Elucidation of Ion-Pairing Elution Behavior of Anions and Cations in Electrostatic Ion Chromatography Using Water as a Mobile Phase

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Abstract
Peculiar ion-pairing elution behavior of analyte anions with co-counter cations in electrostatic ion chromatography (EIC) using water as a mobile phase was theoretically elucidated based on the Donnan membrane equilibrium principle and charge balance condition. New parameters, $f_{\text{anion}}$ and $f_{\text{cation}}$, containing a fluctuating coefficient, $x$, which corresponded to capacity factors, were derived from the Donnan equation. The $f_{\text{anion}}$ and $f_{\text{cation}}$ of the constituent anion and cation in ion-pairing elution were connected with each other by the $x$, and the capacity factor, $k_{\text{ion pair}}$ (of co-eluting anion and cation) could be predicted by solving the relation of $f_{\text{anion}} = f_{\text{cation}}$ for $x$. The elution volumes for various ion pairs thus semi-empirically calculated were in good agreement with those experimentally obtained. In addition, the selective ion-pairing formation in EIC could reasonably be explained by applying the parameters to a modified classical plate theory.

Keywords: Electrostatic ion chromatography; Anion; Cation; Ion-pairing elution; Donnan membrane

1. Introduction
Although more than forty years have passed since the introduction of suppressed ion chromatographic (IC) system by Small in 1975 [1], IC is still of major importance for the determination of ionic species [2,3]. During the past several decades, the original dual-column system has undergone enormous modifications and changes [4], while non-suppressed IC methods comparable to the suppressed IC method have extensively been exploited [5,6].

The use of more dilute eluent that gives the lower background signal is the key to the success in more sensitive detection of ionic species by IC [7,8]. Several promising IC systems using an eluent with the concentration of μM order were presented, resulting in more sensitive conductivity detection and/or indirect UV absorption detection [9,10]. As one of the ultimate cases, our research group succeeded in separating inorganic anions with only water as a mobile phase, and termed this separation system “electrostatic IC (EIC)”, where zwitterionic surfactants immobilized on a reversed-phase column were used as the stationary phase [11,12].

EIC has a potential for highly sensitive detection but concurrently an inevitable problem of peak-split [13]. That is, when water that contains no ionic species except for H$^+$ and OH$^-$ from the dissociation of H$_2$O is used as a mobile phase, target anions must be eluted together with cations in sample solutions to keep the electric charge balance. As a result, each analyte anion provides two or more peaks attributed to possible combinations (for simplicity we will denote these as ion pairs) of anions and cations. In the case of conventional IC, this peak-split problem does not occur or has little influence on the analysis, because the relatively larger amount of counterions in the mobile phase may electrically neutralize the analyte ions.

In order to solve the peak-split problem, we developed an on-line pre-replacement technique for the elution of anions as a particular cation form and achieved the determination of anions in real samples [14,15]. From a theoretical viewpoint, on the other hand, EIC may provide an interesting opportunity to understanding sample component interactions, i.e., the influence of co-existing species on the chromatographic behavior of analyte species. In the present work, thus, the interest is directed towards the clarification of contribution of counter cations to the retention of analyte anions, where the basic idea of a liquid-liquid partition process is used and extended [16,17]. On the basis of Donnan
membrane equilibrium principle and charge balance condition, a new parameter corresponding to capacity factor is brought out. The parameter involving a coefficient is used not only to predict the elution volumes of various ion pairs but also to elucidate the peculiar ion-pairing elution behavior in EIC. Furthermore, the potential and limitation of IC using water eluent is discussed.

2. Theoretical

In EIC using a sulfobetaine-type zwitterionic stationary phase and water eluent, the elution order of anions is consistent with Hofmeister series related to the state of hydration, as reported in previous paper [18]. This fact means that the differences in hydration energy play an important role in separation selectivity for inorganic anions.

When a zwitterionic surfactant-modified ODS column contacts with water, solvent water molecules would be held on the hydrophilic part of the surfactant. In that case, the properties of water molecules near the surfactant probably differ from those of ordinary free water because of the interaction of water with the surfactant. It seems, therefore, reasonable to consider that two aqueous solution phases with different chemical potentials (water-imbibing zwitterionic phase and external bulk water phase as the stationary and mobile phases, respectively) exist in the separation column.

