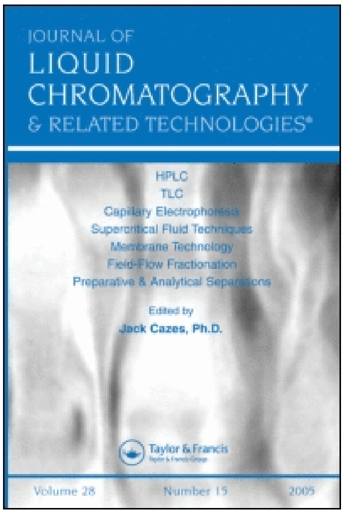

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[a] Aichi Mizuho College, Nagoya, Japan [b] Department of Applied Chemistry, Nagoya Institute of Technology, Nagoya, Japan

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ESTIMATION OF HYDROPHOBICITY USING QUASI-MOLECULAR VOLUME OF QUATERNARY AMMONIUM IONS

Chizuko Ohtsuka,[1] Yasuyuki Mori,[2] Shigeyuki Hayashi,[2]
Takao Tsuda,[2] Hiroko Wada[2]

[1] Aichi Mizuho College
Shunko-cho, Mizuho-ku
Nagoya (Japan)

[2] Department of Applied Chemistry
Nagoya Institute of Technology
Gokiso, Showa-ku
Nagoya (Japan)

ABSTRACT

When the Cu(II), Co(III), Ni(II), and Fe(II) chelates with 2-(5-Nitro - 2-pyridyl - 5 - [*N*-propyl-*N*-sulphopropyl)amino]phenol (Nitro-PAPS) were separated on octadecylsilane (ODS) stationary phase (Kaseisorb ODS Super and Kaseisorb LC ODS-300-5) by the addition of several quaternary ammonium salts (Q^+Br^-) individually to the aqueous ACN mobile phase, a correlation of these hydrophobic cation's character with the retention of the chelates was examined. The degree of the variation of the stationary phase due to the adsorption of quaternary ammonium ion (Q^+) is measured by using a series of elution time of a solute, which was obtained by its successive injection. When the alkyl group of Q^+ is larger, the period for attaining the equilibrium becomes longer. As a concentration of Q^+ is higher, the period becomes longer.

The estimation of quasi-molecular volume is proposed. The estimation volume provides its first order relationship with log *k*' of the metal chelates, regardless of the difference of its charged position in the structure. The degree of the slope of the first order relationship corresponds to the number of Q^+ associated with metal/Nitro-PAPS chelate. The elution order corresponds to the total number of Q^+ and Nitro-PAPS being associated with metal ions.

INTRODUCTION

Reversed phase ion-pair partition (RP-IPP) chromatography, in which the pairing ion is directly added to the aqueous mobile phase and modified on a bonded stationary phase, is preferred because of separating nonionic and ionic compounds simultaneously. Ion-pairing was visualized as masking the ionic portions of the solute molecules, making them hydrophobic. Therefore, elution follows in order of the decrease of ion-pair polarity, typical in a reverse phase mode. Reversed-phase ion-pair partition chromatography is widely applied to the separation of acids, bases,[1,2] and other species such as charged metal chelates.[3] For separation of negatively charged metal chelates, tetraalkylammonium salts have been often employed as ion-pair reagents in extraction and high performance liquid chromatography.[3-6]

For the transfer processes of an ion paired solute in RP-IPP, there are three popular hypotheses, namely the ion-pair model, the dynamic ion-exchange model and the ion-interaction model.[2,7] When the counter ion (paired ion) is hydrophobic, it distributes much in the stationary phase. Thus, the character of the stationary phase may be varied, depending upon both the relative size of the organic portion of the counter ion and its concentration in the mobile phase. The counter ion trapped in the stationary phase interacts generally with a solute. The hydrophobicity of the ion-paired solute has been discussed with regard to van der Waals Volume and related parameter.[8-13] However, the retention of solute, ion-pair with quaternary ammonium ions (Q^+s), has not been well characterized yet with the suitable parameter of the various Q^+s overall.

