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Study on how mobile phase buffer composition effects the retention behavior of the system peak in non-suppressed ion chromatography

Masayuki Nishimura^{a,*}, Morimasa Hayashi^a, Atsushi Yamamoto^b,
Tatsuji Horikawa^c, Kazuichi Hayakawa^d, Motoichi Miyazaki^d

^aAnalytical Applications Department, Shinadzu Corporation, 1 Nishinokyo-kuwabara-cho, Nakagyo-ku, Kyoto 604, Japan

^bToyama Institute of Health, 17-1, Nakataikoyama, Kosugi-machi, Toyama 939-03, Japan

^cTechnological University of Nagaoka, 1603-1, Kamitomioka, Nagaoka 940-21, Japan

^dFaculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920, Japan

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Abstract

The effect of mobile phase buffering on the retention behaviour of a system peak in non-suppressed ion chromatography was studied. When a weak base whose pK_a was near to the pH of the mobile phase was added to the mobile phase as a counter ion, the retention of the system peak selectivity shortened while the retention times of the analyte anions did not change. The cause of this phenomenon was thought to be an effect of mobile phase buffering induced by a weak base. Quantitative interpretation was successfully carried out to control the retention time of the system peak. The results allow the separation of analyte anions and control of the retention time of the system peak to be done independently. This method makes it possible to increase the variety of mobile phase compositions in the ion chromatography of anions.

1. Introduction

From the beginning of the history of non-suppressed ion chromatographic analysis of anions [1], an obviously retained vacant peak, the so-called "system peak", has often been observed when an organic acid ion is used as the eluting ion. In a practical sense, the appearance of a system peak has both merits and demerits. The greatest merit is making possible the detection of weakly retained species such as fluoride by narrowing the water dip peak. Trace levels of fluoride detected from a flat baseline by the

high-volume injection method [2] provide a notable example. However, in general, the appearance of a system peak prolongs the interval possible between injections and is thus the greatest demerit in terms of throughput in routine analyses. Therefore, if it is possible to control the retention of the system peak so as to have it elute within an adequate period, high-performance separations can be consistent with shorter analytical periods.

The retention behaviour of the system peak has been widely studied [3–8] and it has been clarified that the hydrophobicity of the undissociated organic acid affects the retention of the system peak. Recently, we succeeded in predict-

* Corresponding author.

ing the retention behaviour of a system peak by assuming a bi-Langmuir sorption isotherm model [5]. With this model, the capacity factor of a system peak (k'_c) had become predictable when the counter ions were a proton and/or a strong base ion.

On the other hand, it has been reported that k'_c selectivity decreased with the use of a weak base as a counter ion when the pK_a of the conjugate acid was near the pH of mobile phase [9], a linear relationship between pK_a and k'_c being observed. However, a quantitative interpretation had not yet been achieved. If the mechanism underlying the retention behaviour is understood quantitatively, it should become possible to control the retention time of a system peak with the added benefit of regaining the usefulness of a great variety of mobile phases which hitherto have been disregarded only by reason of the inadequate retention behaviour of the system peak in each instance.

The aim of this work was to clarify the mobile phase buffer effect on k'_c and to develop a theory which allows k'_c to be predicted under any mobile phase conditions. The control of k'_c in a two-base system, using strong and weak bases, is discussed.

2. Theory

When an n -basic organic acid (H_nA) is dissolved in pure water and the pH is adjusted with a monoacidic base (B) to prepare a mobile phase solution, the following simultaneous equations apply:

$$K_{ai} = \frac{[H^+]_m [H_{n-i}A^i]_m}{[H_{n-i+1}A^{(i-1)}]_m} \quad (i = 1 \text{ to } n)$$

