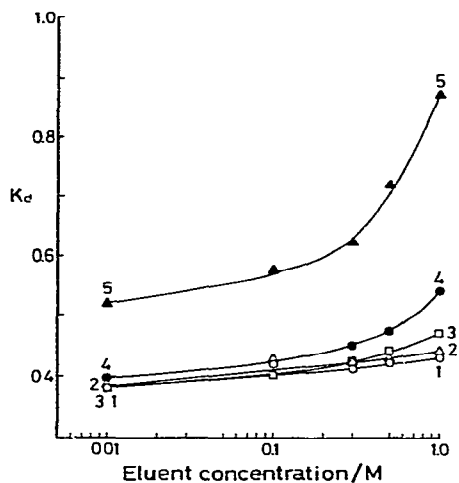


Fig. 2. Effect of eluent concentration on K_d values of TAA ions on Sephadex G-10. Eluent: NaCl solutions of various concentrations at pH 2.3. Temperature: 20°C. Cations: 1 = Me₄N⁺; 2 = Et₄N⁺; 3 = n-Pr₄N⁺; 4 = n-Bu₄N⁺; 5 = n-Pe₄N⁺. The value of n-Bu₄N⁺ (●) at 0.1 M NaCl is overlapped by those of Me₄N⁺ (○) and Et₄N⁺ (Δ).

These facts suggest that the hydrophobic interaction has an important rôle in the behaviour of TAA ions, at least of n-Pr₄N⁺, n-Bu₄N⁺ and n-Pe₄N⁺, in aqueous dextran gel systems.

Effect of organic eluent

When only the molecular sieving effect depending on the solute size is operative in gel chromatography, the K_d values are linearly dependent on the logarithms of the molecular weights (log MW) or of the molar volumes of the solutes¹.

Fig. 3 shows the relationships between log MW of TAA ions and their K_d values on Sephadex G-10 with 0.1 M sodium chloride solution at pH 2.3, 95% (v/v) methanol and 0.1 M lithium chloride in 95% methanol. With 95% methanol the K_d values decrease with increasing log MW. This tendency is more significant with 0.1 M lithium chloride in 95% methanol. In both cases, the sieving effect must be predominant, but the non-linear dependence of the K_d values on log MW indicates probably that side effects occur even with those eluents which are almost free from water. The effect of addition of lithium chloride to 95% methanol may be attributable to the alteration of solvent properties in both the gel and the mobile phase rather than to the suppression of the electrostatic interaction, because carboxylic groups fixed to the gel matrix presumably do not dissociate in the methanol-rich solvents. With the aqueous eluent the K_d values of the TAA ions, except for n-Pe₄N⁺, are almost equal. The notable increase of the K_d value of n-Pe₄N⁺ conflicts with the sieving effect.

These facts support the hypothesis that hydrophobic adsorption is responsible, at least partly, for the anomalous behaviour of TAA ions with the aqueous eluent.

Effect of temperature

An endothermic temperature dependence of K_d values is the most important criterion for detection of hydrophobic interactions, because the hydrophobic affinity

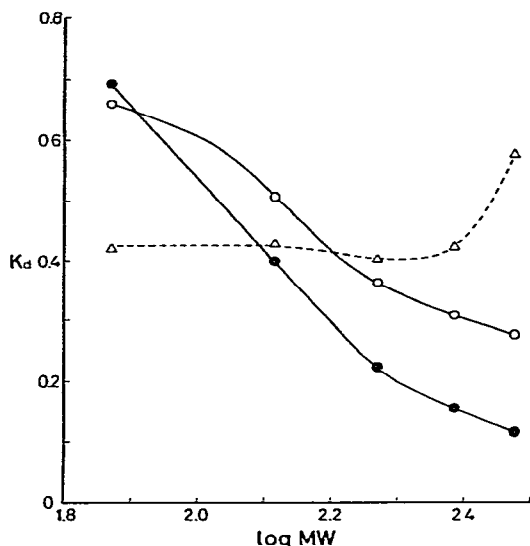


Fig. 3. Effect of organic eluent on K_d values of TAA ions on Sephadex G-10. Eluents: ○, 95% (v/v) methanol; ●, 0.1 M LiCl in 95% methanol; △, 0.1 M NaCl in water at pH 2.3. Temperature: 20°C.

of the solute for the gel matrix arises from an increase in entropy due to partial melting of an ice-like arrangement of the water structure around the solute²⁸. The K_d values of TAA ions at various temperatures are summarized in Table I. The pH of the eluent was adjusted to 2 to suppress the electrostatic interaction as far as possible. It was found that the behaviour of TAA ions on Sephadex G-10 was considerably influenced by the previous usage of the gel column. Therefore, the K_d values at 20°C are somewhat different from those at pH 2 in Fig. 1 because different columns were used in each series of experiments.