In the present study, eight different Q^+s with different charged positions (tetraalkylammonium ions and n-alkyltrimethylammonium ions) were used as counter ions for solutes of negatively charged metal chelates in the chromatographic conditions and clarified the relationships between the proposed quasi-molecular volume for the hydrophobicity of the counter ions and the retention time of metal chelates. Metal chelates with tridentate ligand, Nitro-PAPS (LH_2) investigated in this study, are Cu(II)L, $Co(III)L_2^-$, $Ni(II)L_2^{2-}$ and $Fe(II)L_2^{2-}$. Every metal chelate with Nitro-PAPS is extremely soluble in water and has a quite high molecular absorbancy. Therefore, we could carry the experiment with a low concentration of metal chelate for the study.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of a Shimadzu LC-6A pump (0.3 mL min^{-1}), a Shimadzu SPD-6AV UV-visible spectrometer (560 nm) and Shimadzu C-R3A chromatopac recorder. The sample was injected with a PTFE sample loop (20 µL) by suction.

A Kaseisorb LC ODS Super, ODS-300-5 (both of particle size 5 µm, pore size 120Å and 300Å, carbon content about 15% and 5%, the surface area of the silica 350 m^2 g^{-1} and 100 m^2 g^{-1}, respectively) column (250 mm x 4.6 mm I.D.) from Tokyo Kasei Kogyo was employed. The column was set in a oven (Gulliver series CO-965, Jasco, Tokyo) at 30°C.

Reagents and Procedures

Nitro-PAPS obtained from Dojindo Lab. (Kumamoto) was dissolved in water to give 1 x 10^{-3} M solution. Stock solutions of metal ions were prepared from the nitrates. Tetramethylammonium-(TMA), tetraethylammonium-(TEA), tetrapropylammonium-(TPA), tetrabutylammonium-(TBA), tetra-*n*-amylammonium-(TAA), *n*-hexyltrimethylammonium-(HTA), *n*-octyltrimethylammonium-(OTA) and *n*-dodecyltrimethylammonium-(DTA) bromide were of analytical reagent grade and were dissolved in water. Tris(hydroxymethyl)aminomethane was of analytical reagent grade and was used as a buffer solution with HCl.

The water used was purified by means of a Toraypure LV-10T ultrapure water manufacturing device (Toray). Chelate solutions were prepared to be 1 x 10^{-5} M, 3 x 10^{-5} M and 2 x 10^{-2} M for metal ion, Nitro-PAPS and tris-HCl buffer, respectively.

The mobile phase was aqueous acetonitrile solution with 2 x 10^{-2} M tris-HCl buffer, which also included tetra-alkylammonium ion. The concentration of Q^+ was examined low enough not to form micelle.

In LC, the capacity factor (k') is commonly used to define retention. Proper measurement of the column void volume is essential for the calculation of solute k' values. Sodium nitrate[14] was used in the study for this purpose.

The void volume of the system was also measured as the first distortion of the baseline after injection of water.[15] The void volume measured by $NaNO_3$ was consistent with the first irregularity in the baseline.

RESULTS AND DISCUSSION

The Period Need for Saturation of Stationary Phase with Quaternary Ammonium Ions

When we introduce the new effluent into a column, it takes a reasonable period for column conditioning in general. The length of period depends on compounds included in the effluent, the character of the additive in it, pH, etc. We measured the period necessary to attain the column conditioning. The process of column conditioning is nearly equal to the saturation process of stationary phase with the components between mobile and stationary phase.

In the present paper, for measuring this period, we took a very direct method. Namely a solute is successively injected into a column during the process of column conditioning.

The retention time of the solute is gradually increased and becomes a constant value after a certain time. The period for obtaining a constant retention time of a solute is equal to the necessary period for column conditioning, that is, “the period required for attaining an equilibrium (PRE).” This period is known as PRE. This PRE is obtained as follows: After a column was conditioned with acetonitrile/water and output signal of UV detector (base line) was monitored to become a constant value, the new effluent contained with Q^+Br^- is introduced and the output signal of UV detector is recorded continually. As a marker for checking the condition of the stationary phase the retention of a metal chelate was used. Cobalt chelate is selected as a solute, because it forms the complex with Q^+ in both mobile and stationary phase. We call tetraalkylammonium ion and *n*-alkylktrimethylammonium ion “spherical” and “linear” Q^+, respectively.

