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THEORETICAL CONSIDERATIONS ON THE APPEARANCE OF SAMPLE AND SYSTEM PEAKS IN ION CHROMATOGRAPHY WITH PHOTOMETRIC DETECTION

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SUMMARY

A general model is proposed for interpreting the appearance of sample and system peaks in ion-exchange chromatography with photometric detection. In this theory, a changed fraction of organic acid in the mobile phase resulting from the ion-exchange process with a sample ion migrates through the column from plate to plate in accordance with the ionic and partition equilibria. This perturbation of UV-detectable components yields the sample and system peaks. Simulation of this theory by computer accounts well for the sample peaks observed in real ion chromatography. An equation expressing the sample response–capacity factor relationship at low mobile phase pH was derived.

INTRODUCTION

In the determination of inorganic and organic anions by high-performance liquid chromatography with photometric detection, both reversed-phase and ion-exchange columns have been used. It was observed in the reversed-phase mode with a light-absorbing modifier in a mobile phase that a "system peak" appeared and that the direction of the sample peak changed in relation to it. The closer the sample peak eluted to the system peak, the larger the peak area became. It is generally considered that a system peak appears in liquid chromatography when the mobile phase contains more than one component[1–8]. Recently, Schill and co-workers[9–12] derived a theoretical equation for the interpretation on this phenomenon.

In the ion-exchange mode, in which a light-absorbing organic acid solution at neutral pH was usually used, a system peak was not observed and the direction of the sample peak was always negative[13]. A system peak appeared on decreasing the pH of the mobile phase. When an organic acid solution at acidic pH was used as a mobile phase on a column packed with a hydrophilic material-based anion exchanger, the direction of the sample peak was positive prior to the system peak and negative after it.

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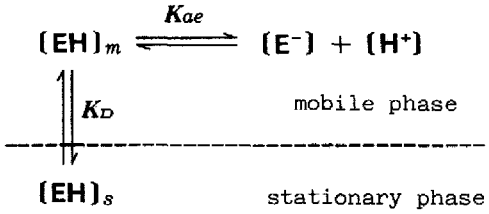
These results were similar to those in the reversed-phase mode as described above. However, neither the formation of sample and system peaks nor the direction and area of the sample peak have been studied theoretically in ion-exchange chromatography with photometric detection, especially at low pH. In this paper, we proposed a general model for the formation of sample and system peaks and derive a theoretical equation for the response of a sample peak with a mobile phase low pH. The present theory explains well the phenomenon described above.

THEORY

In a column packed with hydrophilic material-based ion exchanger, there is a small interaction between an organic acid in the mobile phase and the unfunctionalized region of the packing material[1]. Only the undissociated form has been thought to be adsorbed. The following equilibrium is assumed to hold for mobile phase organic acid in the column: where [EH] and [E^-] are the undissociated and dissociated forms of the mobile phase organic acid, respectively, K_{ae} is its acid dissociation constant, K_D is the ratio of the fractions of the undissociated acid in the two phases and the subscripts m and s represent existence in the mobile and the stationary phase, respectively.



It is assumed that the hydrophilic sample ions are retained on the separation column only by an ion-exchange process. The concentration of sample ion in the mobile phase, [S^-], is deduced from

$$K_{as} = [S^-][H^+]/[SH]$$

$$[ST] = [S^-] + [SH]$$

Therefore,

$$[S^-] = K_{as}[ST]/(K_{as} + [H^+]) \quad (1)$$

where K_{as} is the acid dissociation constant of the sample and [ST] and [SH] are the concentrations of total and undissociated samples in the mobile phase, respectively.