$$K_{aB} = \frac{[H^+]_m [B]_m}{[HB^+]_m}$$

$$[H^+]_m + [HB^+]_m = \sum_{i=0}^n i [H_{n-i}A^i]_m + [OH^-]_m$$

$$[t - H_nA]_m = \sum_{i=0}^n [H_{n-i}A^i]_m$$

$$[t - B]_m = [B]_m + [HB^+]_m$$

where the subscript m represents the bracketed species in the mobile phase, K_{ai} represents the i th dissociation constant of H_nA , K_{aB} represents the K_a of the conjugate acid of B and $[t - H_nA]_m$ and $[t - B]_m$ represent the total concentrations of H_nA and B in the mobile phase, respectively. From the above equations, the concentration of an undissociated organic acid in the mobile phase ($[H_nA]_m$) can be represented by $[H^+]_m$, the K_a values and $[t - H_nA]_m$ as follows:

$$[H_nA]_m = \frac{[H^+]_m^n}{[H^+]_m^n + \sum_{i=1}^n [H^+]_m^{n-i} \prod_{j=1}^i K_{aj}} \cdot [t - H_nA]_m \quad (1)$$

In a system peak zone, $[t - H_nA]_m$ is different from that in the baseline zone. A decrease in $[t - H_nA]_m$ is generally the cause of a decrease in $[H^+]_m$ and $[H_nA]_m$. However, changes in $[H^+]_m$ are suppressed when the solution is strongly buffered, and so the change in $[H_nA]_m$ ($d[H_nA]_m$) becomes small for a given change in $[t - H_nA]_m$ ($d[t - H_nA]_m$).

According to a previous paper [5], the capacity factor of a system peak (k'_c) can be represented as follows:

$$k'_c = \frac{d[H_nA]_m K_d \phi}{\sum_{i=0}^n d[H_{n-i}A^i]_m} = \frac{d[H_nA]_m K_d \phi}{d[t - H_nA]_m} \quad (2)$$

where K_d is the distribution coefficient of the undissociated acid and ϕ represents the phase ratio of the column.

Since K_d is constant while neither the pH of mobile phase nor $[t - H_nA]_m$ changes, k'_c is dependent only on $d[H_nA]_m/d[t - H_nA]_m$. As mentioned above, $d[H_nA]_m/d[t - H_nA]_m$ becomes small when the buffering action in the mobile phase is strong; hence it is obvious that k'_c also becomes small under such conditions. The buffering effect can be changed by selecting a base with a pK_{aB} near to the pH of the mobile phase. Using Eq. 2, it is possible to calculate k'_c if $K_d \phi$ is known. We selected phthalic and salicylic acids as organic acids in the mobile

phase to test the theory in actual analytical systems.

3. Experimental

3.1. Instrumentation and reagents

The ion chromatograph used was an LC-10A (Shimadzu, Kyoto, Japan), which consisted of an LC-10AD pump, a CTO-10A column oven, a CDD-6A electrical conductivity detector, a Rheodyne Model 7125 manual injector and a C-R7A data processor. Shim-pack IC-A1 ($d_p = 10 \mu\text{m}$, $100 \text{ mm} \times 4.6 \text{ mm}$ I.D.) and Shim-pack IC-A3 ($d_p = 5 \mu\text{m}$, $150 \text{ mm} \times 4.6 \text{ mm}$ I.D.) anion-exchange columns designed for ion chromatography (Shimadzu, Kyoto, Japan) were used as analytical columns. The base material of both columns was polymethacrylate resin; the surface of the IC-A3 column is more hydrophobic than that of the IC-A1 column. All reagents were of the highest available purity. Phthalic acid, salicylic acid, tris(hydroxymethyl)aminomethane (Tris), γ -aminobutyric acid (GABA) and other weak bases containing zwitterions (Wako, Osaka, Japan) listed in Table 1 were used as received. Mobile phases were prepared by dissolving the appropriate reagents in purified water from a Toraypure water-purification system (Toray Industries, Tokyo, Japan).

