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Factors affecting selectivity in micelle exclusion chromatography of inorganic anions

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ABSTRACT

Selectivity in micelle exclusion chromatography is influenced by a number of factors, such as adsorption ability of stationary phases, mobile phase additives, pH of mobile phases and the charge density of micelles. These effects on the retention behaviour of inorganic anions are discussed on the basis of a model that permits the determination of partition coefficients of analytes. If needed, the acid–base equilibrium of an analyte can be involved in the model. A derived equation allows the evaluation of the individual contributions of the neutral and the ionic species to the total partition and the determination of the dissociation constant of the analyte in a micellar solution. Stationary phases of low adsorption ability offer unique selectivity and precise partition coefficients.

INTRODUCTION

Micellar mobile phases, which are often substituted for organic solvents in the reversed-phase chromatography of organic compounds [1–10], provide some advantages, such as non-toxicity, non-flammability, unique selectivity, high sensitivity in fluorimetric detection [11,12] and improvement of baseline drift in gradient elution chromatography with electrochemical detection [13], over hydro–organic or aqueous mobile phases. However, micellar mobile phases do not necessarily improve the chromatographic resolution. On the contrary, peak broadening due to slow kinetics of micellar partitioning was pointed out as a disadvantage. Adding organic solvents and/or raising the temperature for overcoming this problem were investigated [4–6]. Khaledi *et al.* [6], for example, reported that a mixture of an organic solvent and a surfactant showed a higher elution strength than expected from the individual elution strengths, and systematically enhanced the selectivity. However, we cannot say that micellar mobile phases are useful because of their separation abilities. The unique selectivity and the facility to evaluate the partition behaviour of solutes are important features of micellar mobile phases. From this point of view, the retention and partition behaviour of organic compounds have been well investigated using various micellar mobile phases [5–10].

In spite of its uniqueness and usefulness, only a few reports have appeared which describe the applicability of micellar mobile phases to inorganic chromatography. Mullins and Kirkbright [14,15] studied the reversed-phase chromatographic separa-

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tion of inorganic anions with cationic micellar mobile phases. However, the chromatographic selectivity was reported to be the same as that of anion-exchange or ion-interaction chromatography. This is due to the strong adsorption ability of the stationary phase; the retention was principally controlled by the partition to the stationary phase. Although they determined partition coefficients for some inorganic anions according to Armstrong's model [1], these values may involve much ambiguity, as will be indicated later. Okada [16,17] developed micelle exclusion chromatography in which size-exclusion chromatographic stationary phases of low adsorption ability were used in place of the usual non-polar stationary phases, and showed that the selectivity of inorganic chromatography can be effectively modified using micellar mobile phases. In micelle exclusion chromatography, the retention of analytes is mainly controlled by the partition of analytes to micellar phases rather than by that to the stationary phase.

In previous work, Okada [16], indicated factors that affect the selectivity of micelle exclusion chromatography, and inferred that some of them affected the partition either to the micellar phase or to the stationary phase, and some influenced both. Actual retention was thought to be determined by a subtle balance of these effects. In this paper, these factors, that is, the adsorption ability of the stationary phase, mobile phase additives, pH of mobile phases and the charge density of micelles, are quantitatively discussed on the basis of a model derived to interpret the retention behaviour of analytes.

EXPERIMENTAL

The chromatographic system consisted of a Tosoh CCPM computer-controlled pump, a Rheodyne injection valve equipped with a 100-μl sample loop, a column oven (CO-8000; Tosoh), a JASCO 875-UV UV–visible detector, a JASCO Model 830-RI refractive index detector and a conductimetric detector (CM-8000; Tosoh). Separation columns were Asahipak GS-300H, GS-310H and GS-320H [250 mm × 7.6 mm I.D., packed with poly(vinyl alcohol) gel with a variety of degrees of saponification, particle size 9 μm]. A Toa Model CM-20S conductimeter was used to determine critical micelle concentrations (CMC) of ionic and mixed ionic–non-ionic micelles. The temperature was maintained at 25°C. The flow-rate was 1 ml/min.

Hexadecyltrimethylammonium bromide (HTAB) of analytical-reagent grade and polyoxyethylene(23)dodecyl ether [POE(23)D; the number in parentheses refers to the average number of repeating oxyethylene units] of amino acid analysis grade were purchased from Nacalai Tesque. Hexadecyltrimethylammonium chloride (HTAC) was prepared by replacing bromide in HTAB solutions with an anion-exchange resin (Dowex 1-X4) in the Cl^- form. POE(23)D solutions were deionized with a mixed-bed ion-exchange resin column.