We now consider the separation mechanism to be a liquid-liquid partition process, in which each ion is partitioned between the two phases and the differences in solvation energy play an important role in separation selectivity. This fact means that the differences in hydration energy play an important role in separation selectivity for inorganic anions.

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The chemical potential, $\mu$, of species $i$ with the charge of $z$ is given by

$$\mu_i = \mu_i^° + k_i F \Phi + RT \ln a_i + zF \Phi$$  \hspace{1cm} (1)

where $\mu_i^°$ is the chemical potential in the standard state, and $R$, $T$, $a$, $F$ and $\Phi$ denote gas constant, absolute temperature, activity, Faraday constant and electrostatic potential, respectively.

When a sample contains two components, $X^{n+}$ and $Y^{m-}$, their chemical potentials are represented as follows;

$$\mu_{X^{n+}} = \mu_{X^{n+}}^° + RT \ln a_{X^{n+}} + nF \Phi$$  \hspace{1cm} (2)

$$\mu_{Y^{m-}} = \mu_{Y^{m-}}^° + RT \ln a_{Y^{m-}} + mF \Phi$$  \hspace{1cm} (3)

$$\mu_{X^{n+}} - \mu_{Y^{m-}} = RT \ln (a_{X^{n+}}/a_{Y^{m-}}) + nF \Phi - mF \Phi$$  \hspace{1cm} (4)

$$\mu_{X^{n+}} - \mu_{Y^{m-}} = RT \ln (\alpha_{X^{n+}}/\alpha_{Y^{m-}}) - mF \Phi + nF \Phi$$  \hspace{1cm} (5)

Thus, the following equations are obtained.

$$\Delta \mu_{X^{n+}} = \mu_{X^{n+}} - \mu_{X^{n+}}^° = RT \ln (a_{X^{n+}}/\alpha_{X^{n+}}) + nF \Phi$$  \hspace{1cm} (6)

$$\Delta \mu_{Y^{m-}} = \mu_{Y^{m-}} - \mu_{Y^{m-}}^° = RT \ln (a_{Y^{m-}}/\alpha_{Y^{m-}}) - mF \Phi$$  \hspace{1cm} (7)

$$\Delta \mu_{X^{n+}} - \Delta \mu_{Y^{m-}} = RT \ln (a_{X^{n+}}/\alpha_{Y^{m-}}) - mF \Phi + nF \Phi$$  \hspace{1cm} (8)

$$\Delta \mu_{X^{n+}} = \mu_{X^{n+}} - \mu_{X^{n+}}^° = RT \ln (a_{X^{n+}}/\alpha_{X^{n+}}) + nF \Phi$$  \hspace{1cm} (9)

$$\Delta \mu_{Y^{m-}} = \mu_{Y^{m-}} - \mu_{Y^{m-}}^° = RT \ln (a_{Y^{m-}}/\alpha_{Y^{m-}}) - mF \Phi$$  \hspace{1cm} (10)

$$\Delta \Phi = \Phi - \Phi$$  \hspace{1cm} (11)

We now assume for simplicity that activity coefficients of the ions are equal in the two phases, i.e.,

$$\alpha_{X^{n+}}/\alpha_{X^{n+}}^° = \alpha_{Y^{m-}}/\alpha_{Y^{m-}}^° = \tau$$  \hspace{1cm} (12)

where $x$ is the concentration given by

$$c_x = N_x/V_m$$  \hspace{1cm} (13)

In the eqs 14 and 15, $V_m$ and $V_s$ are the volumes of mobile and stationary phases, while $N_x$ and $\bar{N}_x$ denote the quantities of ion $i$ in the mobile and stationary phases, respectively.

Eliminating $\Delta \Phi$ from the eqs 8 and 9, and using the eqs 13-15, we obtain

$$(k_{X^{n+}})'^{n+} = k_{X^{n+}}^° + RT \ln (a_{X^{n+}}/\alpha_{X^{n+}}) + nF \Phi$$  \hspace{1cm} (16)

$$f_{X^{n+}} = \bar{N}_{X^{n+}}^{°} / N_{X^{n+}}$$  \hspace{1cm} (17)

$$f_{Y^{m-}} = \bar{N}_{Y^{m-}}^{°} / N_{Y^{m-}}$$  \hspace{1cm} (18)

$$f_{X^{n+}} = (V_m/V_s) \exp (-\Delta \mu_{X^{n+}}/RT)$$  \hspace{1cm} (19)

$$f_{Y^{m-}} = (V_m/V_s) \exp (-\Delta \mu_{Y^{m-}}/RT)$$  \hspace{1cm} (20)