The K_d values of all TAA ions increase with increasing temperature, but only slightly for those of Me_4N^+ and Et_4N^+ . These data gave the thermodynamic functions $\Delta H_{\text{GC}}^\circ$ and $\Delta S_{\text{GC}}^\circ$, where $\Delta H_{\text{GC}}^\circ$ is the standard enthalpy change for transfer of the solute from outside the gel beads to the gel phase at infinite dilution. Positive $\Delta H_{\text{GC}}^\circ$ and $\Delta S_{\text{GC}}^\circ$ values were obtained for all TAA ions except Me_4N^+ , as shown in Fig. 4a. This again suggests that the hydrophobic interaction has an important rôle in the gel chromatography of TAA ions.

Enthalpy-entropy compensation test

The enthalpy-entropy compensation test is a diagnostic tool used to differentiate between mechanistically different separation processes. It has been successfully applied to the elucidation of the behaviour of organic and biological solutes in reversed-phase chromatography²⁹⁻³¹ and to that of aliphatic *n*-alcohols in gel chromatography¹⁰.

Compensation behaviour had been tested by the linearity of a plot of ΔH° against ΔS° values for analogous substances, until Krug *et al.*^{32,33} proposed a plot of ΔG° against ΔH° in order to minimize statistical compensation effects due to errors in ΔH° and ΔS° values. Fig. 4a shows a conventional plot of $\Delta H_{\text{GC}}^\circ$ against $\Delta S_{\text{GC}}^\circ$, and Fig. 4b a plot of $\ln K_{d(20)}$ against $\Delta H_{\text{GC}}^\circ$ according to the procedure of Krug *et al.*; the

TABLE I
 K_d VALUES OF TAA IONS AT VARIOUS TEMPERATURES

Gel: Sephadex G-10. Eluent: 0.1 M NaCl at pH 2.

Ion	Temperature ($^{\circ}$ C)	K_d
Me_3N^+	10.73	0.366
	16.05	0.371
	20.57	0.374
	25.66	0.377
	30.86	0.382
Et_3N^+	10.50	0.353
	15.95	0.360
	20.63	0.378
	25.46	0.393
	29.97	0.401
$n\text{-Pr}_3\text{N}^+$	10.70	0.300
	15.45	0.320
	19.50	0.352
	25.57	0.374
	29.93	0.402
$n\text{-Bu}_3\text{N}^+$	10.65	0.273
	15.50	0.323
	19.55	0.366
	25.57	0.419
	29.93	0.471
$n\text{-Pe}_3\text{N}^+$	10.70	0.327
	15.50	0.419
	19.25	0.481
	25.57	0.621
	29.95	0.706

quantity $\ln K_{d(20)}$ was employed since it is proportional to ΔG_{GC}° and has the minimum statistical errors. The first plot gives an excellent linear relationship, in contrast to the second, indicating the validity of Krug's proposal. We conclude that the behaviour of the TAA ions did not obey a common physico-chemical mechanism under the present experimental conditions.

Separation mechanism

The electrostatic interaction is unlikely to be operative with the eluent at pH 2, because the charge of the gel matrix would be zero or negligibly small²⁶ and the surface charge densities of TAA ions would be much smaller than those of alkali metal and halide ions, because of their larger ionic radii³⁴.

The TAA ions did not exhibit common enthalpy-entropy compensation in their partitioning processes between the mobile and the gel phase. This suggests that the behaviour of individual TAA ions is governed by a number of factors whose individual importance may vary. One such factor is probably the hydrophobic interaction; this is strongly supported by the effects of eluent concentration, organic eluent and temperature on the K_d values. Further supporting evidence is provided by the fact