Standard solutions of inorganic anions were prepared by dissolving potassium salts, which were dried at 110°C under vacuum, in water. Distilled, deionized water was used throughout.

RESULTS AND DISCUSSION

Basic aspects of micelle exclusion chromatography

Fig. 1 represents a scheme of operative equilibria for the micelle exclusion chromatographic retention of an anion (A^-) with an alkylammonium micellar mobile phase: V_e, V_i and V_s denote the volume of the external solvent into which micelles permeate, the volume of the inner solvent into which micelles do not permeate but monomeric surfactants do permeate, and the volume of the stationary phase, respectively; K_{MW}, K_{SW} and K_d represent the partition coefficients of analytes between the micellar and the external solvent phase, between the inner solvent and the stationary phase and between the inner and the external solvent phase, respectively. K_d can be regarded as unity for small analytes such as simple inorganic anions which can permeate the entire inner pores of the stationary phase without restriction. Anionic analytes are partitioned both to the micellar phase and to the stationary phase by electrostatic interactions similarly to anion exchange.

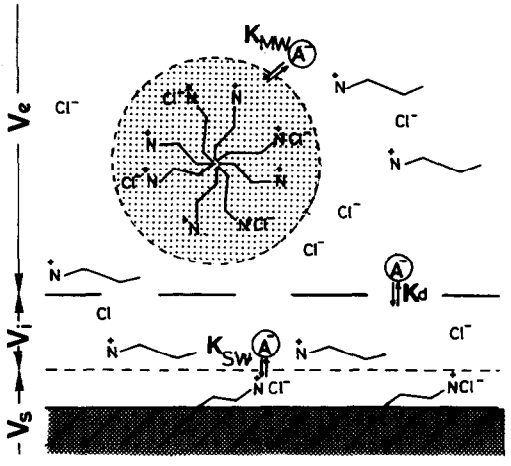

Fig. 1. Schematic representation of operative equilibria in micelle exclusion chromatography. Symbols are given in the text.

We can derive the following equation that describes the retention behaviour of analytes in micelle exclusion chromatography, if a pseudo-phase model is applicable to micelles [1,16]:

$$1/(V_r - V_e) = [(K_{KW} - 1)\bar{v}C_m + 1]/(V_i + V_sK_{SW}) \quad (1)$$

where $\bar{v}$, C_m and V_r represent the partial molal volume of micelles, the concentration of micelles (which is equal to the difference between the concentration of the surfactant used in the mobile phase and the CMC) and the retention volume of the analyte, respectively. Armstrong and Nome [1] reported a similar equation for interpreting the retention of organic compounds in reversed-phase chromatography with micellar

mobile phases. V_i will be negligibly small for the usual reversed-phase columns, but should be taken into account in micelle exclusion chromatography. According to eqn. 1, we can calculate K_{MW} and K_{SW} values from plots of $1/(V_r - V_e)$ *vs.* C_m. An analyte that is not retained on the stationary phase ($K_{SW} = 0$) will be eluted between V_e and $V_e + V_i$. The stationary phases employed in this study are weak adsorbents in comparison with the usual reversed-phase materials. However, the adsorption of surfactants is not negligible when discussing the retention behaviour of anions. Adsorbed surfactants act as anion-exchange sites and widen the effective elution window, as can be envisaged from eqn. 1. However, large K_{SW} values diminish the uniqueness in the selectivity, as shown later, because the partition to the micellar phase becomes less important.

It should be noted that V_e is used instead of the void volume of the stationary phase. The stationary phases used in this study exclude molecules with molecular weights >40 000. The aggregation number of an HTA^+ micelle was reported to be 78 [18]; although the molecular sieve effects depends not only on the molecular weights but also on the molecular shapes, HTAC micelles are thought to permeate part of the stationary phase. Fig. 2 shows the change in the elution volume of HTAC micelles with concentration (C_{HTAC}). The elution time decreases with increasing C_{HTAC}, and becomes constant when C_{HTAC} reaches 0.01 *M*. The constant elution time can be regarded as the intrinsic elution time of HTAC micelles. On the other hand, the monomeric surfactants mainly determine the elution times of HTAC solutions, when C_{HTAC} is lower than 0.01 *M*. This phenomenon depicts the dynamic aspect of the micellar system.