3.2. Calculation of isotherm

The isotherm for phthalic acid on a Shim-pack IC-A1 column was determined using k'_c values measured for several mobile phase conditions which included proton and/or a strong base as counter ions. The experimental results were fitted to the function $[\text{H}_2\text{A}]_m$ according to the method established in Ref. [5].

3.3. Analytical conditions

Phthalic acid systems

Ten kinds of bases, as listed in Table 1, were each added to separation solutions of 2.5 mM phthalic acid to adjust the pH to 4.08 in each

Table 1
 $\text{p}K_{\text{aB}}$ of the bases added to the mobile phase in the phthalic acid system

Base	$\text{p}K_{\text{aB}}$
Glycine	2.35
β -Alanine	3.60
γ -Aminobutyric acid (GABA)	4.20
ϵ -Aminocaproic acid	4.37
Aniline	4.60
Pyridine	5.22
<i>p</i> -Aminophenol	5.82
Bis-Tris ^a	6.55
Imidazole	7.20
Tris(hydroxymethyl)aminomethane	8.08

^a Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane.

case. A Shim-pack IC-A1 column was employed as the analytical column. The flow rate was 1.5 ml/min and all flow lines including the detector cell were maintained at 40°C by the column oven. A 5- μl volume of pure water was injected for each different mobile phase composition and the retention time of the system peak (negative peak) was measured.

Salicylic acid systems

A 5 mM salicylic acid solution was prepared and divided into two equal portions. To one portion was added Tris to adjust the solution pH to 4.17 (solution A). The remaining portion was also adjusted to pH 4.17 by addition of GABA to give solution B. Aliquots of solutions A and B were then combined with stirring in the following ratios (v/v): A:B = 100:0, 87.5:12.5, 75:25, 50:50 and 0:100. Except for the analytical column (Shim-pack IC-A3) and flow-rate (1.2 ml/min), all other conditions were the same as those used for the phthalic acid systems described above.

4. Results and discussion

4.1. Phthalic acid systems

Because the $\text{p}K_{\text{a}_1}$ and $\text{p}K_{\text{a}_2}$ values of phthalic acid, H_2A , are known ($\text{p}K_{\text{a}_1} = 2.9$, $\text{p}K_{\text{a}_2} = 5.2$,

calculated from data given in Ref. [10]), $[H_2A]_m$ can be calculated for any phthalate-buffered mobile phase which includes a weak base B by solving Eq. 2. $K_a\phi$ was calculated using the bi-Langmuir isotherm equation evaluated previously, as follows:

$$\Gamma_{H_2A} = \frac{570[H_2A]_m}{1 + 30700[H_2A]_m} + \frac{14[H_2A]_m}{1 + 680[H_2A]_m} \quad (3)$$

where Γ_{H_2A} represents the total amount of H_2A adsorbed on the column. Fig. 1 represents the relationship between pK_{aB} and $\log k'_e$. The solid line is the calculated interpolation and the closed squares are the experimental results. Good agreement between the calculated and experimental results confirms that k'_e directly reflects the buffering activity of the mobile phase and is predictable. Moreover, the observed phenomenon that the retention time of a system peak showed a minimum value when the mobile phase was buffered to around pH 5.2 [5,6] can be understood with our model, since the mobile phase buffering action became maximum at pH 5.2, i.e., at the pK_{a_1} of phthalic acid.

4.2. Salicylic acid systems

Salicylic acid also has been widely employed as an organic acid in mobile phases. The high hydrophobicity of salicylic acid makes it possible to elute analyte ions at low concentrations. This acid also provides good baseline stability with low background conductivity. Moreover, large peak areas can be obtained even under higher pH (>5) conditions because the acid is monobasic. In a salicylic acid system, however, the retention time of the system peak is usually long so that the injection intervals are also long. Because of this, the use of salicylic acid systems has been limited. The proposed technique should be effective in practice. In addition, prediction of k'_e will become easier in this system rather than the phthalic acid system since salicylic acid is a fairly strong monobasic acid.