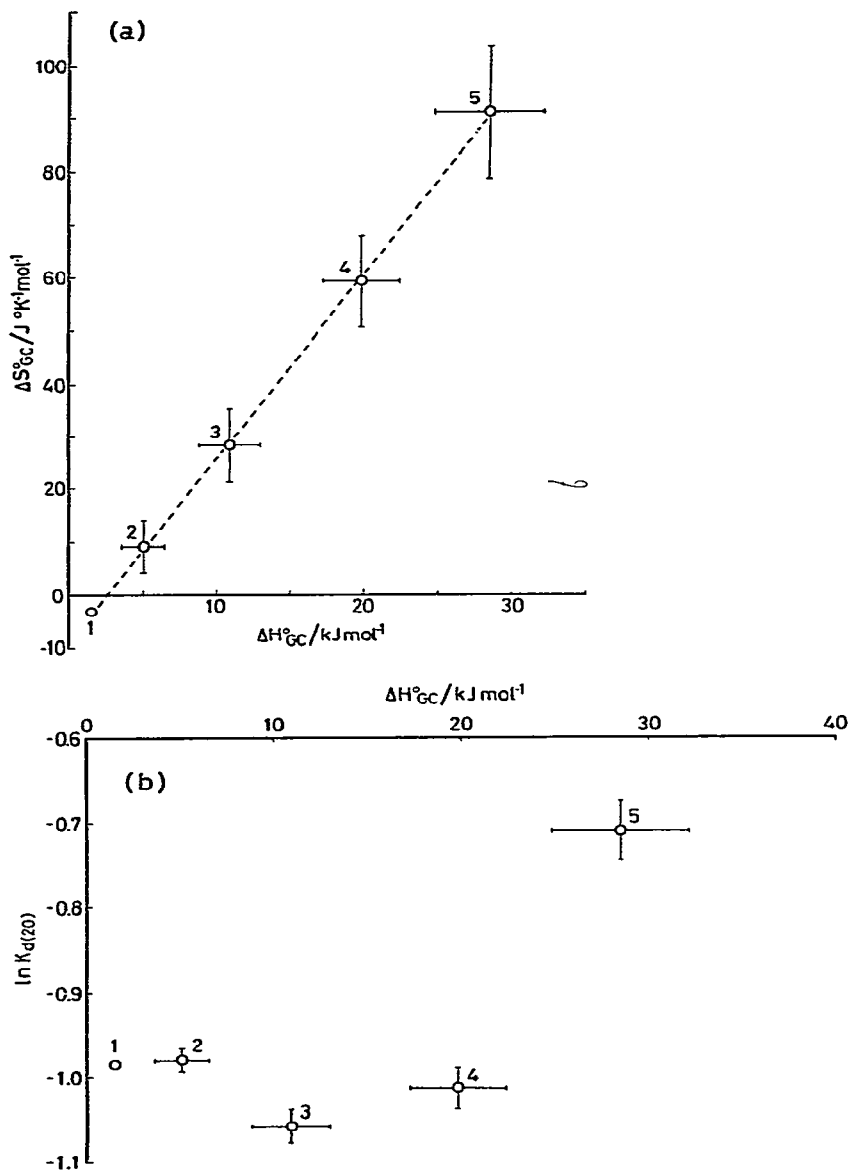


Fig. 4. Plots for enthalpy-entropy compensation test. Cations as in Fig. 2. The vertical and horizontal lines through the circles indicate the 0.95 confidence intervals of the thermodynamic functions. a, Conventional plot of ΔS_{GC}° against ΔH_{GC}° ; b, Krug plot of $\ln K_d(20)$ against ΔH_{GC}° .

that both the ΔH_{GC}° and ΔS_{GC}° values of all the TAA ions, except ΔS_{GC}° of Me_4N^+ , are positive. This is similar to the case of aliphatic *n*-alcohols in aqueous dextran gel systems, where the linear correlations of the ΔS_{GC}° values of the alcohols with their ΔS_{HY}° values indicated the occurrence of hydrophobic interactions¹⁰.

However, no ΔS_{HY}° values of TAA ions have been reported. We have pro-

posed³⁵ the use of $(B_\eta - 0.0025 \bar{V}^\circ)$ values as a substitute for the $\Delta S_{\text{HV}}^\circ$ of hydrophobic solutes, where B_η is the second virial coefficient and \bar{V}° the partial molar volume of the solute in infinite dilute solution. Accordingly, the $\Delta S_{\text{GC}}^\circ$ values of TAA ions are plotted against their $(B_\eta - 0.0025 \bar{V}^\circ)$ values in Fig. 5. The excellent linear relationship obtained indicates that the separation mechanism of TAA ions can be interpreted in terms of a hydrophobic interaction.