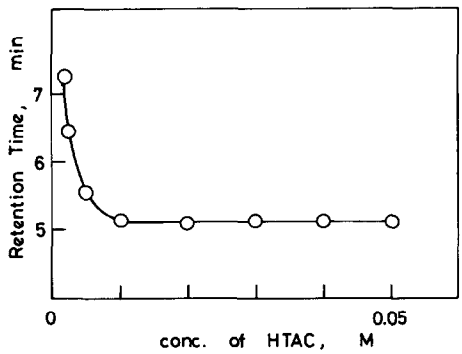

Fig. 2. Variation in the retention times of HTAC micelles with concentration. Stationary phase, GS-320H.

Adsorption ability of the stationary phase

The poly(vinyl alcohol) stationary phases used in this study have variable adsorption ability. The amounts of HTAC adsorbed on these stationary phases are listed in Table I together with other column parameters. GS-320H is the weakest and GS-300H is the strongest adsorbent. Fig. 3 shows a comparison of chromatograms obtained with these three stationary phases. The usual elution order of anions in anion-exchange or ion-interaction chromatography is $IO_3^- < NO_2^- < NO_3^- < I^-$ [16]. This order cannot be changed by varying the anion-exchange capacity, the structure of anion-exchange sites or the concentration of non-micellar mobile phases.

TABLE I

ADSORPTION ABILITY AND OTHER PARAMETERS OF STATIONARY PHASES

Stationary phase	Amount of HTAC adsorbed (mmol per column)	V_e (ml)	V_i (ml)	V_s (ml)
GS-300H	0.76[a]	5.7	1.4	4.3
GS-310H	0.66[a]	5.7	2.2	3.5
	0.96[b]			
	1.09[c]			
	1.20[d]			
GS-320H	0.21[a]	5.1	1.9	4.4

[a,b,c,d] Adsorption measured using 0.01 *M* HTAC solutions containing 0, 0.1, 0.2 and 0.3 *M* NaCl, respectively.

However, in micelle exclusion chromatography, the elution order varies with the adsorption ability of the stationary phases and the concentrations of micelles in the mobile phase. The selectivity obtained with GS-320H especially is very unusual, *i.e.*, I^- is eluted most rapidly and the elution order of NO_2^- and NO_3^- is also reversed.

Partition of analytes to the stationary phase is governed by electrostatic interactions similarly to the usual anion exchange. According to the Donnan–Gibbs theory, an ion-exchange equilibrium constant (K_{IE}) between univalent anions, A and B, is given by the following equation [19]:

$$A(b) + B(f) \rightleftharpoons A(f) + B(b)$$

$$\ln K_{IE} = P(\bar{v}_A - \bar{v}_B)/RT + \ln(\gamma_A/\gamma_B)_{(b)} - \ln(\gamma_A/\gamma_B)_{(f)} \quad (2)$$

where P, R and T represent the swelling pressure of the resin, the gas constant and absolute temperature, respectively, $\bar{v}_A$ *and* $\bar{v}_B$ are the partial molal volumes of A and B,

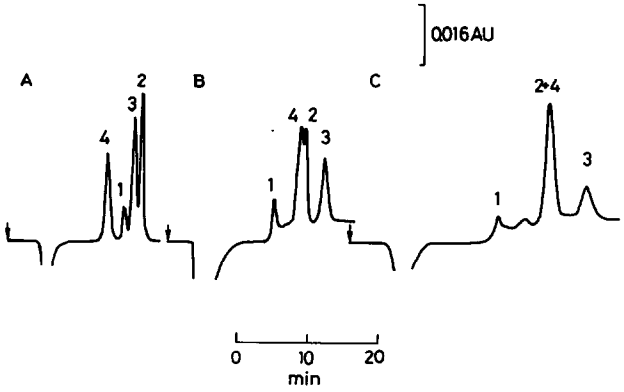

Fig. 3. Comparison of micelle exclusion chromatograms of anions. Stationary phase: (A) GS-320H; (B) GS-310H; (C) GS-300H. Mobile phase, 0.05 *M* HTAC for GS-300H and GS-310H, 0.013 *M* HTAC for GS-320H. Peaks: (1) IO_3^- (10 ppm); (2) NO_2^- (5 ppm); (3) NO_3^- (5 ppm); (4) I^- (10 ppm). Detection, UV (220 nm). Other conditions are given in the text.