Similarly, [E^-] is deduced from

$$[ET] = [E^-] + [EH]_s + [EH]_m$$

$$K_{ae} = [E^-][H^+]/[EH]_m$$

$$K_D = [EH]_s/[EH]_m$$

Therefore,

$$[E^-] = K_{ae}[ET]/\{K_{ae} + (K_D + 1)[H^+]\} \quad (2)$$

where [ET] is the concentration of total organic acid. From the charge balance, the following equation holds:

$$[H^+] + [Na^+] = [E^-] + [S^-] + [OH^-]$$

where $[Na^+]$ is the concentration of sodium ion as a counter ion. Substitution of eqns. 1 and 2 into this equation gives

$$[H^+] = K_{ae}[ET]/\{K_{ae} + (K_D + 1)[H^+]\} + K_{as}[ST]/(K_{as} + [H^+]) + \\ + K_w/[H^+] - [Na^+] \quad (3)$$

where K_w is ionic product of water. The equilibrium proceeds in all the theoretical plates in the column to satisfy eqn. 3 accompanied by a difference in the concentrations of the sample and mobile phase acid.

The system peak observed in ion chromatography (IC) with an acidic mobile phase is considered to appear as a result of the elution of adsorbed mobile phase acid from the column[1,2]. Its capacity factor (k'_e) is the ratio of the changed fractions between the undissociated acid adsorbed on the stationary phase and the total acid in the mobile phase:

$$k'_e = [dEH]_s/([dEH]_m + [dE^-]) \quad (4)$$

When the sodium ion is free in the mobile phase, as $[E^-] = [H^+]$, eqn. 4 can be rewritten as

$$k'_e = 2[E^-]K_D/(2[E^-] + K_{ae}) \quad (5)$$

Eqn. 5 indicates that k'_e is constant despite the difference in sample species and the K_D value can be determined experimentally.

By applying this constant k'_e, the behaviours of the sample and mobile phase acid in the column under conditions with free sodium ions are calculated based on a Craig-type repetitive distribution[14]. The ideal transfer of sample through a Craig-type repetitive distribution instrument can be described by

$$[ST]^{n,p} = [S]_m^{n,p} + [S]_s^{n,p} = [S]_m^{n-1,p-1} + [S]_s^{n-1,p}$$

where $[S]_m^{n,p}$ is the fraction of sample in the mobile phase that is in contact with plate p when the nth plate has been equilibrated. Similarly, $[S]_s^{n,p}$ is the fraction of sample in the stationary phase and $[ST]^{n,p}$ is the sum of both fractions. The following relationship holds between k'_s and the distribution equilibrium of the sample:

$$k'_s = [S]_s^{n,p}/[S]_m^{n,p}$$

By using this equation, a general equation is deduced:

$$[ST]^{n,p} = (n - 1)!\, k_s^{n-p}/(p - 1)!\,(n - p)!\,(1 + k_s')^{n-1}$$

The organic acid in the mobile phase is substituted quantitatively by the sample ion according to a mechanism of one-to-one ion exchange. Similarly, by using $[dE]_m^{n,p}$ and $[dE]_s^{n,p}$, the fractions of the excess or deficiency of organic acid in the mobile phase and in the stationary phase, respectively, $[dET]^{n,p}$, the sum of both fractions, can be described as follows:

$$[dET]^{n,p} = [S]_s^{n,p} - [S]_s^{n-1,p} + [dE]_m^{n-1,p-1} + [dE]_s^{n-1,p}$$

where $[dE]_s = [dEH]_s$ and $[dE]_m = [dEH]_m + [dE^-]$. By using eqn. 4, a general equation is deduced:

$$[dET]^{n,p} = (n - 1)!\, k_e'^{n-p} k_s'/(p - 1)!\,(n - p)!\,(1 + k_e')^{n-1}\,(k_s' - k_e') -$$

$$(n - 1)!\,(1 + k_e')\,k_s'^{n-p+1}/(p - 1)!\,(n - p)!\,(1 + k_s')^n\,(k_s' - k_e')$$

Now, consider the quantitative ratio between the sample and the changed organic acid in the mobile phase:

$$[dE]_m^{n,p}/[S]_m^{n,p} = \{k_e'^{n-p}(1 + k_s')^n/k_s'^{n-p}(1 + k_e')^n - 1\}k_s'/(k_s' - k_e')$$