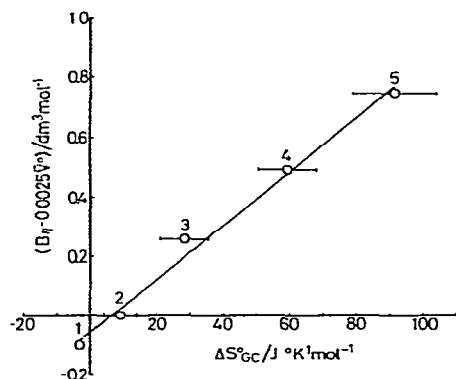


Fig. 5. Plot of $\Delta S_{\text{GC}}^\circ$ against $(B_\eta - 0.0025 \bar{V}^\circ)$ for TAA ions. Cations as in Fig. 2.

As discussed previously¹⁰, the entropy of a system, including the gaseous hydrophobic solute and the solvent, decreases upon dissolution of the solute in water because of the formation of hydrophobic hydration shells, where the water structure is more ordered. This entropy loss will be linearly dependent on the degree of ordering of the water structure, which can be estimated from the $(B_\eta - 0.0025 \bar{V}^\circ)$ value of the solute³⁵. It may also be partly compensated by the permeation of the solute from the bulk to the gel phase and by the local sharing of the hydration shell of the solute with that of the hydrophobic site of the gel matrix, resulting in decrease in the degree of ordering of the water structure. The larger $(B_\eta - 0.0025 \bar{V}^\circ)$ value of the solute results in the higher entropy compensation. This may account for the hydrophobic interaction in the separation mechanism of TAA ions, at least of $n\text{-Pr}_4\text{N}^+$, $n\text{-Bu}_4\text{N}^+$ and $n\text{-Pe}_4\text{N}^+$, as discussed later.

Another important factor is probably the sieving effect. In order to confirm this, Van 't Hoff plots of the K_d values in Table I were carried out. On extrapolation of the straight lines in Fig. 6, it is found that the K_d values decrease in the order of Me_4N^+ , Et_4N^+ , $n\text{-Pr}_4\text{N}^+$, $n\text{-Bu}_4\text{N}^+$ and $n\text{-Pe}_4\text{N}^+$ below 0°C , suggesting that the sieving effect may operate principally in this temperature range. In contrast, the K_d values increase in the same order above 30°C , where the hydrophobic interaction may be predominant.

According to Desnoyers and Perron³⁶, Et_4N^+ having a $(B_\eta - 0.0025 \bar{V}^\circ)$ value of zero is neither a structure-maker nor a structure-breaker for water and Me_4N^+ having a negative $(B_\eta - 0.0025 \bar{V}^\circ)$ value is a structure-breaker. Since hydrophobic solutes have been regarded as structure-makers, the behaviour of Me_4N^+ and Et_4N^+ can hardly be discussed in terms of the hydrophobic interaction. Nevertheless, TAA ions, including Me_4N^+ and Et_4N^+ , yield an excellent linear correlation of $\Delta S_{\text{GC}}^\circ$ with

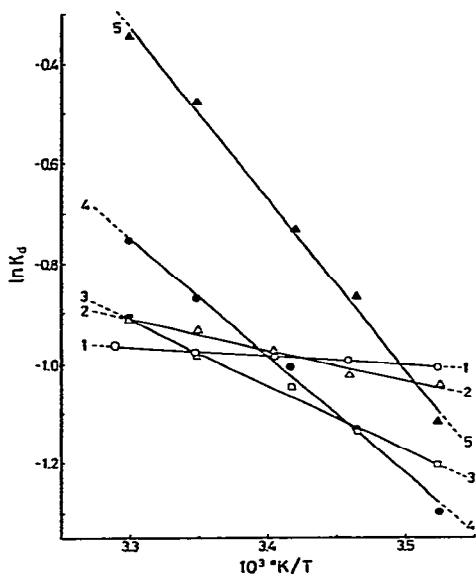


Fig. 6. Van 't Hoff plots of K_d values of TAA ions in Table I. Cations as in Fig. 2.

$(B_\eta - 0.0025 \bar{V}^\circ)$ values, as shown in Fig. 5. The reason for this has not yet been ascertained.

In conclusion, both the hydrophobic interaction and the sieving effect may contribute to various extents to the behaviour of individual TAA ions in the temperature range studied.