Due to the charge balance requirement, on the other hand, ions do not migrate independently of one another. Then, the conditions of electroneutrality in the stationary and mobile phases should be imposed as follows;

$$\bar{N}_{X^{n+}}^{°} = mN_{X^{n+}}$$  \hspace{1cm} (21)

$$\bar{N}_{Y^{m-}}^{°} = mN_{Y^{m-}}$$  \hspace{1cm} (22)

, where $\bar{N}_{X^{n+}}$ and $\bar{N}_{Y^{m-}}$ denote the quantities of positive and negative charges of the zwitterionic stationary phase, respectively.

Dividing of the eq 21 by the eq 22 leads to

$$k_{X^{n+}} = k_{Y^{m-}} = \bar{N}_{X^{n+}}^{°}/\bar{N}_{Y^{m-}}^{°}$$  \hspace{1cm} (23)

Since the quantity of $\bar{N}_{X^{n+}}$ is equal to that of $\bar{N}_{Y^{m-}}$ in this sulfobetaine-type stationary phase, we have the relationship

$$k_{X^{n+}} = k_{Y^{m-}} = \kappa$$  \hspace{1cm} (24)

Here we define $\kappa$ as the capacity factor of an ion pair, $mX^{n+} - nY^{m-}$, again, as shown in parentheses. By substituting the $k_{X^{n+}}$ and $k_{Y^{m-}}$ for the eq 16, an equation relating anion and cation has been derived.

$$k_{X^{n+}} = k_{Y^{m-}} = \kappa$$  \hspace{1cm} (25)

3 Experimental

3.1. Apparatus

The EIC system used to obtain several experimental data was almost the same as in previous work [14]. The instrumental components and operating conditions of the system are summarized in Table 1. The separation column was prepared by adsorbing zwitterionic surfactants on the ODS column through hydrophobic interaction. The amount of the surfactants adsorbed was calculated by a breakthrough curve method [18]. The conductivity detector, UV detector and ICP-AES were assembled in series through PTFE tubing. The ICP-AES instrument with a time-sequential detection program allowed simultaneous multielement chromatogram measurements.
Table 1. Instrumental components of EIC system.

| Pump: | Model LC-10AD (Shimadzu, Kyoto, Japan) |
|------|----------------------------------------|
| Injector: | Model 7725 injector with a 20 μl sample loop (Rheodyne, CA) |
| Column: | ODS column (4.6 mm i.d. × 250 mm long; Chemicals Evaluation and Research Institute, Tokyo) modified with a sulfobetaine-type zwitterionic surfactant (the amount of the surfactants adsorbed on the ODS: 1.1 mmol) |
| Eluent: | Pure water prepared by a Milli-Q deionization system (Nihon Milipore Kogyo, Tokyo) |
| Detection: | (1) Non-suppressed conductivity by Model CDD-6A (Shimadzu) |
|                | (2) UV absorption at the wavelength of 210 or 230 nm by Model 870-UV (Jasco, Tokyo) |
|                | (3) Atomic emission at the wavelength of 589.0 (Na+), 766.4 (K+), 279.0 (Mg2+), and 317.9 nm (Ca2+) by Model Plasma AtomComp MKII (Jarrell-Ash, MA) |

3.2. Reagents

The sulfobetaine-type zwitterionic surfactant used here was tetradecyldimethyl(3-sulfopropyl)ammonium hydroxide purchased from Aldrich Japan (Tokyo). Inorganic salts used as the analytes were of analytical reagent grade from Wako Pure Chemicals (Osaka, Japan). These reagents were used without further purification.

4 Results and discussion

4.1. Division of \( k_{mX^\text{+}_nY^\text{-}n} \) between \( f_{X^\text{+}} \) and \( f_{Y^\text{-}} \)

Table 2 shows some \( k_{mX^\text{+}_nY^\text{-}n} \) values for sodium salts and nitrates, which were experimentally obtained from the retention volume of 10 mM of each single salt and the void volume of this system. According to the eq 25, on the other hand, \( k_{mX^\text{+}_nY^\text{-}n} \) is given by a charge-weighted mean of the constituent \( f_{X^\text{+}} \) and \( f_{Y^\text{-}} \). If the \( f \) value of an ion is arbitrarily set at \( x \), then the \( f \) values of all ions tested will be represented as a parameter involving the \( x \) by using the eq 25. Thus, the division of experimental \( k_{mX^\text{+}_nY^\text{-}n} \) values into the constituent anion and cation contributions (i.e., \( f_{X^\text{+}} \) and \( f_{Y^\text{-}} \)) was attempted.

