

## Chromatographic Simulation of the Effect of a Polyvalent Counter Ion on Retention Behavior and Peak Intensity of Sulfate

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The effect of a mobile phase polyvalent counter ion on the retention behavior and peak intensity of sulfate in non-suppressed ion chromatography was quantitatively studied using a chromatogram simulation method. In this study, both indirect photometric detection (IPD) method and conductometric detection (CD) method were evaluated. With the IPD method, the retention of sulfate was observed to decrease in the presence of the polyvalent counter ion (Trien) without any change in peak intensity. With the CD method, however, peak intensity of sulfate decreased and, under some conditions, gave a negative peak in simulation as was also observed in actual experiments. The present work demonstrates the possibility of predicting the effect of a polyvalent counter ion on sulfate retention without resorting to physical experiments.

**Keywords** Ion chromatography, polyvalent cation, retention behavior, simulation, sulfate, counter ion

There are many kind of anions which can be determined with anion chromatography. Among them, fluoride, chloride, nitrate and sulfate are determined very often. Generally, sulfate divalent anion interacts with an anion exchanger more strongly than the monovalent anions mentioned. The analysis time for the simultaneous determination of all of these species is determined by the retention time of sulfate. Moreover, when concentration of a competing anion is decreased to achieve good separation of monovalent anions, the retention time of sulfate is extremely increased, which increases the analysis time. Therefore, it is very important to find appropriate analytical conditions which allow analysis to be as short as possible while maintaining good resolution of all peaks of interest.

Recently, we found that the addition of a polyvalent cation such as triethylenetetramine (Trien) into the eluant could selectively shorten the retention times of di- and polyvalent anions. The complexation of a polyvalent cation and a di- or polyvalent anion appeared to account for the phenomenon as discussed in a previous paper.<sup>1</sup> In that work, the stability constants of divalent sulfate-trivalent Trien and divalent sulfate-tetravalent Trien were estimated to be 936 and 4213 (40°C), respectively, from the retention behavior of sulfate as obtained experimentally.

We have already investigated a chromatographic

simulation method based on plate theory.<sup>2,3</sup> With this method, the retention time and peak area of an analyte ion with both indirect photometric detection (IPD) method and conductometric detection (CD) method can be estimated.

In the present study, we applied the simulation using our complexation model for predicting the retention time and peak area of sulfate with both IPD and CD methods. The simulated retention times and peak areas were close to the experimental results and appear to validate our complexation model.

### Theory

To formulate the simultaneous equations of equilibrium for our model, we made these assumptions.

- 1) Tri- or tetravalent Trien and sulfate form one-to-one complexes.
- 2) No species interact with the stationary phase except sulfate and the competing ion (*p*-toluenesulfonate; *p*-TSA).
- 3) The partition factor of sulfate ( $k'_s$ ) is a constant, because the kind of the eluting ion and its concentration does not change.
- 4) The acid-base reaction of the complexes is negligible.

Using the above assumptions, the following equations of equilibrium were formed.

The total amount of sulfate per theoretical plate ( $C_{Ts}$ )

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is defined as

$$C_{TS} = C_{SM} + C_{STM3} + C_{STM4} + C_{SS}\phi \quad (1)$$

where  $C_{SM}$ ,  $C_{STM3}$  and  $C_{STM4}$  represent concentrations of sulfate, trivalent Trien-sulfate complex and tetravalent Trien-sulfate complex, respectively, in the mobile phase.  $C_{SS}$  represents the concentration of sulfate in the stationary phase and  $\phi$  is the phase ratio. Since both tri- and tetravalent Trien-sulfate complexes as well as Trien are all cations at pH 4.2, these exist only in the mobile phase.

The total amount of Trien ( $C_{TT}$ ) can thus be expressed as

$$C_{TT} = C_{TM3} + C_{TM4} + C_{STM3} + C_{STM4} \quad (2)$$

where,  $C_{TM3}$  and  $C_{TM4}$  respectively represent the concentration of tri- and tetravalent Trien in the mobile phase. Also, the relationship between  $C_{TM3}$  and  $C_{TM4}$  can be described as

$$C_{TM3} = (K_{a1}/C_{HM})C_{TM4} \quad (3)$$

where  $C_{HM}$  and  $K_{a1}$  represent the concentration of proton in the mobile phase and the dissociation constant of tetravalent Trien, respectively.