$[S]_m^{n,p}$ has its maximum at $n = p(1 + k_s')$:

$$[dE]/[S]_{max} = \{k_e'^{pk_s'}(1 + k_s')^{p(1+k_s')}/k_s'^{pk_s'}(1 + k_e')^{p(1+k_s')} - 1\}k_s'/(k_s' - k_e')$$

In a real system, a large value for p is required for the separation of the peaks:

$$\lim_{p \to \infty} [dE]/[S]_{max} = k_s'/(k_e' - k_s') \qquad (k_e' \neq k_s') \tag{6}$$

The concentration of the mobile phase organic acid in the sample zone can be calculated by eqn. 6. If the sample amounts are equal, the relative peak area (A_s/A_t) of a given sample (S) to the standard sample (T) is expressed by the following equation:

$$A_s/A_t = k_s'(k_e' - k_t')/k_t'(k_e' - k_s') \tag{7}$$

EXPERIMENTAL

The ion chromatographic system included a Shimadzu (Kyoto, Japan) LC-6A pump, a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector, a Shimadzu SPD-2A UV detector and a Shimadzu C-R3A Chromatopac calculator. The anion-exchange columns used were a Tosoh (Tokyo, Japan) TSK gel IC-Anion-PW (5 cm × 4.6 mm I.D., polyacrylate based, particle size 10 μm), a Tosoh TSK gel IC-Anion-SW (5 cm × 4.6 mm I.D., silica, 5 μm) and a Shimadzu Shim-pack IC-A1 (10 cm × 4.6 mm I.D.,

polyacrylate, 12.5 μm). Mobile phases were prepared by dissolving salicylic acid, *o*-nitrobenzoic acid (ONB), phthalic acid and sulphanilic acid individually in distilled, deionized water. Sodium hydroxide solution was used for pH adjustment, if necessary.

The elution behaviour of samples in ion chromatography (IC) was confirmed by the analysis of samples in eluate fractions. Acetic and formic acids were determined by gas chromatography–mass spectrometry (GC–MS) and phosphoric acid was determined by the method of Baba *et al.*[15]. GC–MS was performed with a Shimadzu QP-1000 gas chromatograph–mass spectrometer. The operating conditions were as follows: column, Gasukuro Kogyo (Tokyo, Japan) Gaskuropack 54 (1.6 m × 3 mm I.D.); column temperature, 170°C; carrier gas, Helium at a flow-rate of 30 ml/min; separator and source temperature, 250°C; and ionization energy, 70 eV. The UV spectra were measured with a Hitachi (Tokyo, Japan) UV-2000 spectrophotometer.

Adsorption isotherms were prepared using the unfunctionalized IC-Anion-PW packing material (30–60 μm) provided by Tosoh, to which the bulk solutions were added. After equilibration for 6 h at 25 ± 1°C, the decrease in concentration was measured.

Calculations were made on an NEC (Tokyo, Japan) PC-9801 UX personal computer.

RESULTS AND DISCUSSION

In the stoichiometric study of chromatographic processes, photometric detection has the great advantage over conductivity detection that the absorbance difference between the mobile phase and sample is constant when samples are not light absorbing, whereas the conductance difference is affected by changes in the sample species. A sample response model for photometric detection with a mobile phase of neutral pH has been proposed[13,16], but it is inapplicable at other pH values. Fig. 1 shows a typical chromatogram of five organic acids by using acidic sulphanilic acid solution as the mobile phase. Two characteristic features were observed. The first was that sample zones of acetic and laevulinic acids eluting prior to the system peak formed

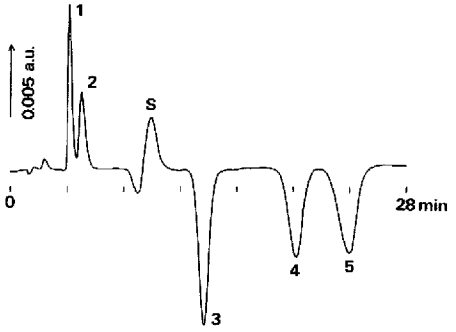


Fig. 1. Ion chromatogram for a system with an organic acid solution at low pH as the mobile phase. Column, TSK gel IC-Anion-PW; mobile phase, 0.5 m*M* sulphanilic acid (pH 3.5); flow-rate, 0.8 ml/min; column temperature, 40°C; wavelength of UV detection, 271 nm; injection volume, 10 μl. Peaks: 1 = acetate (0.7 m*M*); 2 = laevulinate (0.25 m*M*); S = system peak; 3 = lactate (0.25 m*M*); 4 = formate (0.25 m*M*); 5 = succinate (0.25 m*M*).