In Figure 1 and 2, the typical relationships between the elution time of Co/Nitro-PAPS *vs*. the introduction period of a new effluent or alternative effluent are shown. The expression “elution time” is intended to be distinguished from “retention time” used for the saturated stationary phase.

In Figure 1, we present results for the variation of retention time of Co/Nitro-PAPS with spherical Q^+ salts on ODS Super in the 40% acetonitrile aqueous solution. The periods of spherical Q, tetra-methyl, ethyl, propyl, butyl, and amyl are fully 10 min, 10 min, 10 min, 40 min, and 70 min, respectively. Similar results of the linear Q^+, HTA, OTA and DTA are 10 min, 10 min, and 50 min, respectively.

In Figure 2, the periods of different concentration for spherical Q^+, TAA 3×10^{-4} M and 1×10^{-4} M in the 40% acetonitrile aqueous solution are 80 min

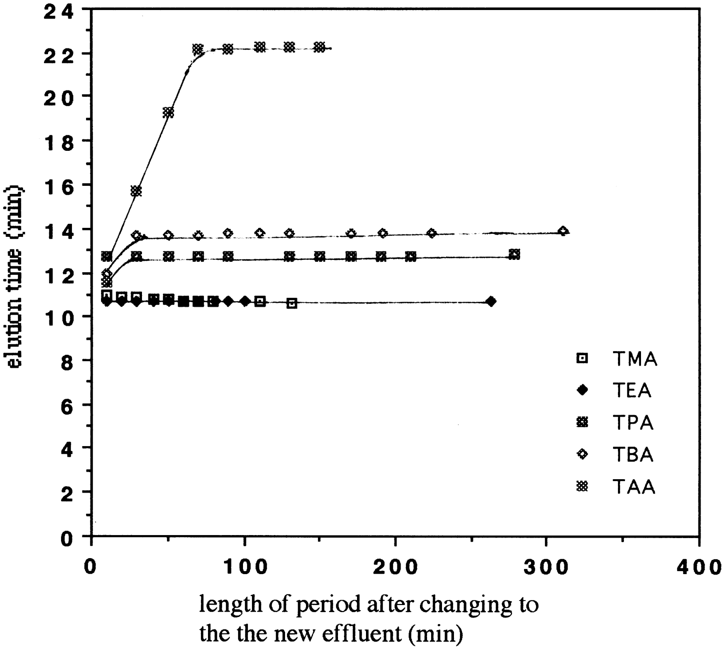


Figure 1. Dependence of the elution time of Co/Nitro-PAPS on a series of quaternary ammonium ions. Effluent: 2 x 10^{-2} M tris-HCl buffer and 1 x 10^{-4} M tetraalkylammonium ion (TMA, TEA, TPA, TBA and TAA) in aceto-nitrile/water (40:60, v/v).

and 140 min, respectively. Similar results of different concentrations for linear Q^+, DTA 1 x 10^{-3} M and 1 x 10^{-4} M are obtained at 50 min and 130 min, respectively.

The effects of hydrophobicity and concentration of Q^+ on the period of the distribution process of Q^+ on ODS-300-5 exhibited the same as those on ODS Super. As the alkyl group of Q^+ is larger, the period becomes longer for attaining the equilibrium. The covering with larger Q^+ on the surface of the stationary phase might need a longer period. As the concentration of hydrophobic counter ion is higher, the period becomes the shorter. It might be ascribed to the increase of the probabilities of collision between counter ion and site on the stationary phase.

Column conditioning is an important procedure to obtain reproducible analyses. Therefore, samples should be allowed to be injected after sufficient column conditioning.