A two-base system, using strong and weak bases, was employed with regard to practical usage since it is easier to control the mobile phase buffer activity by changing the concentration of a selected weak base than by attempting to give an adequate base every time.

Using salicylic acid and two bases, a strong

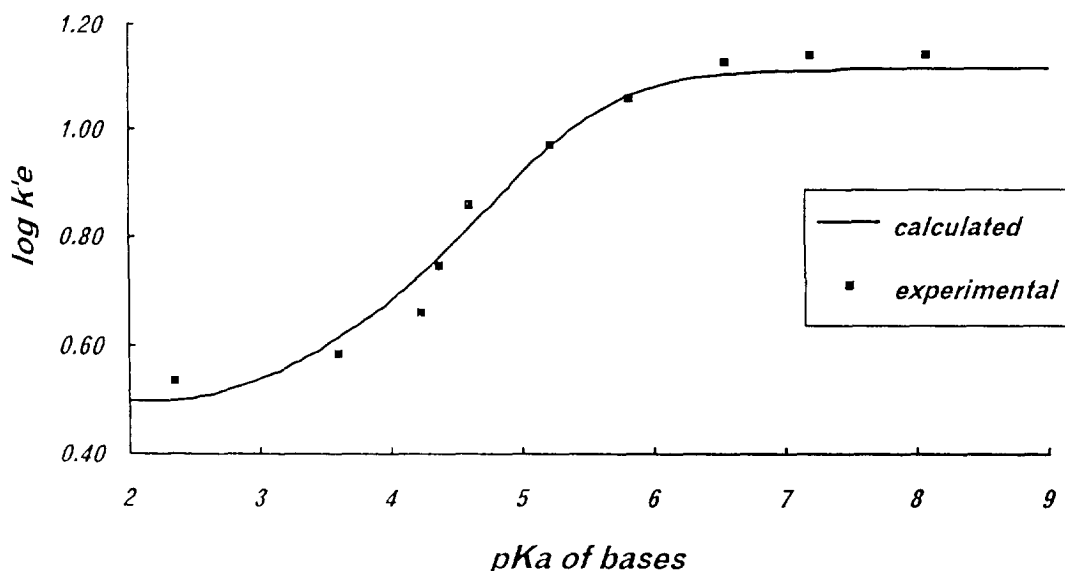


Fig. 1. Relationship between pK_a of a conjugate acid of the added base and the capacity factor of a system peak (k'_e). Solid line, calculated; squares, experimental results.

base, e.g., Tris; B1, and a weak base, e.g., GABA; B2, the equation of charge balance was obtained using known values as follows:

$$[\text{H}^+]_m + [t - \text{B1}]_m + \frac{[\text{H}^+]_m[t - \text{B2}]_m}{K_{a\text{B2}} + [\text{H}^+]_m} = \frac{K_a[t - \text{HA}]_m}{K_a + [\text{H}^+]_m} + \frac{K_w}{[\text{H}^+]_m} \quad (4)$$

where K_w is the ionic product of water, but was neglected because the mobile phase used was acidic. Eq. 4 was rewritten with $[\text{H}^+]_m$ as a cubic equation. However, the term in $[\text{H}^+]_m^3$ was neglected as the $\text{p}K_a$ of salicylic acid (2.98 [11]) is more than ten times larger than $[\text{H}^+]_m$. The equation was therefore solved directly using a quadratic equation. In this study, we calculated $K_d\phi$ for conditions at which the concentration of salicylic acid was known and the pH was adjusted to desired value with only B1. In this case, because $[t - \text{B2}]_m$ was equal to zero, Eq. 4 was rewritten as a quadratic equation for $[\text{H}^+]_m$, and so $[\text{H}^+]_m$ was strictly calculated. Moreover, $d[\text{HA}]_m/d[t - \text{HA}]_m$ could be directly calculated to be 0.830 in this case also. Hence $K_d\phi$ was calculated to be 35.1 with $d[\text{HA}]_m/d[t - \text{HA}]_m$ and the experimental k'_c (29.2) using Eq. 1.