positive peaks, whereas sample zones of lactic, formic and succinic acids eluting after the system peak gave negative peaks, despite the formation of negative peaks with all of these samples using a sulphanilic acid mobile phase of neutral pH. A similar phenomenon was observed on combination of other anion-exchange columns with hydrophilic packing materials and other organic acids tested as mobile phases in the work. The second feature was that the area of the sample zone increased as its elution time became closer to that of the system peak.

In order to detect the elution of samples directly, GC–MS and spectrophotometry were used. Fig. 2 shows that the total amount of a sample injected is restricted to the corresponding positive peak. As these sample ions show no absorption at the detection wavelength of 310 nm, the positive peaks indicate that sample ions were eluted from the column accompanied by light-absorbing mobile phase organic acid. In this instance, the system peak became negative to compensate for the deficit of the mobile phase organic acid coeluted with sample ions.

At acidic pH, both dissociated and undissociated forms of the mobile phase organic acid coexist. Fig. 3 shows adsorption isotherms for organic acids used as mobile phases on the unfunctionalized IC-Anion-PW packing material of the separation column. In contrast to non-adsorption of the dissociated acids in the presence of high bulk solution concentrations of sodium chloride[17], neutral acids were substantially adsorbed according to the Langmuir model. As these adsorption isotherms might be considered to be straight lines below the mobile phase concentration and were not affected by the coexistence of samples, Scheme I has been found to hold and K_D can be considered to be constant.

The behaviour of sample ions in an ion-exchange column can be considered on the basis of the theory of Gjerde *et al.*[18]. If the sample ions are retained on the column only by an ion-exchange process, there is a constant relationship between the concentration of the eluting solution and the capacity factor of the sample. Plots of the logarithm of salicylate ion concentration in the mobile phase *vs.* log k'_s for four anions

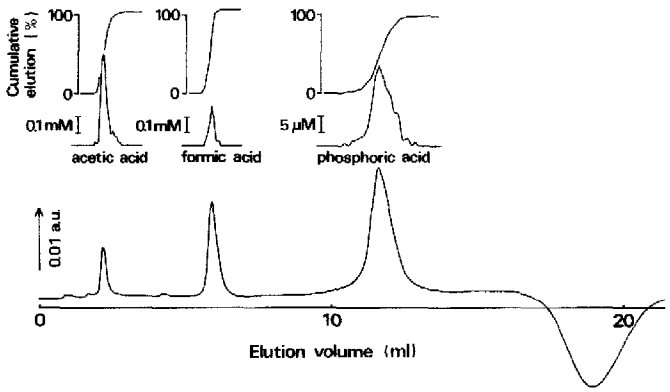


Fig. 2. Verification of the correspondence of sample elutions (upper) to the positive peaks in the ion chromatogram (lower). Sample elutions were analysed by GC–MS (acetic and formic acids) and by spectrophotometry using a molybdenum reagent (phosphoric acid). Mobile phase, 0.5 m*M* salicylic acid; column temperature, ambient; wavelength of UV detection, 310 nm. Samples: acetic acid (6 m*M*), formic acid (2 m*M*) and phosphoric acid (2 m*M*). Other conditions as in Fig. 1.

