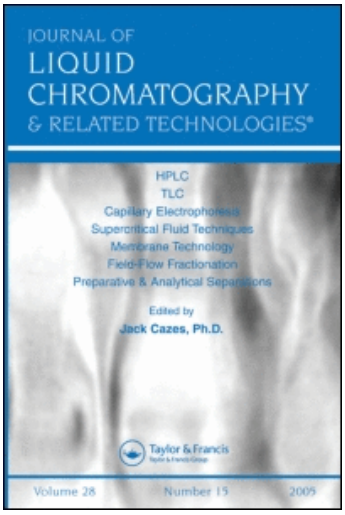

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A Novel Ion Chromatographic Method Using Zwitterionic Surfactants as the Stationary Phase and Water as the Mobile Phase

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A NOVEL ION CHROMATOGRAPHIC METHOD USING ZWITTERIONIC SURFACTANTS AS THE STATIONARY PHASE AND WATER AS THE MOBILE PHASE

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ABSTRACT

Zwitterionic surfactants immobilized on the surfaces of octadecylsilica (ODS) are used for the stationary phase and water as the mobile phase for the ion chromatography (IC) of target analytes. The creation of an electrical double layer (EDL), when a zwitterionic stationary phase is in contact with the analyte ions,

is proposed to explain the separation mechanism. When an EDL is created using a zwitterionic stationary phase (ZWEDL), its properties differ considerably to those of a single charge-fixed stationary phase created EDL. For a ZWEDL, (i) the electrostatic field is increased, resulting in the simultaneous retention and separation of both cations and anions; (ii) the electrostatic affinity between the analytes in the ZWEDL and the stationary phase is extremely weak. This results in the "effective" distribution of the analytes between the stationary phase and the mobile phase without the need for ion-exchange. Since only water is used for the mobile phase, the sensitivity of detection by conductivity is vastly improved and the direct determination (without pre-concentration) of inorganic ions at ultra low levels is possible. Furthermore, since both positive and negative electrostatic fields are produced simultaneously, both analyte cations and anions are retained and separated in a single stage of the stationary phase. This provides the basis for a simple and rapid chromatographic method for the simultaneous analysis of cations and anions.

INTRODUCTION

For the analysis of inorganic ions by ion chromatography (IC), separation and detection are normally achieved in series. Usually, ion exchangers are used in the stationary phase to achieve the separation while conductometry is used for detection. The development of "suppression"[1] and "non-suppression"[2,3] ion exchange chromatographic techniques has enabled the successful separation and detection of a large number of ionic species. However, although these techniques provide high precision and good detection limits, increased resolution and sensitivity are difficult to obtain for a number of reasons:

1. The residual (in suppression IC) or the original (in non-suppression IC) background electrical conductance of the mobile phase contributes to the noise level and reduces the signal to noise ratio (S/N). This adversely affects the overall detection limits of IC, and restricts the minimum detection limits of most ionic species to the ppb region.

2. The overlapping of analyte species is common resulting in information loss and a decrease in resolution. Under certain conditions, with respect to the column and mobile phase, the retention intervals between the analyte species may be constant and can result in elution-

overlapping. For example, when two analyte species are eluted in series, one being higher in concentration than the other, the elution containing the lower concentration of analytes are often partly or completely overlapped by the elution containing the higher concentration of analytes.

3. For ion-exchange chromatography two separation procedures are necessary. One each for the separation of cations and anions; this reduces the number of samples that can be analyzed as a function of time.

Since these problems are largely associated with the use of ion-exchanges, they will never be effectively overcome unless alternative and suitable methods are developed. One solution is to use zwitterionic surfactants for the stationary phase. When a small amount of aqueous solution containing analyte cations and anions, is passed through a zwitterionic surfactant-immobilized stationary phase, the cations and anions receive an electrostatic attraction and repulsion from the stationary phase, simultaneously. As a result, the "net" electrostatic affinity between the analyte ions and the stationary phase is extremely low compared to the electrostatic affinity observed when using a single charged fixed stationary phase. The appropriate distribution of the analyte ions between the stationary phase and the mobile phase, which is critical for separation, is achieved by using water as the mobile phase. Furthermore, since negative and positive electrostatic fields are produced simultaneously, if a zwitterionic stationary phase is used, both the analyte cations and anions are retained and/or separated with a single stationary phase. This method of separating ions based on simultaneous electrostatic attraction and repulsion interactions has been termed "electrostatic ion chromatography (EIC)".[4,5]

In EIC, water is used as the mobile phase; therefore, the problem of low sensitivity, normally associated with conventional IC, is completely removed. EIC is less time consuming since the analyte cations and anions are separated within a single stage. Furthermore, the intervals between the retention time of the analyte ions can be extended by the adjustment of sample conditions.[6] Hence, the problems of overlapping eluents and incomplete separation of the target analytes are easily overcome.