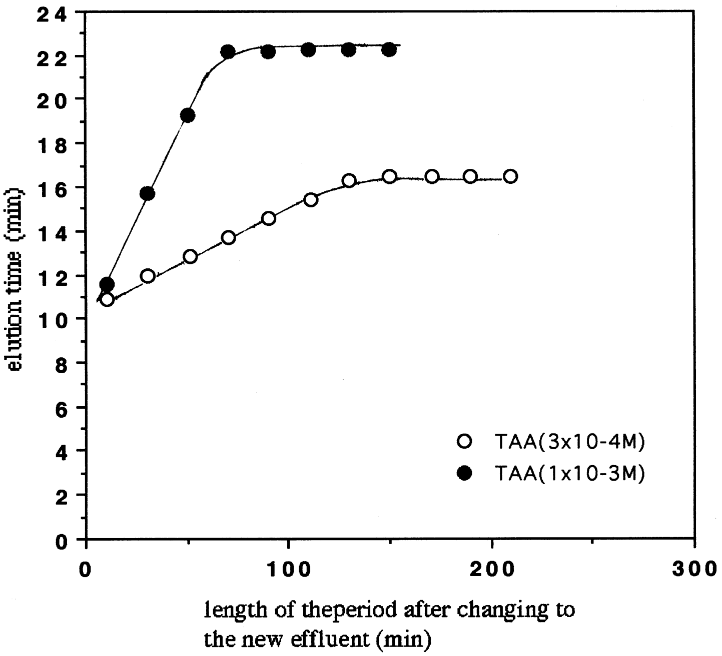


Figure 2. Dependence of the elution time of Co/Nitro-PAPS on the concentration of TAA in the effluent. Effluent: 2 x 10^{-2} M tris-HCl buffer and 1 x 10^{-4} M or 1 x 10^{-3} M tetraalkylammonium ion in acetonitrile/water (40:60, v/v).

Proposal Quasi-Molecular Volume Model

We propose the quasi-molecular volume for the estimation of hydrophobicity of Q^+. The reason of the proposal is described below.

In reversed-phase liquid chromatography, the hydrophobicity of the solute can be correlated with the retention time. The hydrophobicity has been studied on physical parameters such as structure-activity,[8] van der Waals volume,[9,11] van der Waals surface area,[10,11] solvent cavity surface area,[10,12] and molecular connectivity index.[11,13] In this article, the evaluation of the hydrophobicity for Q^+ is attempted.

Although the number of carbon atoms of a Q^+, van der Waals volume, and van der Waals surface area are generally used for the estimation of the hydrophobicity, we were not successful in finding a good correlation for the relationships between these parameters and retention time. The above estimated values are expressed as the sum of the individual volume or surface area of atoms. Therefore, they do not distinguish the structural difference of molecules such as spherical and linear Q^+.

We have found a most reliable estimation by the quasi-molecular volume of Q^+ defined in this work for its relationships with logarithm of capacity factor of the metal chelate shown in Figure 3 (correlation coefficients for Cu(II), Co(III), Ni(II), and Fe(II) chelates; 0.9297, 0.9347, 0.9908, 0.9919) and Figure 4 (correlation coefficients for Cu(II), Co(III), Ni(II), and Fe(II) chelates; 0.9710, 0.9555, 0.9856, 0.9871).

The packing materials both ODS super and ODS-300-5 showed very similar results except that the former shows larger *k*' compared to the latter. Log *k*' is a first-order function of the $(NB)^3$,