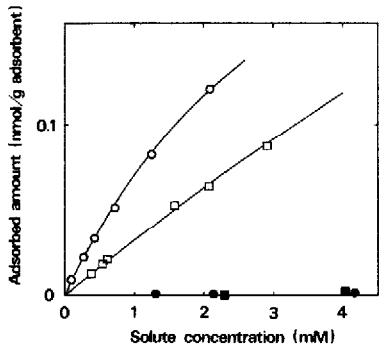


Fig. 3. Adsorption isotherms for mobile phase organic acids on unfunctionalized IC-Anion-PW packing material. Conditions: salicylic acid at pH < 0.7 (○) and at pH > 7 and $Na^+ = 0.2\ M$ (●); phthalic acid at pH < 0.7 (□) and at pH > 7 and $Na^+ = 0.2\ M$ (■).

on IC-Anion-PW are shown in Fig. 4. As these sample ions and salicylate act as monovalent anions, the slopes of these lines must be -1, a deviation from -1 indicating that the ratio of the retention on the column by the ion-exchange process to that by adsorption is decreased. It is clear from the results of adsorption experiments that chloride is not adsorbed on the packing material at all but other sample acids are adsorbed slightly. Consequently, sample ions that follow the theory of Gjerde *et al.* result in chromatograms compatible with our theory.

The simulation of a chromatogram was performed on the basis of a Craig-type repetitive distribution[14] to satisfy eqn. 3 in the column and by considering the acid equilibrium with the delocalized sample in the column effluent[19]. Fig. 5 shows the results of the simulation for 1 mM salicylic acid as the mobile phase. The lower chromatogram was simulated by the K_D of salicylic acid, which was determined from eqn. 4 (upper chromatogram). The two chromatograms are in good agreement.

The simulations made by altering the pH of the mobile phase were performed by

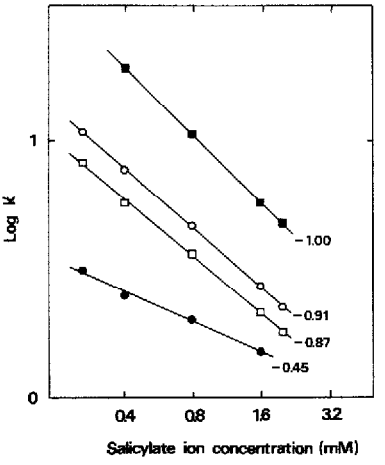


Fig. 4. Log (ionic concentration of salicylic acid) *vs.* log (capacity factor) at pH 3.6 on IC-Anion-PW. ■ = Chloride; ○ = formate; □ = lactate; ● = acetate. The numbers on the lines represent the slopes.

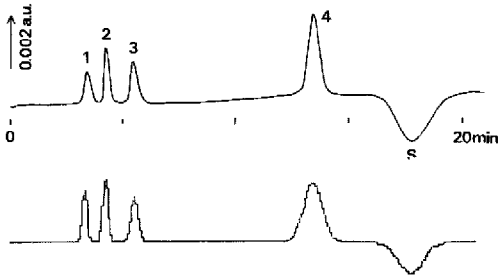


Fig. 5. Comparison between actual (upper) and computer-simulated (lower) chromatograms. Conditions for the actual chromatogram: mobile phase, 1 m*M* salicylic acid; wavelength of UV detection, 319 nm; other conditions as in Fig. 1. Peaks: 1 = lactate (0.5 m*M*); 2 = formate (0.5 m*M*); 3 = pyroglutamate (0.5 m*M*); 4 = chloride (0.2 m*M*); S = system peak. The lower trace represents the elution of mobile phase simulated by using $K_D = 31.0$ and $K_w = 10^{-14}$ in eqn. 3.

use of this K_D value. In this instance, the pK_a values of organic acids were assumed not to be affected, as the local variation of the ionic strength is small. For chloride and lactate, a comparison of the peak areas in the actual and simulated chromatograms is shown in Fig. 6. The solid lines represent the calculated peak areas and the open and closed circles are the actual peak areas of chloride and lactate, respectively. The ordinate indicates the relative peak areas in relation to that of chloride in a mobile phase of neutral pH. The results show clearly that their peaks are positive at low pH (less than *ca.* 3.5), that they reverse with increase in pH and finally converge under standardized conditions[13,16].