$k'_s$  is expressed by the following formula;

$$k'_s = C_{SS}\phi / C_{SM} (= \text{constant}) \quad (4)$$

The stability constants of trivalent Trien-sulfate complex ( $K_{23}$ ) and tetravalent Trien-sulfate complex ( $K_{24}$ ) are described as

$$K_{23} = C_{STM3} / (C_{SM}C_{TM3}) \quad (5)$$

and

$$K_{24} = C_{STM4} / (C_{SM}C_{TM4}) \quad (6)$$

Finally, the charge balance of the mobile phase is described as

$$2C_{SM} + C_{EM} = 3C_{TM3} + 4C_{TM4} + C_{STM3} + 2C_{STM4} + C_{HM} + C_{BM} \quad (7)$$

where,  $C_{EM}$  and  $C_{BM}$  are the concentration of *p*-TSA and a monovalent counter ion, respectively, in the mobile phase. According to assumptions 1), 2) and 4),  $C_{HM}$  and  $C_{BM}$  are considered to be constant.

Rearranging for  $C_{TM3}$ ,  $C_{TM4}$ ,  $C_{STM3}$  and  $C_{STM4}$  gives

$$C_{TM3} = K_{a1}C_{TT}/A \quad (8)$$

$$C_{TM4} = C_{HM}C_{TT}/A \quad (9)$$

$$C_{STM3} = K_{a1}K_{23}C_{TT}C_{SM}/A \quad (10)$$

$$C_{STM4} = C_{HM}K_{24}C_{TT}C_{SM}/A \quad (11)$$

where  $A = K_{a1} + C_{HM} + (K_{a1}K_{23} + C_{HM}K_{24})C_{SM}$ .

Substitution in Eq. (1) with Eqs. (4), (10) and (11) gives

$$C_{TS} = C_{SM} + (K_{a1}K_{23} + C_{HM}K_{24})C_{TT}C_{SM}/A + k'_sC_{SM} \quad (12)$$

Rearranging for  $C_{SM}$ , we get

$$C_{SM} = \{ (1 + k'_s)(K_{a1} + C_{HM}) + (K_{a1}K_{23} + C_{HM}K_{24}) \times (C_{TT} - C_{TS}) \} / B + [ \{ (1 + k'_s)(K_{a1} + C_{HM}) + (K_{a1}K_{23} + C_{HM}K_{24})(C_{TT} - C_{TS}) \}^2 + 2B(K_{a1} + C_{HM})C_{TS} ]^{1/2} / B \quad (13)$$

where  $B = 2(1 + k'_s)(K_{a1}K_{23} + C_{HM}K_{24})$ .

Substitution in Eq. (7) with Eqs. (8) to (11) and (13) and rearranging for  $C_{EM}$  gives

$$C_{EM} = \{ 3K_{a1} + 4C_{HM} + (K_{a1}K_{23} + 2C_{HM}K_{24})C_{SM} \} C_{TT}/A + C_{HM} + C_{BM} - 2C_{SM} \quad (14)$$

From Eq. (14),  $C_{EM}$  can be calculated for any given plate and any time. In this model, since a strong monobasic acid ion (*p*-TSA) is used as the competing ion, a chromatogram with the IPD method directly represents the change in  $C_{EM}$ . Thus, plotting  $C_{EM}$  versus time can simulate a chromatogram.

Using CD, the equivalent conductivity values and concentrations of all ionic species in the mobile phase should be considered since the conductivity of a solution is equal to the sum of the conductivities of all ions present. The concentrations of each ion in Eq. (7) were calculated from Eqs. (8)–(11). The equivalent conductivity values were taken from reference 4. Some unknown values were experimentally determined by conductometric measurements.