γ_A and γ_B are the activity coefficients of A and B and (b) and (f) denote bound and free anions, respectively. Eqn. 2 shows that ions with small hydrated volumes will be more strongly bound by the resin as P increases. Therefore, such analytes become more distributable to the stationary phase with increasing P than ions of large hydrated volumes. In this study, an increase in the swelling pressure is roughly related to an increase in the adsorption ability of the stationary phases. K_{SW} values for NO_2^- and NO_3^- determined with varying C_{HTAC} in the mobile phase are listed in Table II. A $K_{SW}(NO_3^-)/K_{SW}(NO_2^-)$ ratio becomes large with increasing adsorption ability of the stationary phase; partitioning of NO_3^- is more enhanced than that of NO_2^-. This effect obviously diminishes the uniqueness in the selectivity. This result shows that the use of the stationary phase of low adsorption ability is essential to obtain unique selectivity.

TABLE II

COMPARISON OF K_{SW} VALUES OBTAINED FOR THREE STATIONARY PHASES

Stationary phase	K_{SW}[a] (relative value)	
	NO_2^-	NO_3^-
GS-300H	28 (0.25)	110 (1)
GS-310H	17 (0.26)	66 (1)
GS-320H	6.4 (0.44)	14 (1)

[a] K_{SW} values were determined on the basis of eqn. 1 by varying the concentration of HTAC in the mobile phase.

Effect of mobile phase additives

Most mobile phase additives affect the partitioning of analytes both to micelles and to the stationary phase. As reported previously [16], organic solvents, for example, decrease the amount of surfactants adsorbed on the stationary phase by lowering the permittivity and increasing the hydrophilicity of mobile phases. These effects lower the K_{SW} values and decrease the retention. In addition, organic solvents permeate to the micelles, stabilize the monomeric surfactants in solutions and finally prevent micellization [20], thus lowering the K_{MW} value and increasing the retention. The actual retention of analytes is determined by a subtle balance of both effects. Although effects of organic solvents are important from fundamental and practical viewpoints, a quantitative discussion is not included in this paper because of its extreme difficulty.

Addition of salts also affects partitioning both to the stationary phase and to micelles. Mass action reduces the partitioning to both phases; both K_{MW} and K_{SW} become small. On the other hand, the salting-out effect promotes the adsorption of surfactants and enhances K_{SW} values. The changes in the amounts of adsorbed HTAC on GS-310H with sodium chloride concentration are given in Table I. However, it was found in ion-interaction chromatography that the mass action effect was more important than the salting-out effect [21] in the determination of the retention. The salting-out effect also influences the micellization and lowers the CMC. This effect is negligible, however, because the increase in the micelle concentration is marginal.

TABLE III

K_{MW} AND K_{SW} VALUES OBTAINED FOR SOLUTIONS OF VARIOUS SALINITY USING GS-310H AS THE STATIONARY PHASE

[NaCl] (M)	NO_2^-		NO_3^-		I^-	
	K_{MW}	K_{SW}	K_{MW}	K_{SW}	K_{MW}	K_{SW}
0.05	74.3	6.89	189	19.6	850	68.1
	(1.7)[a]	(0.09)	(10)	(0.7)	(73)	(5.0)
	$r = 0.999$[b]		$r = 0.999$		$r = 1.000$	
0.1	37.8	3.86	100	11.6	615	53.9
	(2.3)	(0.13)	(3.7)	(0.3)	(26)	(2.0)
	$r = 0.998$		$r = 1.000$		$r = 1.000$	
0.15	29.8	2.82	76.8	8.65	438	40.4
	(1.4)	(0.04)	(2.2)	(0.13)	(21)	(1.6)
	$r = 0.998$		$r = 1.000$		$r = 1.000$	
0.2	23.6	2.34	56.6	7.14	341	35.1
	(0.4)	(0.33)	(2.3)	(0.13)	(18)	(1.5)
	$r = 0.999$		$r = 0.999$		$r = 1.000$	
0.3	16.2	1.55	43.1	5.17	226	25.1
	(0.7)	(0.01)	(1.6)	(0.08)	(4.4)	(0.3)
	$r = 0.999$		$r = 1.000$		$r = 1.000$	

[a] Standard deviations in parentheses (n = 5–9).
[b] r = Correlation coefficient of plots based on eqn. 1.