In a divalent mobile phase, the charge balance is indicated by

$$[H^+] + [Na^+] = [EH^-] + 2[E^{2-}] + [S^-] + [OH^-]$$

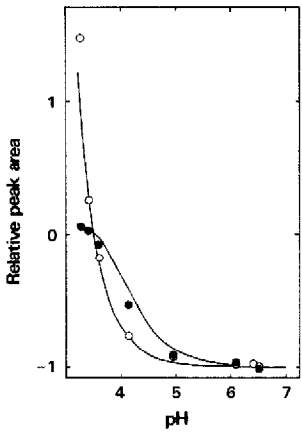


Fig. 6. Comparison of relative peak areas simulated by eqn. 3 (solid lines) with actual values for (○) chloride and (●) lactate with 1 m*M* salicylate as the mobile phase on IC-Anion-PW.

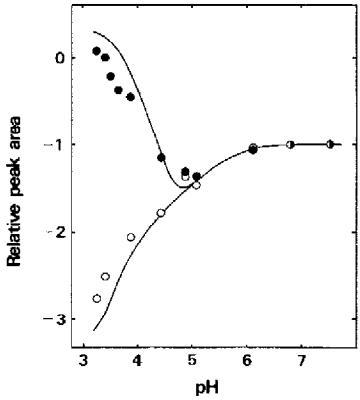


Fig. 7. Comparison of relative peak areas simulated by eqn. 8 with actual values with 1 m*M* phthalate as the mobile phase on IC-Anion-PW. Symbols as in Fig. 6.

Therefore,

$$[H^+] = (K_{ae1}[ET]\,[H^+] + 2K_{ae1}K_{ae2}[ET])/\{K_{ae1}K_{ae2} + K_{ae1}[H^+] + + (K_D + 1)\,[H^+]^2\} + K_{as}[ST]/(K_{as} + [H^+]) + K_w/[H^+] - [Na^+] \quad (8)$$

where K_{ae1} and K_{ae2} represent the first and second acid dissociation constants, respectively, of the mobile phase.

Similarly, the K_D value in 1 m*M* phthalic acid as the mobile phase was determined and simulation of the chromatogram based on eqn. 8 was carried out. The result obtained are illustrated in Fig. 7. Chloride gives a negative peak at all pH values and lactate shows an inversion of peak shape as the pH increases. Their behaviour was elucidated well in this simulation. The discrepancies below pH 4 suggest that the fraction of divalent phthalate in the stationary phase is larger than the calculated value, and this will be investigated in the future.

TABLE I

COMPARISON OF EXPERIMENTAL, COMPUTER-SIMULATED AND CALCULATED SAMPLE PEAK AREAS WITH 1 m*M* ONB AS MOBILE PHASE ON IC-ANION-PW

Sample	*k'*	*Relative peak area (chloride = −1)*		
		Experimental	*Simulated by eqn. 3*	*Calculated by eqn. 7*
Acetate	1.61	0.04	0.15	0.15
Lactate	3.54	0.32	0.37	0.43
Formate	5.60	0.87	0.92	1.02
Pyroglutamate	7.29	2.02	1.85	2.26
System peak	9.71	—	—	—
Phosphate	15.6	−1.32	−1.60	−1.99
Bromate	33.7	−1.03	−1.03	−1.05
Chloride	39.0	−1	−1	−1

In a mobile phase with free sodium ions, the relative peak area is calculated by eqn. 7. The comparison of the experimental results with simulated and calculated values is shown in Table I. The calculated results are in agreement with the experimental values in a similar manner to the simulated values, although small deviations are observed. Consequently no troublesome simulations are required under these conditions.

Eqn. 6 most closely resembles the response–capacity factor relationship of Schill and co-workers[9–11] in reversed-phase liquid chromatography, despite the difference in retention mechanisms. It seems that similar interactions between the sample and components of the mobile phase occur in the column.

The results indicate that new acid and partition equilibria arised in the column between the sample and the mobile phase organic acid if the sample is retained and eluted only by an ion-exchange process in IC. This perturbation of the light-absorbing mobile phase organic acid gives the sample and system peaks.

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