The k'_c value was then predicted for a two-base system. For this purpose, $d[\text{HA}]_m/d[t - \text{HA}]_m$ should be solved strictly. First, a new function, $f([\text{H}^+]_m)$, is introduced as follows:

$$f([\text{H}^+]_m) = \frac{[\text{H}^+]_m}{[\text{H}^+]_m + K_a}$$

Then $[\text{HA}]_m$ is expressed as follows:

$$[\text{HA}]_m = f([\text{H}^+]_m)[t - \text{HA}]_m$$

with this technique, $d[\text{HA}]_m/d[t - \text{HA}]_m$ can be calculated directly as follows:

$$\frac{d[\text{HA}]_m}{d[t - \text{HA}]_m} = \frac{df([\text{H}^+]_m)}{d[\text{H}^+]_m} \cdot \frac{d[\text{H}^+]_m}{d[t - \text{HA}]_m} \cdot [t - \text{HA}]_m + f([\text{H}^+]_m) \quad (5)$$

in Eq. 5, $d[\text{H}^+]_m/d[t - \text{HA}]_m$ is easily calculated because $[\text{H}^+]_m$ can be represented as a function of $[t - \text{HA}]_m$. With Eq. 5 and the previously obtained $K_d\phi$ value, k'_c for any ratio of B1 and B2 was calculated.

Fig. 2 shows the relationship between the concentration of GABA and k'_c . It is obvious that calculated k'_c (solid line) agreed with the

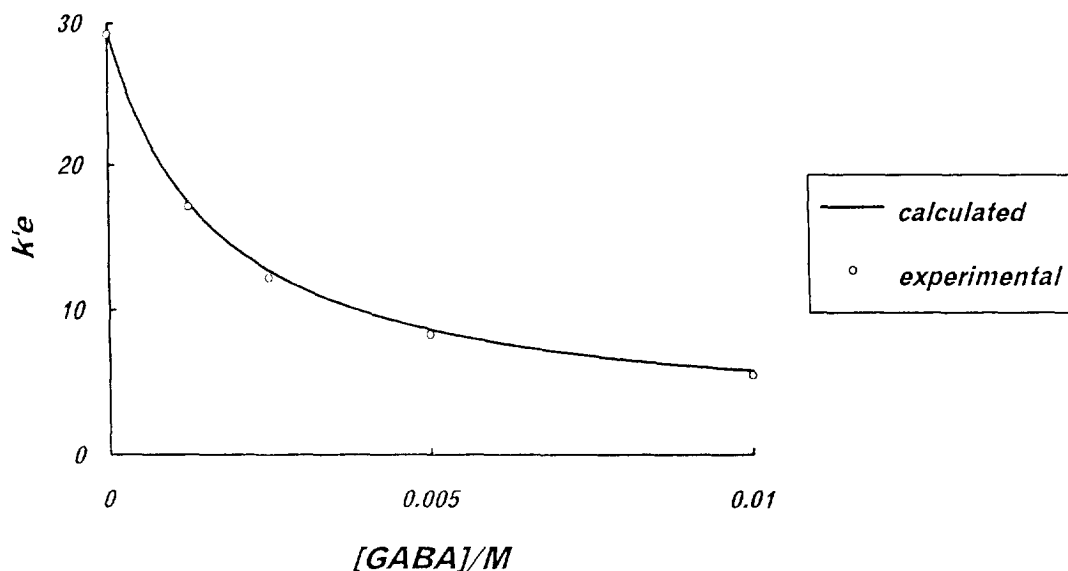


Fig. 2. Relationship between the concentration of GABA and k'_c .