To further elucidate the separation mechanism involved in EIC, an electrical double layer (EDL) model, proposed in previous study,[7] has been further investigated in this research. The initial results of identical samples which were analyzed in duplicate, using EIC and conventional IC methodologies, are also presented to enable comparison. The advantages of EIC over conventional IC are discussed.

EXPERIMENTAL

Apparatus

The HPLC system used in this study is the same as described in previous research[7] and consisted of the following. A Shimadzu (Kyoto, Japan) LC-6A system equipped with a pump (LC-7A), an auto-injector (SIL-6A) with a sample injection volume of 100 μL. A system controller (SCL 6A), and a conductivity detector (CDD-6A) interfaced with a photodiode array UV visible detector (SPD-M6A). The HPLC system was coupled to an inductively coupled plasma atomic emission spectrometry (Model 075 Plasma Atomcomp MKII, Thermo Jarrell-Ash, Franklin, MA) for the identification of the analyte cations. Three ODS-packed columns (L-Column, 250 x 4.6 mm I.D.; Chemical Inspection and Testing Institute, Tokyo, Japan), the first coated with CHAPS micelles, the second with Zwittergent-3-14 micelles, and the third coated with ammonium sulfobetaine-3 micelles, were used as the separation columns (zwitterionic columns). A commercial anion-exchange column (Shim-pack IC-A3; 150 x 4.6 mm I.D., Shimadzu, Kyoto, Japan) was also used for conventional IC measurements.

Reagents

The zwitterionic surfactants, CHAPS, Zwittergent-3-14 and ammonium sulfobetaine-3 were used to produce the zwitterionic stationary phases. These were obtained from Dojin (Kumamoto, Japan), Calbiochem (La Jolla, CA, USA), and Janssen Chimica (2340 Beerse, Belgium), respectively. The inorganic salts, which were used as the standard analytes, were purchased from Wako (Osaka, Japan) and all reagents were used as received. Pure water (conductivity, 0.01 - 0.03 μS/cm) obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA) was used throughout.

RESULTS AND DISCUSSION

The Separation Mechanism in EIC

As with all types of chromatography, ion chromatography requires an "effective" distribution of the analyte ions between the stationary phase and the mobile phase to achieve separation of the analytes. In conventional IC, when a single charge-fixed stationary phase is used the separation of anions requires

the use of a positive charge-immobilized stationary phase, while for cations, a negative charge-immobilized stationary phase is necessary. The effective distribution of the analyte ions between the stationary phase and the mobile phase is achieved by ion-exchange. The ion exchange process can be better understood by the use of a stoichiometric model:

$$jA^{i+}_{(s)} + iR^{j+}_{(m)} \Leftrightarrow jA^{i+}_{(m)} + iR^{j+}_{(s)} \quad (1)$$

$$jA'^{i-}_{(s)} + iR'^{j-}_{(m)} \Leftrightarrow jA'^{i-}_{(m)} + iR'^{j-}_{(s)} \quad (2)$$

Equation's 1 and 2 represent the cation and anion-exchange, respectively. Where i and j denote the ion charge, $A^{i+}(s)$ and $A'^{i-}(s)$ denote the analyte ions, which are attracted by the charged stationary phase, and $R^{j+}(m)$ and $R'^{j-}(m)$ represent the ions present in the mobile phase, which are used for ion-exchange, i.e., the replacing ions. Further understanding of the stoichiometric model can be gained from the analysis of two monographs contributed by Helfferich[8] and Haddad and Jackson.[9] Since the stoichiometric model only allows for a quantitative investigation of the ion-exchange process, further illustration of the ion-exchange concept can be made via the introduction of the Stern model. For a single charge-fixed surface (the stationary phase in IC) in contact with an electrolytic aqueous solution, the ions of the electrolytes, having the opposite charge to the stationary phase, are surrounded by the charged stationary phase, creating an electrical double layer (EDL). The Stern model is shown in Figure 1. The analyte ions in the double layer receive their electrostatic attraction from the stationary phase. Therefore, to achieve an "effective" distribution of the analyte ions between the stationary phase (electrical double layer) and the mobile phase (bulk solution), namely, to release the analyte ions from the electrical double layer to the bulk solution, a procedure of ion exchange is required. To achieve ion separation when using a single charge-fixed stationary phase, the mobile phase must contain replacing ions in order to facilitate ion-exchange. Further clarification of the electrical double layer model, as applied to IC, is given in previous literature.[10-22]

It was initially proposed in previous research, that for a zwitterionic stationary phase in contact with ions, an "electrical double layer (ZWEDL)" is also created.[7] However, the properties of a ZWEDL differ to those of an electrical double layer created with a single charge-fixed stationary phase. These are illustrated in Figure 1 and are briefly described below.