K_{MW} and K_{SW} values for NO_2^-, NO_3^- and I^- determined in 0.05–0.3 M sodium chloride solutions are given in Table III, together with standard deviations. K_{MW} values, which are not affected by the adsorbed surfactant concentration in the stationary phase, are decreased by the mass action of Cl^-. Despite the increase in the amount of surfactant adsorbed, the K_{SW} values decrease with increasing salt concentration. This indicates that mass action is the primary factor determining the retention change induced by adding salts.

Decreases in K_{SW} and K_{MW} values by adding salts to the mobile phase bring about an advantage. The determination of precise K_{MW} values will be of fundamental importance in analytical, solution and micellar chemistry. Micellar chromatography generally facilitates the determination of a K_{MW} value, which is calculated from the ratio of the slope to the intercept of the $1/(V_r - V_e)$ *vs.* C_m plot. In this instance, the standard deviation of the K_{MW} value is calculated on the basis of the theory of the method of least squares. A Jacobian matrix (**J**) can be derived from eqn. 1:

$$\mathbf{J} = [\partial f(C_{m_j})/\partial K_{MW},\ \partial_f(C_{m_j})/\partial I]_n \tag{3}$$

$$f(C_{m_j}) = [(K_{MW} - 1)\bar{v}C_{m_j} + 1]I$$

$$I = 1/(K_{SW}V_s + V_i)$$

where n represents the number of measurements. The standard deviation (σ) of the K_{MW} is given by

$$\sigma = \left\{\sum_j S_j^2 r^2 / I^2 \bar{v}^2 \left[\sum_j C_{m_j}^2 \sum_j S_j^2 - \left(\sum_j C_{m_j} S_j\right)^2\right](n-2)\right\}^{1/2} \quad (4)$$

$$S_j = (K_{MW} - 1)\bar{v} C_{m_j} + 1$$

where r^2 represents the residual sum of squares. Ambiguity of K_{MW} originates from deviations of both the slope and the intercept, and increases as the intercept (I) approaches the origin. This indicates that large intercepts, which result from low K_{SW} values, are required for the determination of reliable K_{MW} values. As shown in Table III, except for accidental errors, a near-zero intercept is generally accompanied by a large standard deviation.

Further, lack of distinction of the CMC of a surfactant employed in a mobile phase causes serious errors. Partitioning of analytes to micellar phases has been regarded as the primary separation mode in micellar chromatography. However, some studies have shown that retention behaviour is not necessarily changed at the CMC determined in water [7,22]. This may be caused by a difference between the CMC measured in water and that in a chromatographic system. This ambiguity in the CMC also becomes serious as the intercept approaches the origin. Negative intercepts, which obviously have no physical meaning, were often observed for plots based on eqn. 1 or equivalent equations when analytes show large K_{SW} values [16,17]. This is due to the lack of distinction of CMC in the chromatographic system and the near-zero intercept of the plot. From these viewpoints, a stationary phase of low adsorption ability should be used to study micellar partitioning on the basis of K_{MW} values. The adsorption abilities of the stationary phases used in this study were not low enough for the purpose. However, addition of salts to the mobile phases lowers the K_{SW} values (in other words, it increases the intercept), and makes it possible to discuss K_{MW} values precisely and systematically.

Some workers studied partitioning of ions by applying an ion-exchange model to micellar systems [23]. Also, Hux and Cantwell [24] derived an equation that describes the ion-exchange distribution (or partition) coefficients ($= K_{SW}$) for a univalent anion:

$$K_{SW} = \sigma_0 K_{IE} A_{Sp} \cdot 10^3 / 2Fc \quad (5)$$

where σ_0 is the surface charge density, F is the Faraday constant, c is the ionic strength of the bulk solution and A_{Sp} is the specific surface area of the ion exchanger. If the ionic distribution to micelles obeys an ion-exchange model, and the ion-exchange equilibrium constant at the micellar surface is not affected by the ionic strength of the bulk solution, an equivalent equation can be derived:

$$K_{MW} = \sigma_0^m K_{IE}^m A_{Sp}^m \cdot 10^3 / 2Fc \quad (5a)$$

where the superscript m denotes a micelle. In this case, the K_{MW} values are proportional to the reciprocal of the ionic strength of the bulk solution (c). Ionic micelles usually imbibe most of the counter ions; for example, 80% of Br^- are bound by HTAB micelles [20]. Thus, in the presence of large excess of an added salt, the concentration of the salt dominates the ionic strength of the solution. In fact, this consideration is

applicable to the K_{MW} values listed in Table III. The relationships between K_{MW} values and $1/c$ are as follows:

$$K_{MW}(NO_2^-) = 3.4(1/c) + 5.6 (r = 0.998)$$
$$K_{MW}(NO_3^-) = 8.7(1/c) + 14.7 (r = 0.999)$$
$$K_{MW}(I^-) = 57.7(1/c) + 44.3 (r = 0.998)$$