ACKNOWLEDGEMENTS

The authors thank Professor S. Ohashi and Dr. N. Yoza of Kyushu University and Associate Professor T. Deguchi of Kumamoto University for valuable discussions and encouragement, and Mr. K. Yoshitake for technical collaboration.

REFERENCES

- 1 T. Kremmer and L. Boross, *Gel Chromatography*, Wiley, New York, 1979.
- 2 N. V. B. Marsden, *Ann. N.Y. Acad. Sci.*, 125 (1965) 428.
- 3 W. Brown, *J. Chromatogr.*, 59 (1971) 335.
- 4 W. Brown and Ö. Andersson, *J. Chromatogr.*, 67 (1972) 163.
- 5 N. V. B. Marsden, *Acta Univ. Upsaliensis*, 123 (1972) 1.
- 6 J. H. Knox and G. Vasvari, *J. Chromatogr.*, 83 (1973) 181.
- 7 M. Janado, R. Nakayama, Y. Yano and H. Nakamori, *J. Biochem. (Tokyo)*, 86 (1979) 795.
- 8 M. Janado, K. Takenaka, H. Nakamori and Y. Yano, *J. Biochem. (Tokyo)*, 87 (1980) 57.
- 9 M. Janado, Y. Yano, H. Nakamori and T. Nishida, *J. Chromatogr.*, 193 (1980) 345.
- 10 K. Ujimoto and H. Kurihara, *J. Chromatogr.*, 208 (1981) 183.
- 11 F. Franks (Editor), *Water*, Vols. 2 and 3, Plenum, New York, 1973.
- 12 M. Nakagaki (Editor), *Mizu no Kozo to Bussei*, Nankodo, Tokyo, 1974.
- 13 K. Ujimoto, T. Yoshimura, I. Ando and H. Kurihara, *J. Chromatogr.*, 174 (1979) 123.
- 14 A. J. W. Brook and S. Housley, *J. Chromatogr.*, 41 (1969) 200.
- 15 A. J. W. Brook and S. Housley, *J. Chromatogr.*, 42 (1969) 112.

- 16 J. Vejrosta and J. Málek, *J. Chromatogr.*, 109 (1975) 101.
- 17 K. Ujimoto, I. Ando, T. Yoshimura, K. Suzuki and H. Kurihara, *J. Chromatogr.*, 190 (1980) 161.
- 18 M. D. Griesser and D. J. Pietrzyk, *Anal. Chem.*, 45 (1973) 1348.
- 19 C.-H. Chu and D. J. Pietrzyk, *Anal. Chem.*, 46 (1974) 330.
- 20 H. Takahagi and S. Seno, *J. Chromatogr.*, 108 (1975) 354.
- 21 D. J. Pietrzyk and C.-H. Chu, *Anal. Chem.*, 49 (1977) 757.
- 22 D. J. Pietrzyk and C.-H. Chu, *Anal. Chem.*, 49 (1977) 860.
- 23 D. J. Pietrzyk, E. P. Kroeff and T. D. Rotsh, *Anal. Chem.*, 50 (1978) 497.
- 24 E. P. Kroeff and D. J. Pietrzyk, *Anal. Chem.*, 50 (1978) 502.
- 25 Cs. Horváth, W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
- 26 K. Ujimoto, I. Ando, T. Yoshimura, K. Suzuki and H. Kurihara, *Fukuoka Univ. Sci. Rep.*, 9 (1979) 125.
- 27 T. Deguchi, personal communication.
- 28 A. Ben-Naim, *Hydrophobic Interactions*, Plenum, New York, 1980.
- 29 W. Melander, D. E. Campbell and Cs. Horváth, *J. Chromatogr.*, 158 (1978) 215.
- 30 W. Melander, B.-K. Chen and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 99.
- 31 Gy. Vigh and Z. Varga-Puchony, *J. Chromatogr.*, 196 (1980) 1.
- 32 R. R. Krug, W. G. Hunter and R. A. Gieger, *J. Phys. Chem.*, 80 (1976) 2335.
- 33 R. R. Krug, W. G. Hunter and R. A. Gieger, *J. Phys. Chem.*, 80 (1976) 2341.
- 34 E. R. Nightingale, Jr., *J. Phys. Chem.*, 63 (1959) 1381.
- 35 K. Ujimoto and H. Kurihara, *Fukuoka Univ. Sci. Rep.*, 11 (1981) 39.
- 36 J. E. Desnoyers and G. Perron, *J. Solution Chem.*, 1 (1972) 199.