(i) For a zwitterionic stationary phase, both positive and negative electrostatic fields are produced simultaneously, introducing both the analyte cations and anions in to the EDL. As a result, both of the analyte cations and anions are retained/separated with a single

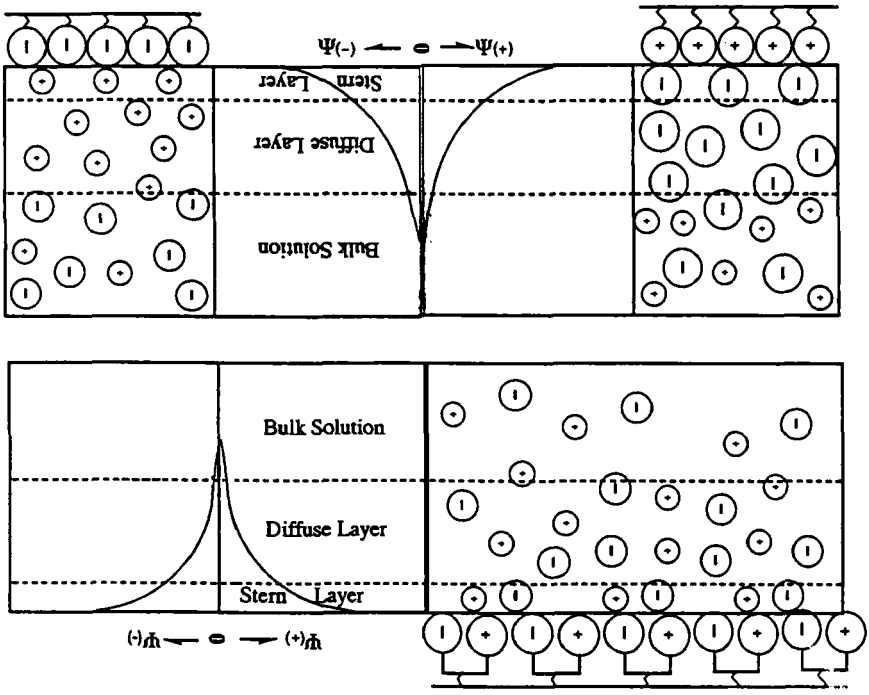


Figure 1. Electrical double layers created with a positive charge-fixed and a negative charge-fixed stationary phase (left side) and a positive/negative charge-fixed (zwitterionic) stationary phase (right side). The potential (ψ) of the electrostatic field produced by the charged stationary phase decreases with increasing the distance from the charged stationary surface to the solution. Ψ decreases up to zero in the bulk solution. Analyte ions are retained by the electrostatic field, creating an electrical double layer.

stationary phase. However, when a single charge-fixed stationary phase is used, the electrical double layer involves either the analyte cations, for a negative-charge-fixed stationary phase, or the anions, for a positive charge-fixed stationary phase. Therefore, for the separation of cations and anions, two stages (stationary phases), one for retaining/separating cations, one for retaining/separating anions, are required.

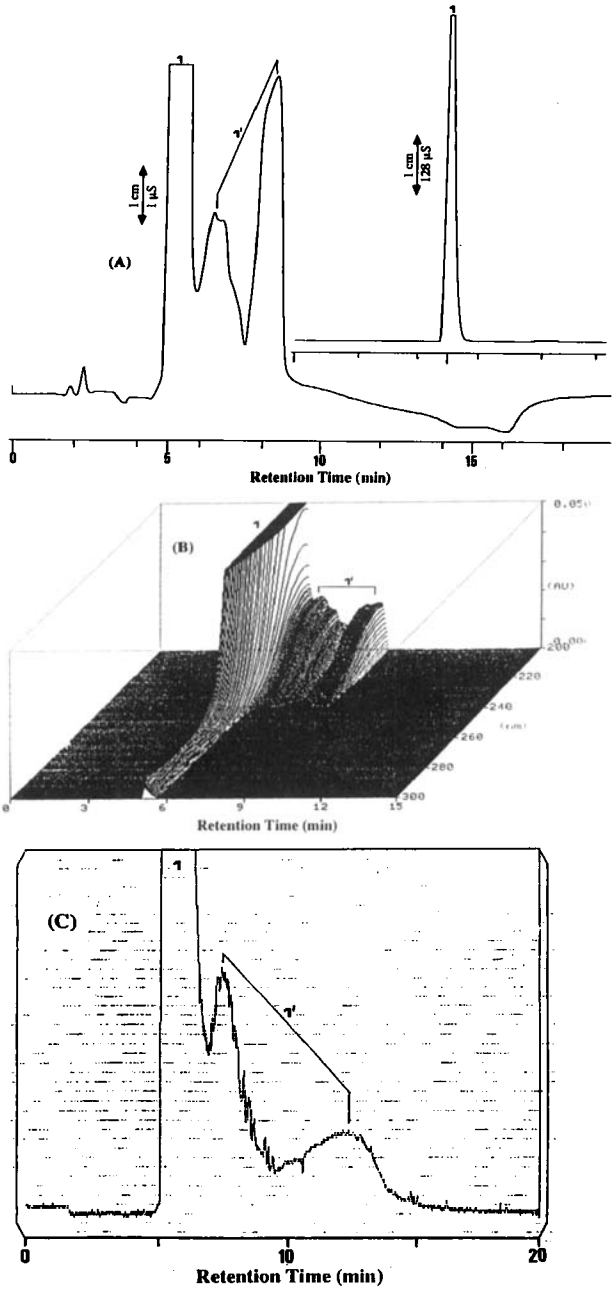
(ii) For a zwitterionic stationary phase, the distribution of the analyte ions between the electrical double layer and the bulk solution is achieved without the need for ion-exchange. This is because the positive and the negative charges are fixed in close proximity to each other in the