Table 2. Capacity factors of ion pairs experimentally obtained from each single-salt elution.\(^{39}\)

| Ion pair          | Elution volume / ml | \( k_{b} \) |
|-------------------|---------------------|------------|
| Na^-I             | 2.44                | 0.31       |
| 2Na^+-SO_4^-      | 2.48                | 0.34       |
| Na^-I0_3          | 2.49                | 0.34       |
| Na^-Cl^-          | 2.89                | 0.56       |
| Na^-NO_3^-        | 3.17                | 0.71       |
| Na^-Br^-          | 3.91                | 1.11       |
| Na^-NO_2^-        | 4.45                | 1.40       |
| Na^-I^-           | 9.49                | 4.11       |
| Na^-SCN^-         | 17.50               | 8.43       |
| K^+-NO_3^-        | 4.48                | 1.42       |
| Mg_2^+-2NO_3^-    | 6.60                | 2.56       |
| Ca_2^+-2NO_3^-    | 6.70                | 2.61       |
| Sr_2^+-2NO_3^-    | 6.74                | 2.63       |
| Ba_2^+-2NO_3^-    | 6.88                | 2.71       |

a) 10 mM of each single-salt solution was used.

b) The void volume was 1.86 ml.

Table 3 shows an example, in which \( f_{SO_4^-} \) is provisionally regarded to be \( x \). Each \( f_{X^\text{+}_n} \) value was first derived from the corresponding \( k_{X^\text{+}_nYO_3^-} \). Subsequently, the obtained \( f_{Na^+} \) value (1.95/\( x \)) and experimental \( k_{mNa^+_nYO_3^-} \) values were used to obtain each \( f_{Y^\text{-}_n} \) value. Thus, the \( f \) value of each ion, was represented as a parameter involving a coefficient, \( x \).

Table 3. Intrinsic capacity factors of individual anions and cations in EIC using an ODS column modified with zwitterionic surfactants.

| Anion          | \( f_{anion} \) |
|----------------|-----------------|
| NO_3^-         | 0.01            |
| SO_4^-         | 0.05            |
| F^-            | 0.06            |
| Cl^-           | 0.16            |
| I^-            | 0.25            |
| SCN^-          | 0.63            |

Table 4. Experimental and calculated values of elution volumes of ion pairs.

| Ion pair          | Calculated \( k \) | Calculated E.V. / ml | Experimental E.V. / ml |
|-------------------|--------------------|----------------------|------------------------|
| K^+-Cl^-          | 0.56               | 2.90                 | 2.92                   |
| K^+-I^-           | 4.17               | 9.59                 | 9.75                   |
| K^+-SCN^-         | 8.55               | 17.71                | 17.68                  |
| Mg_2^+-2Cl^-      | 0.75               | 3.25                 | 3.20                   |
| Ca_2^+-2Cl^-      | 0.77               | 3.28                 | 3.24                   |
| Mg_2^+-2I^-       | 10.81              | 21.91                | 22.09                  |
| Ca_2^+-2I^-       | 11.03              | 22.31                | 22.48                  |
| Mg_2^+-2SCN^-     | 28.16              | 54.09                | 53.86                  |
| Sr_2^+-2Cl^-      | 0.77               | 3.29                 | 3.29                   |
| Sr_2^+-2I^-       | 11.12              | 22.49                | 22.74                  |
| Sr_2^+-2SCN^-     | 28.98              | 55.61                | 55.20                  |
| Ba_2^+-2Cl^-      | 0.79               | 3.33                 | 3.34                   |
| Ba_2^+-2I^-       | 11.44              | 23.08                | 23.26                  |
| Ba_2^+-2SCN^-     | 29.81              | 57.15                | 56.77                  |
| Mg_2^+-SO_4^-     | 0.41               | 2.62                 | 2.54                   |
It should be mentioned here that the calculated $k_{mX^+}^{nY^-}$ values can also be obtained by solving the relation of $f_{X^+} = f_{Y^-}$ for $x$, since the ion-pairing elution implies the $f_{X^+}$ value is equal to the $f_{Y^-}$ value. This manner would be more essential.