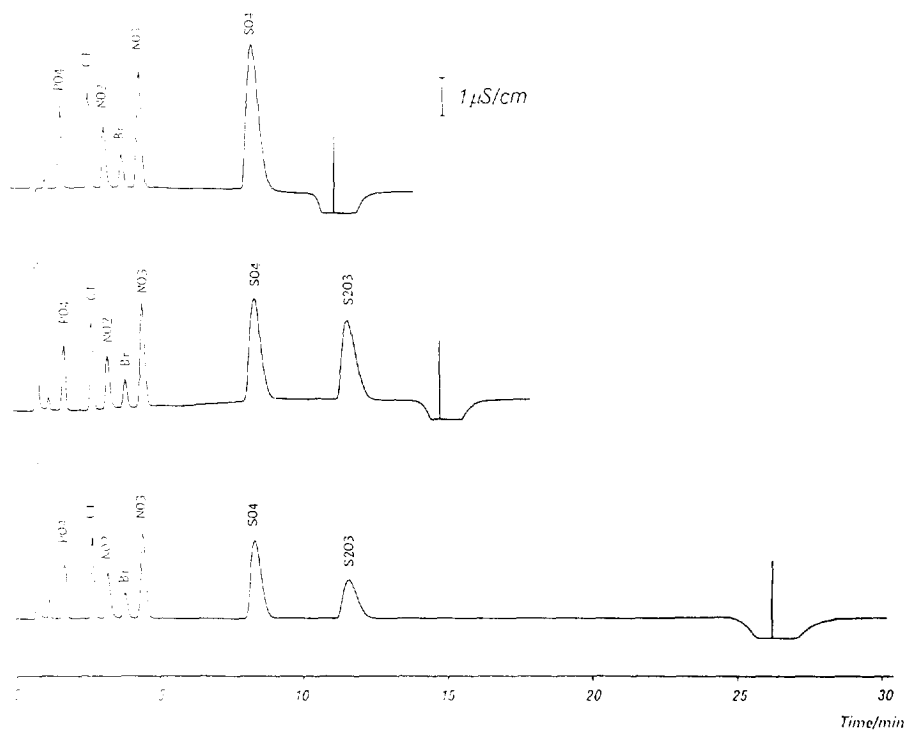


Fig. 3. Comparison of chromatograms obtained under the following conditions: mobile phase, 2 mM salicylic acid (pH 4.0) containing Tris and/or GABA (see text); column, Shim-pack IC-A3; flow-rate, 1.5 ml/min; temperature, 40°C; detection, conductivity; injection volume, 10 μ l. The injected samples contained the following anions: PO_4^{3-} 50, Cl^- 10, NO_2^- 12, Br^- 10, NO_3^- 30, SO_4^{2-} 40 and $\text{S}_2\text{O}_3^{2-}$ 50 mg/l.

experimental k'_c (open circles) very well over the range between 0 and 10 mM of GABA added. In this case, it was possible to control k'_c freely from 5 to 30.

Quantitative interpretation of the last aspect of the retention behaviour of a system peak was successfully performed. Especially the use of the salicylic acid system led to very easy calculations and extended the controllable range of k'_c . Using these results, optimization of the separation of analyte ions and control of k'_c were investigated independently. Fig. 3 presents typical examples of the utility of the present method. The three chromatograms were obtained with mobile phases in which 2 mM salicylic acid was included and the pH values were adjusted to 4.0 with Tris and/or GABA. In the bottom chromatogram, 1.6 mM Tris was added to adjust the pH of the mobile phase. Under the conditions applied, k'_c was as large as 28.1. The top chro-

matogram was obtained with a mobile phase in which 3.0 mM GABA was employed as the only base. k'_c was so small that the determination of thiosulfate ($\text{S}_2\text{O}_3^{2-}$) was impossible owing to interference from the system peak. In the middle chromatogram, 0.8 mM Tris and 1.5 mM GABA were added to the mobile phase and k'_c was adequate under these conditions. On the chromatograms, the predicted retention times of the system peaks are masked with solid vertical lines. These results indicated that an improvement in ease of use and an extension of the limits of ion chromatography can be realized with the present method.

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