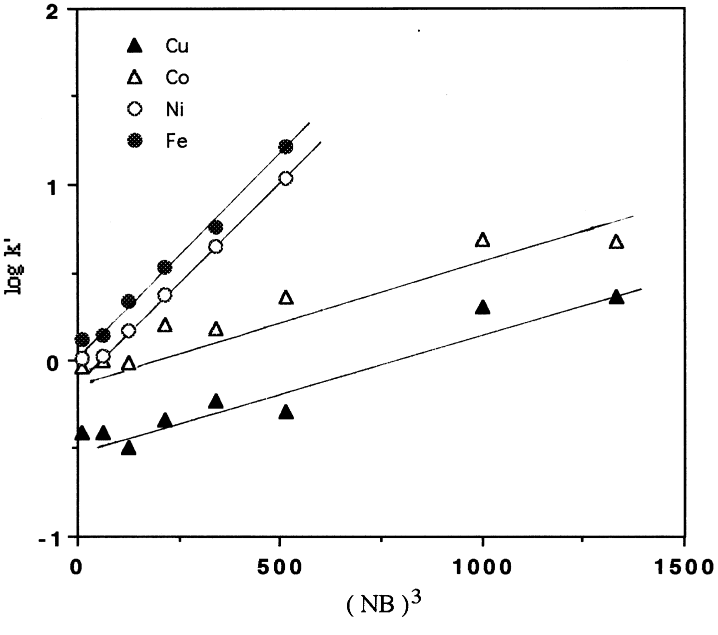


Figure 3. Plots of the logarithm of the capacity factor, *k*' of Cu(II), Co(III), Ni(II) and Fe(II) chelates with Nitro-PAPS as a function of $(NB)^3$ of TMA, TEA, TPA, TBA, TAA, HTA, OTA and DTA on ODS super. Effluent: 2×10^{-2} M tris-HCl buffer and 3×10^{-3} M quaternary ammonium ion in acetonitrile/water (35:65, v/v).

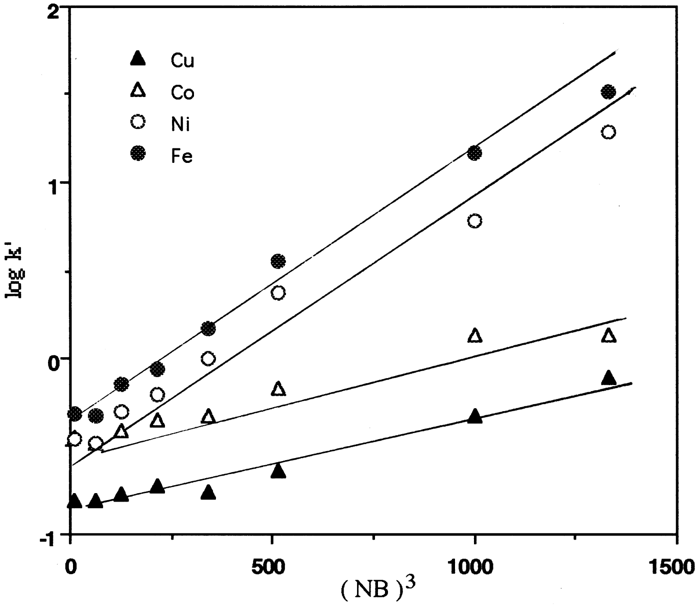


Figure 4. Plots of the logarith of the capacity factor, k', of Cu(II), Co(II), Ni(II), and Fe(II) chelates with Nitro-PAPS as a function of $(NB)^3$ of TMA, TEA, TPA, TBA, TAA, HTA, OTA, and DTA on ODS-300-5. Effluent: 2 x 10^{-2} M tris-HCL buffer and 3 x 10^{-3} M quaternary ammonium ion in acetonitrile/water (35:65, v/v).

$$\log k' \propto (NB)^3$$

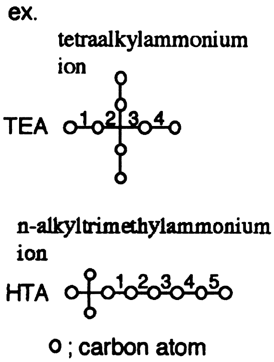
where in spherical Q^+, NB is C-C and C-N bonds from the end to other end shown in Table 1. While in linear Q^+, NB is C-C bonds from the carbon next to a nitrogen atom to the end in long-chain shown in Table 1.

As a result, the proposed $(NB)^3$ for the apparent estimation of structural volume is well suited for the estimation of the interaction between solute and ODS stationary phase. Q^+ is adsorbed onto the alkyl chains bonded to silica-gel with its ionic portion oriented at the surface. A difference of the interaction with the bonded alkyl chains is caused by the shape of Q^+, spherical or linear. Namely, the ionic portion of spherical Q^+ is in the interior of the structure. Therefore, the whole of spherical ion could be able to contribute to hydrophobic interaction. While the ionic portion of linear Q^+ lies so far to the end of the structure. only the residual hydrophobic portion could contribute to hydrophobic interaction. Therefore, it gives rise to the different definition of NB for