It should also be emphasized that the $k_{mX^+}^{nY^-}$ values thus obtained are independent of the choice of standard ion. That is, when $f_{Na^+}$ is set at $x$ as a standard, the expression of each ion defined in Table 3 is changed, but the $k_{mX^+}^{nY^-}$ values calculated by the aforementioned manner are consistent with those shown in Table 4.

### 4.3. Simulation of selective ion-pair formation in EIC

In EIC, anions have some preference in co-eluting with counter cations, as described in our previous paper [15]. Fig. 1(A) shows the typical chromatogram for a mixture containing four components (Na+, Mg$^{2+}$, Cl$^-$ and NO$_3^-$), measured with a conductivity detector. Under the sample condition, the possible combinations of anions and cations are as follows; Na$^+$-Cl$^-$, Na$^+$-NO$_3^-$, Mg$^{2+}$-2Cl$^-$, Mg$^{2+}$-2NO$_3^-$. As expected, the peaks attributed to the four ion pairs were certainly observed on the chromatogram, but the peak intensities were quite different. That is, ion pairs of Na$^+$-Cl$^-$ and Mg$^{2+}$-2NO$_3^-$ were preferentially formed.

Such selective ion-pair formation may significantly relate to the separation process. In order to clarify the mechanism, the behavior of each ion was examined in detail by using a classical plate theory that can show the migration of analytes in the separation column.

Fig. 2 shows the schematic illustration of the distribution of each ion in a hypothetical column subdivided into a number of stages consisting of mobile and stationary phase cells. If an ionic species is distributed between the mobile and stationary phase cells independently of the other species, then it will be eluted at the intrinsic elution time according to its distribution ratio. This is, of course, impossible because of the electroneutrality requirement. That is, when an ion goes into the stationary phase, it must be accompanying with the counter ion so that both the stationary and mobile phases keep the electroneutrality. Thus, a charge balance condition that the quantities of positive and negative charges are equal in each cell was imposed.

The relationship is represented as follows;

**Electric charge balance in the stationary phase cell**,

$$w_{Na^+} \times f_{Na^+} + 2w_{Mg^{2+}} \times f_{Mg^{2+}} = w_{NO_3^-} \times f_{NO_3^-} + w_{Cl^-} \times f_{Cl^-}$$  \(26\)

**Electric charge balance in the mobile phase cell**,

$$w_{Na^+} \times f_{Na^+} + 2w_{Mg^{2+}} \times f_{Mg^{2+}} = w_{NO_3^-} \times f_{NO_3^-} + w_{Cl^-} \times f_{Cl^-}$$  \(27\)

where $w_i$ denotes the amount of each component at the $i^{th}$ stage, top; mobile phase cell, bottom; stationary phase cell.

By solving the eq 26 or 27 (for $x$) in which the corresponding $f$ values (shown in Table 3) were put, the quantity of each ion in each cell could be calculated. As a matter of course, the $x$ value obtained from eq 26 is consistent...
Table 5. Simulated results\(^a\) for the distribution of each ion in the hypothetical column.

|                      | 1\(^\text{st}\) cell | 2\(^\text{nd}\) cell | 3\(^\text{rd}\) cell |
|----------------------|----------------------|----------------------|----------------------|
| \(x = 2.92\)        |                      |                      |                      |
| mobile phase         | Na\(^+\) 1.69       | Mg\(^{2+}\) 2.55     | Cl\(^-\) 6.82       |
| stationary phase     | 4.00                | 3.31                 | 7.45                 |
| \(x = 2.66\)        |                      |                      |                      |
| mobile phase         | Na\(^+\) 0.98       | Mg\(^{2+}\) 2.04     | Cl\(^-\) 2.23       |
| stationary phase     | 2.31                | 2.54                 | 0.95                 |
| \(x = 2.51\)        |                      |                      |                      |
| mobile phase         | Na\(^+\) 0.64       | Mg\(^{2+}\) 1.54     | Cl\(^-\) 0.68       |
| stationary phase     | 0.74                | 1.69                 | 3.87                 |
| \(x = 2.95\)        |                      |                      |                      |
| mobile phase         | Na\(^+\) 0.69       | Mg\(^{2+}\) 1.01     | Cl\(^-\) 3.10       |
| stationary phase     | 1.81                | 1.32                 | 1.47                 |
| \(x = 3.54\)        |                      |                      |                      |
| mobile phase         | Na\(^+\) 0.28       | Mg\(^{2+}\) 0.13     | Cl\(^-\) 2.86       |
| stationary phase     | 1.34                | 0.38                 | 0.47                 |