The K_{MW} value for I^- determined in a 0.05 *M* sodium chloride solution was omitted from this relationship, because it includes much ambiguity. Non-zero intercepts of these relationships arise because the concentrations of the monomeric surfactants and the micelles were not involved in ionic strength. Except for this point, this result justifies the applicability of an ion-exchange model to micellar systems.

K_{MW}/K_{SW} ratios tend to decrease with increasing sodium chloride concentration. This indicates that mass action decreases both K_{MW} and K_{SW} values almost equally but that, on the other hand, the salting-out effect increases only the K_{SW} values. Thus, adding a salt permits us to discuss systematically changes in K_{MW}, but diminishes the unique selectivity.

pH of mobile phases

The pH of the mobile phase also affects the retention behaviour of weak acids. Arunyanart and Cline Love [4] investigated the effects of pH on the retention behaviour in micellar reversed-phase chromatography using a model developed for cyclodextrin mobile phases [25]. They assumed intrinsic capacity factors for neutral and ionic species to derive equations describing the retention behaviour. This model has some advantages, but simultaneously involves some disadvantages; for example, 1:1 stoichiometry between a surfactant molecule in micelles and an analyte was assumed, and individual contributions of neutral or ionic species to the total partition to micelles cannot be evaluated. Okada [26] modified eqn. 1 and derived the following equation to describe the retention behaviours of analytes involving acid–base equilibria:

$$1/(V_r - V_e) = [(K_{MW}^T - 1)\bar{v}C_m + 1]/(V_s K_{SW}^T + V_i) \quad (6)$$

$$K_{MW}^T = \alpha_1 K_{MW1} + \alpha_0 K_{MW0} = \alpha_1(K_{MW1} - K_{MW0}) + K_{MW0} \quad (7)$$

$$K_{SW}^T = \alpha_1 K_{SW1} + \alpha_0 K_{SW0} = \alpha_1(K_{SW1} - K_{SW0}) + K_{SW0} \quad (8)$$

where α_1 and α_0 are the fractions of ionic and neutral species with respect to the total concentration of the analyte, and partition coefficients accompanied by subscripts 0 and 1 are for neutral and ionic species, respectively.

In this study, the retention of NO_2^- was influenced by the pH of the mobile phase. To evaluate the effect of pH on the retention of NO_2^-, it was assumed that HNO_2 was not distributable to the micellar phase. In such a case, K_{MW}^T can be simply replaced by $\alpha_1 K_{MW1}$. The dissociation constant of an ionizable compound at the micellar surface is known to be different from its intrinsic value. It is known that, if the surface potential (ψ) is the only factor affecting an acid–base equilibrium, the apparent

K_a value in a micellar systems (K^m) can be related to the intrinsic K_a value (K_a^i) as follows [27]:

$$pK_a^m - pK_a^i = -F\psi/2.3RT$$

A low permittivity at the micellar surface also influences K_a^m values, although this effect is not contained in the above equation. Before eqn. 6 is applied to HNO_2, the dissociation constant in HTAC solutions should be determined. The dissociation constant (K_a^m) can be determined from a plot of $1/K_{MW}^T$ *vs.* $[H^+]$ in mobile phases according to

$$1/K_{MW}^T = [H^+]/K_{MW1}K_a^m + 1/K_{MW1} \quad (9)$$

This equation can be derived from $K_{MW}^T = \alpha_1 K_{MW1}$. For NO_2^- in 0.1 *M* sodium choride medium, the following relationship was found between $1/K_{MW}^T$ and $[H^+]$:

$$1/K_{MW}^T = 45.6[H^+] + 0.0266 \, (r = 0.991)$$

K_a^m for NO_2^- was calculated as $5.8 \cdot 10^{-4}$ from this relationship. This value is close to $7.1 \cdot 10^{-4}$, the K_a^i value in aqueous solution. A positive surface potential of HTAC micelles decreases pK_a, but the low dielectric constant at the micellar surface leads to an increase in pK_a. These opposite effects results in the above K_a value for HNO_2.