Table 1

Number of the Bonds in Quaternary Ammonium Ions

	NB	$(NB)^3$
TMA	2	8
TEA	4	64
HTA	5	125
TPA	6	216
OTA	7	343
TBA	8	512
TAA	10	1000
DTA	11	1331



spherical and linear Q^+. When σ is now assumed to be a diameter of the imaginary spherical molecule,

$$\sigma = NB \bullet \text{ (bond length)}$$

where bond length is assumed to be 1.5Å for both C-C and C-N.

$$\log k' \propto (NB)^3 \propto \sigma^3$$

Log *k*' becomes proportional to the volume of the imaginary spherical molecule and not the counter ion itself. We call $(NB)^3$ corresponding to the volume of the imaginary spherical molecule, $(\pi/6) \bullet \sigma^3$, as quasi-molecular volume.

It seems reasonable to concluded that $(\pi/ 6) \bullet \sigma^3$ followed with rotation is the maximum volume being able to interact with alkyl chains bonded to silica-gel by hydrophobic interaction. The most likely description might be a measure of adsorbability.

The introduction of quasi-molecular volume of Q^+ provided a linear correlation with log *k*' and gave a reasonable explanation for the effect of the number of Q^+ on log*k*' of the metal chelates. The slopes shown in Figure 3 and 4 can be classified into two groups; one of which is consisted of CuL and CoL_2^- and the other is consisted of NiL_2^{2-} and FeL_2^{2-}. The former group shows gentle slope; log *k*' / $(NB)^3$ = 0.7/1000 and log *k*' / $(NB)^3$ = 0.5/1000 on ODS super and ODS-300-5, respectively. The latter group shows the steep slope; log *k*' / $(NB)^3$ = 2.1/1000 and log *k*' / $(NB)^3$ = 1.5/1000 on ODS Super and ODS-300-5, respectively. The latter also shows a 3 times steeper slope compared to the former. The difference of the slopes might be ascribed to the number of Q^+ associated with negatively charged metal chelates shown in Table 2. CoL_2^- forms with one Q^+, $CoL_2^-Q^+$, while NiL_2^{2-} and FeL_2^{2-} form with two Q^+s, $NiL_2^{2-}Q_2^{2+}$, and $FeL_2^{2-}Q_2^{2+}$.

Now, neutral metal chelate, CuL should not depend upon a counter ion in reversed-phase ion-pair liquid chromatography. Namely, the addition of Q^+ in the effluent substantially alters the retention of the charged species CoL_2^-, NiL_2^{2}, and FeL_2^{2-} but not the neutral species, such as CuL.[7] However, log *k'* of CuL was observed experimentally to be dependent on Q^+. It could be interpreted as follows below.

The coordination number of Cu(II), four, might make CuL formed with Br, $CuLBr^-$, a square-plane structure in the experimental condition. If $CuLBr^-$

Table 2

Composition of the Ion-Pairs

Ion-Pair	**L^{2-}**	**Q^{+a}**	**Total Number[b]**
$[Cu(II)LBr]^-Q^+$	1	1	2
$[Co(III)L_2]^-Q^+$	2	1	3
$[Ni(II)L_2]^{2-}Q_2^+$	2	2	4
$[Fe(II)L_2]^{2-}Q_2^+$	2	2	4

---

[a] **Being concerned in the dependence of log k' on $(NB)^3$ in counterion.**

[b] **Being concerned in the retention strength of ion-pair.**

might exist, it forms an ion-pair with one Q^+, $CuLBr^-Q^+$. So, CuL shows similar dependence to CoL_2^- on Q^+; nearly the same slopes for the relation between log *k*' and quasi-molecular volume. The elution order of the metal chelates was found to be followed with the total hydrophobicity presumed by the total number of both ligand, Nitro-PAPS(L^{2-}) and counter ions (Q^+): $CuLBr^-Q^+$ (1+1) < $CoL_2^-\,Q^+$ (2+1) < $NiL_2^{2-}Q_2^{2+}$ (2+2) and $FeL_2^{2-}Q_2^{2+}$ (2+2), where values in parentheses are referred to Table 2.

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