## Experimental

### Instrumentation and reagents

The ion chromatograph used was an LC-10A ion chromatograph (Shimadzu, Kyoto, Japan), which consisted of an LC-10AD pump, an SIL-10A auto injector (50  $\mu$ l loop), a CTO-10A column oven, a CDD-6A conductivity detector, an SPD-10A UV-VIS detector, an SCL-10A system controller and a C-R7A data processor. The analytical column was a Shim-pack IC-A3 (4.6 mm i.d.  $\times$  150 mmL; Shimadzu). Pure water was prepared with a Toraypure Apparatus (Toray Industries, Tokyo, Japan). All reagents used were the same as those described in the previous paper.<sup>1</sup>

### Preparation of mobile phase and analytical conditions

A 3 mM aqueous solution of *p*-TSA was prepared by dissolving a weighed amount of the substance into pure water. Two aliquots were taken; one was adjusted to pH 4.2 with only a monoacidic base, *i.e.*, tris(hydroxymethyl)aminomethane (eluant 1), and the other one to the same pH value with 0.9 mM Trien (eluant 2). The wavelength of the UV-VIS detector was set at 250 nm. Other analytical conditions were the same as given previously.<sup>1</sup>

### Simulation of chromatogram

The personal computer used was a DESKPRO 4/66i (Compaq Computer Corporation, USA). The equation solving functions Excel V5.0 (Microsoft Corporation, USA) were applied without the need for additional programming. All calculation results were saved on the hard disk and were later recalled by Excel's graphics utility to plot the simulated chromatograms.

The simulations were performed under the same conditions described in *Experimental* and the simulated chromatograms were arbitrarily obtained for the 64th theoretical plate in order to reduce the number of iterations (*i.e.*, calculation time) to a reasonable minimum.

## Results and Discussion

The experimental chromatograms using IPD are shown in Fig. 1. The left chromatogram is the result with eluant 1 and the right one is the result with eluant 2. It is clear in the right chromatogram that the retention time of sulfate selectively shortened without affecting the retention times of the other peaks, and, as evidenced in the chromatogram report (not shown), without a change in the peak area. Figure 2 shows the simulated chromatograms as a change in the competing ion concentration at the 64th plate. The *X*-axis represents the number of repetitions of the calculations. The capacity factor ( $k'$ ) and the peak area for sulfate, as obtained from the numerical data (not shown) for the chromatograms in Figs. 1 and 2, are given in Table 1. The simulated  $k'$  closely approximated the observed  $k'$ ; as in the experimental results, the simulated peak areas with eluant 1 and 2 were equal to each other.

The peak area of sulfate using CD method was also evaluated. The simulated chromatograms for CD method are shown in Fig. 3. The equivalent conductivity values used for the calculation are listed in Table 2. In the simulation, sulfate gave a negative peak with the Trien system as in the experimental results. The experimental and the calculated relative peak areas are listed in Table 1. The simulated and the experimental values are in good agreement. These results suggest that our model of complexation with the assumptions given appears appropriate for predicting both the capacity factor and peak area of sulfate in a variety of eluant systems.

Our assumptions about the unusual retention behavior

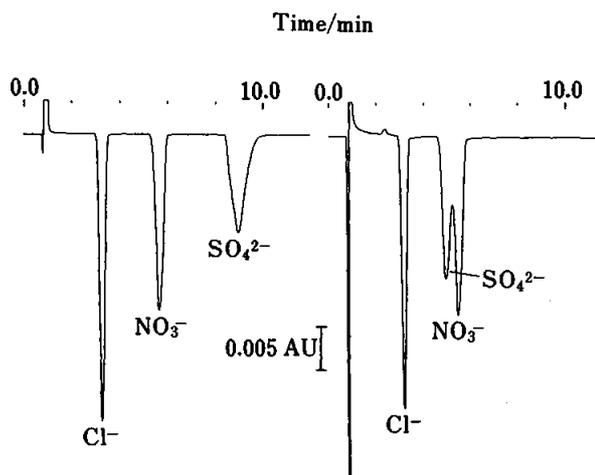


Fig. 1 Comparison of counter ion effects between a monoacidic base (Tris) (left-hand side) and Trien (right-hand side) on retention times of analyte anions on indirect photometric detection method (see text for the analytical conditions). Sample: 20  $\mu$ l of a solution containing 10 ppm of each of the indicated anions.