Substituting α_1, which can be calculated from K_a^m and $[H^+]$, in eqns. 7 and 8 permits us to calculate K_{MW} and K_{SW} values for both NO_2^- and HNO_2. Fig. 4 shows the changes in K_{MW}^T and K_{SW}^T with α_1. According to eqn. 7 and 8, the intercepts of these plots are equal to K_{MW0} and K_{SW0} and the slopes correspond to $K_{MW1} - K_{MW0}$ and $K_{SW1} - K_{SW0}$. Although both linear relationships included relatively large deviations, approximate partition coefficients can be calculated as $K_{MW0} \approx 0$, $K_{MW1} = 38$, $K_{SW0} = 9.8$ and $K_{SW1} = 3.9$. The assumption that HNO_2 is not partitioned into micelles proves to be reasonable. Surprisingly, HNO_2 is more strongly retained on the

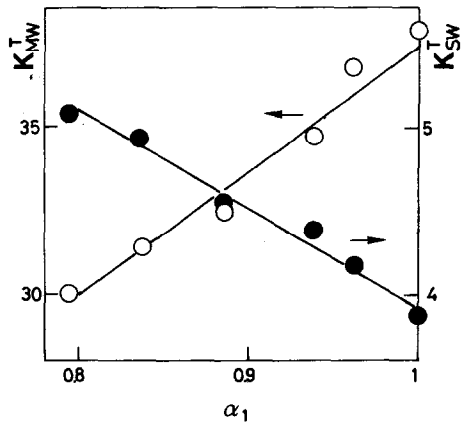

Fig. 4. Plots of (○) K_{MW}^T and (●) K_{SW}^T for NO_2^- *vs.* α_1. Stationary phase, GS-310H.

stationary phase than NO_2^-. Although the details have not been elucidated, specific interactions of HNO_2 with the stationary phase matrix may occur.

The change in the retention of nitrite with the pH of mobile phases containing 0.01 *M* HTAC is plotted as circles in Fig. 5. If the specific retention of HNO_2 did not exist, the retention of nitrite should decrease with decreasing pH, as shown by the broken line. However, the interaction of HNO_2 with the stationary phase leads to the opposite dependence of the retention on pH, *i.e.*, the retention increase with decreasing pH. The disagreement clearly indicates that the specific retention of HNO_2 should be taken into account. In fact, the experimental data are well explained by the calculation using the partition coefficients reported above, as shown by the solid line in Fig. 5.

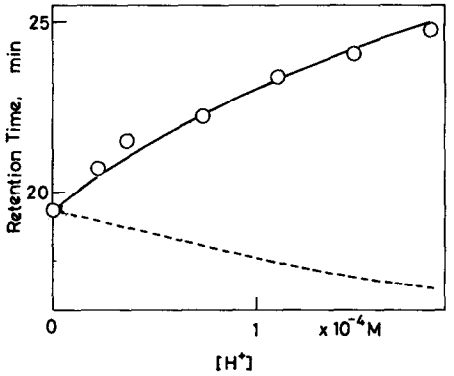

Fig. 5. Change in the retention of NO_2^- with $[H^+]$. ○, Experimental values obtained with mobile phases containing 0.01 *M* HTAC; solid line, calculated on the basis of eqns. 6–8; broken line, calculated by assuming the absence of the specific retention of HNO_2 ($K_{SWO} = 0$). Stationary phase, GS-310H.

Charge density of micelles

The effect of the charge density on the selectivity in ion-exchange resins has been theoretically and experimentally investigated [28,29]. In this study, we can discuss this effect by varying the charge density of micelles instead of an ion-exchange resin, *i.e.*, mixed micelle formation of HTAC and POE(23)D allowed us to change systematically the charge density of micelles (σ_0^m). In a particular mixed micellar system, heterogeneous mixed-micelle formation has been reported [30]. However, HTAC and POE(23)D are expected to form homogeneous mixed micelles [31], and permit the systematic evaluation of the effect of σ_0^m on K_{MW} values.