\(N\) \text{plate}/(1+k) \quad \cdots \quad (28)

\(^a\) A unit amount of a mixture containing four components (in this case, for example, 10 mM Na\(^+\), 5 mM Mg\(^{2+}\), 10 mM NO\(_3\)^\(-\) and 10 mM Cl\(^-\)) was placed in the first mobile phase cell and transferred in the hypothetical column.

with that from eq 27. Table 5 represents a part of the result for a virtual mixture containing the same components as in Fig. 1, and the resultant simulated chromatogram of each ion (Na\(^+\), Mg\(^{2+}\), Cl\(^-\) and NO\(_3\)^\(-\)) after 1000 transfers is presented in Fig. 3. As clearly seen from Figs. 1 and 3, the simulated chromatograms were in good agreement with the real chromatogram. This agreement may support the validity of our ion-pairing migration model based on charge balance condition.

It should be mentioned here that, in plural ion-pair forming mode, the distribution ratio of each ion differs among stages, as can be seen in Table 5. This can be a serious problem because it means that the elution volumes (which are criteria of identification) of ion pairs vary with the sample composition change. In order to evaluate the degree of the variation caused by sample composition change, the peak top positions of the four ion pairs were compared between single ion-pair forming mode (meaning that only a single ion pair is formed) and plural ion-pair forming mode. Under the sample condition in Fig. 3 (in plural ion-pair mode), the peaks for Na\(^+\)-Cl\(^-\), Mg\(^{2+}\)-2Cl\(^-\), Na\(^+\)-NO\(_3\)^\(-\) and Mg\(^{2+}\)-2NO\(_3\)^\(-\) appeared at 642, 563, 419 and 281, respectively.

In the case of single ion-pair mode, on the other hand, the ion pair is distributed between the mobile and stationary phases at the constant ratio \(k\), and the peak top position is simply calculated from the following equation:

\[ N\text{plate}/(1+k) \]

where \(N\text{plate}\) denotes the plate number. The positions thus independently obtained for 1000 transfers were 642 (\(k\text{Na}^+-\text{Cl}^- = 0.559\)), 570 (\(k\text{Mg}^{2+}-2\text{Cl}^- = 0.754\)), 417 (\(k\text{Na}^+-\text{NO}_3^- = 1.396\)) and 281 (\(k\text{Mg}^{2+}-2\text{NO}_3^- = 2.560\)) for Na\(^+\)-Cl\(^-\), Mg\(^{2+}\)-2Cl\(^-\), Na\(^+\)-NO\(_3\)^\(-\) and Mg\(^{2+}\)-2NO\(_3\)^\(-\), respectively. These values in single ion-pair mode are nearly equal to those in plural ion-pair mode. Thus, it was confirmed that the elution volume of each ion pair could be considered to be practically invariable under various sample conditions.

![Fig. 3. Simulated chromatograms for the mixture obtained from the modified plated theory.](image)

5 Conclusion

Theoretically meaningful equations based on Donnan membrane theory and charge balance condition were constructed to elucidate the peculiar ion-pairing elution behavior in EIC. Some characteristic phenomenon in EIC could reasonably be explained by using new parameters \(f_Xn^+\) and \(f_Ym^-\) containing a coefficient, \(x\). Although our approach using chemical potential could not give any concrete information about what factors affect the retention difference, it provided quite significant information on the contribution of coexisting species to the retention of analyte species. Furthermore, it clearly appears from this theoretical consideration that the use of water as a mobile phase is not necessarily ideal for ion chromatographic separations because of the inevitable peak split problem. But of course,
in some cases, target anions are conveniently eluted with particular cations. For example, Br\(^{-}\) in seawater samples is exclusively eluted with Mg\(^{2+}\)/Ca\(^{2+}\) and can be readily determined [19]. In addition, it should be mentioned that the peak-split problem is practically resolved by using an electrolyte solution of the order of 10\(^{-5}\) mol/L as a mobile phase, with preserving the unique separation selectivity of zwitterionic stationary phase [20].

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