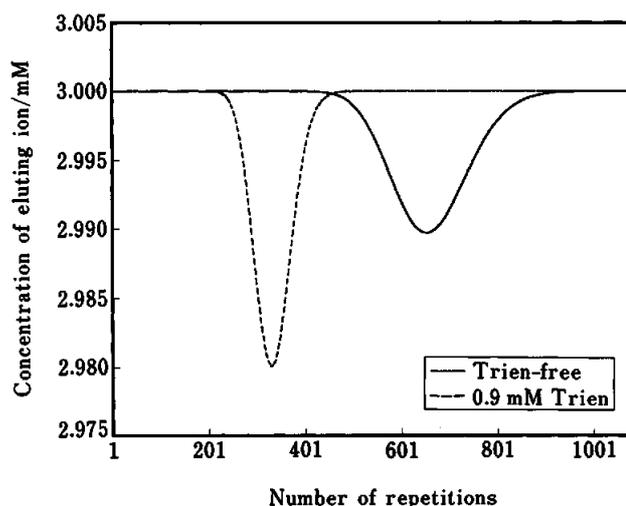


Fig. 2 Simulated chromatograms of sulfate for indirect photometric detection method given as the change of competing ion concentration at the 64th theoretical plate. The solid line and the dotted line correspond to the left-hand side chromatogram and right-hand side chromatogram in Fig. 1, respectively.

Table 1 Comparison of experimental and simulated ratio of capacity factors  $k'$  and peak areas of sulfate between Trien free and Trien systems on IPD and CD methods

	Experimental	Simulated
$k'$	0.509	0.479
Area (IPD)	1.03	1.04
Area (CD)	-0.61	-0.63

Eluant 1: 3.0 mM *p*-TSA pH 4.2 with tris(hydroxymethyl)aminomethane.

Eluant 2: 3.0 mM *p*-TSA pH 4.2 with 0.9 mM Trien.

Ratio was calculated as eluent 1/eluant 2.

Simulated  $k$ 's and areas were calculated at the 64th theoretical plate.

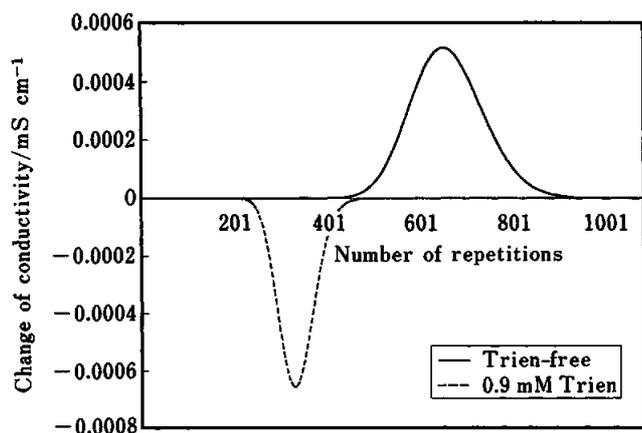


Fig. 3 Simulated chromatograms of sulfate for conductometric detection method at the 64th theoretical plate.

of sulfate were supported by the simulated chromatograms that were calculated according to the Trien-sulfate complexation model developed in an earlier work.<sup>1</sup> In this study, we have successfully shown that the retention behavior of sulfate, in the case when Trien exists in the eluant, can be predicted by simulation. These results suggest a method for the optimization of the concentration of Trien at which monovalent anions are well resolved and sulfate (or possibly other di- or

Table 2 Equivalent conductivities of ions in mobile phase

Proton	350
Tris(hydroxymethyl)aminomethane	30
<i>p</i> -Toluenesulfonate	30
Sulfate	160
Trivalent Trien	160
Tetravalent Trien	220
Trivalent Trien-sulfate	15
Tetravalent Trien-sulfate	30

polyvalent anion species) is rapidly and quantitatively eluted in minimal analysis time.

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