In this study, CMCs of mixed micelles were determined conductimetrically. Fig. 6 shows examples of changes in the specific equivalent conductance (Λ) with the concentration of HTAC in HTAC–POE(23)D mixed micelles. The applicability of conductimetric measurements was limited because of the extremely low CMCs of POE(23)D-rich micelles. However, as ambiguity of low CMC values does not cause serious errors in K_{MW} values, the CMCs of POE(23)D-rich micelles were assumed to be zero. This assumption led only a 2% error even for K_{MW} values determined for HTAC-rich micelles such as HTAC–POE(23)D (10:1) mixed micelle. It is known that

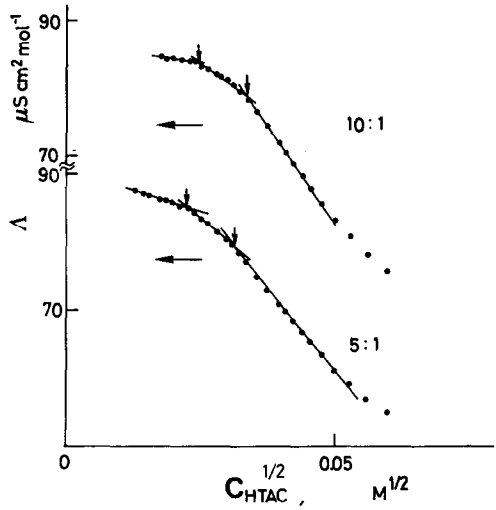

Fig. 6. Conductimetric determination of CMC of HTAC–POE(23)D (10:1 and 5:1) mixed micelles. Small arrows represent break points.

conductimetric and surface tension measurements on HTAB–POE(23)D mixed micelles give two breakpoints [31], which are due to the micellization and the transition of the spherical micelles to the asymmetric micelles. The same phenomena can be seen in Fig. 6. In this study, it was assumed that both spherical and asymmetric mixed micelles would show identical partition behaviour. This assumption is reasonable because the partitioning of anionic compounds to micelles is strongly attributed to the electrostatic attraction; on the other hand, there may be a difference in partition behaviour between these two micelles when the partitioning is determined by dissolving analytes in the micelle cores.

Fig. 7 shows the changes in K_{MW} values with ratio of HTAC concentration to the

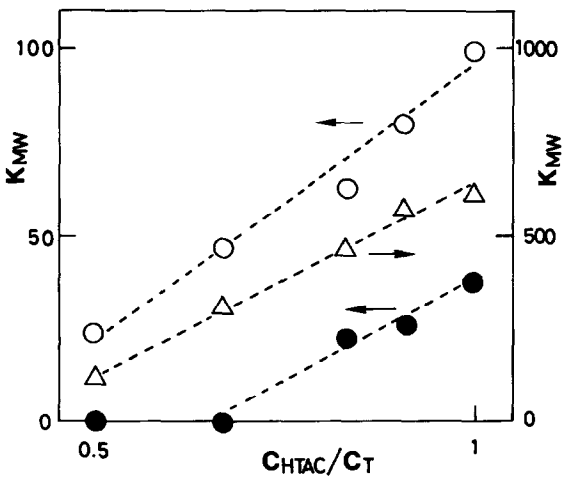

Fig. 7. Changes in K_{MW} values with C_{HTAC}/C_T. ○, NO_3^-; ●, NO_2^-; △, I^-. K_{MW} values were determined with GS-310H as the stationary phase.

total surfactant concentration [C_T, sum of the concentrations of HTAC and POE(23)D]. All mobile phases contained 0.1 M sodium chloride. When C_T is much higher than the CMC, the composition of the surfactants in micelles is the same as that of the surfactants initially mixed in the mobile phases.

According to eqn. 5a, if the changes in the A_{Sp}^{m} values of mixed micelles and in K_{IE}^{m} values are negligibly small, a linear relationship between K_{MW} and σ_0^m exists. K_{MW} values decrease almost linearly with decreasing C_{HTAC}/C_T, as shown by the broken lines in Fig. 7. Partitioning of NO_2^- to mixed micelles cannot be detected when the concentration of POE(23)D increases up to $1/3C_T$.

In conclusion, the usefulness of micelle exclusion chromatography lies in the unique selectivity and the facility for the evaluation of K_{MW} values. In order to employ these features efficiently, K_{SW} values should be kept as low as possible by using a stationary phase of low adsorption ability and adding optimum amounts of salt to the mobile phase. It is believed that micellar chromatography will continue to make significant contributions to micellar chemistry.

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