stationary phase. Therefore the analyte ions are receiving the effect of electrostatic attraction and repulsion simultaneously. Hence, the "net" electrostatic affinity between the stationary phase and the analyte ions in the electrical double layer is extremely weak. Adversely, for a single charge fixed stationary phase, the electrostatic affinity between the stationary phase and the analyte ions in the electrical double layer is 'strong' since the analyte ions are only subjected to electrostatic attraction. Therefore, to release the analyte ions from the electrical double layer into the bulk solution, ion-exchange is necessary.

The Elution of Identical Analyte Ions from the Stern Layer and the Diffuse Layer

When water is used as the mobile phase in EIC, identical analyte ions from the Stern and the diffuse layers elute at slightly different retention times. Although the analyte ions from the diffuse layer constitute the main detectable peak, identical analyte ions from the Stern layer are eluted as two small secondary peaks which appear as shoulders on the main peak. This is typified in Figure 2 (A, B and C) which shows the chromatogram of an aqueous solution containing 1.0 mM $Ba(NO_3)_2$. The chromatogram shown in Figure 2A was obtained using conductometric detection. While, the chromatogram shown in Figure's 2B and C were obtained using a photodiode array UV-Vis detection system and an ICP-AES detection system respectively. These three detection systems were used in series and the ratio of the peak areas, i.e. secondary peak: main peak, in each case, was found to be approximately 3:1000.

For each chemical species (salt) examined in this study, the peak areas of both the main peak, Am, and the secondary peaks, As, are proportional to the concentration of the analytes in the sample solutions. When the concentration of the analytes in the sample solution were relatively high, (mM levels), most of the analytes were eluted from the diffuse layer, i.e., the ratio of As/Am is very small. On the contrary, when the concentration of the analytes in the original sample solution were relatively low (μM levels), most of the analytes were eluted from the Stern layer, i.e., the ratio of As/Am is very large. The secondary peaks become critical when the concentration of the analytes in the original solution is sufficiently low. The actual critical concentration depends on the chemical species of the analytes and the conditions of the zwitterionic stationary phase. The hydrophobic analytes and/or stationary phase give larger critical concentrations than the hydrophilic analytes and/or the stationary phase.



In conventional IC, no differences are observed in the elution retention time for the analyte ions from the Stern layer and the diffuse layer when using a mobile phase which contains the replacing ions. The reason for this being that the analyte ions involved in the Stern layer and in the diffuse layer are replaced, almost simultaneously, when high levels of the replacing ions are present in the mobile phase. Naturally, when only water is used as the mobile phase, the analyte ions involved in the Stern layer exhibit stronger attraction properties than identical analyte ions in the diffuse layer, by means of the stationary phase. Therefore, the analyte ions involved in the Stern layer are eluted with a longer retention time. Furthermore, the analyte ions in the Stern layer may also be affected if both the analyte ions and the zwitterionic stationary phase possess strong hydrophobic properties. However, with the addition of a "sacrifice" species to the original sample solution, the analyte ions in the Stern layer can be persuaded to migrate to the diffuse layer.[7] Eventually, a single elution, and therefore a single peak, equal to the total number of target analyte ions is obtained.

Comparison of EIC and Conventional IC Detection Abilities

The background conductance and the noise level of the mobile phase are the two major factors which determine the detection limits when using conductometry for detection . Water is considered to be an ideal mobile phase for acquiring the lowest detection limits since its conductance and noise levels are low.[23] Previous research dealing with the separation of ions using water as the sole component of the mobile phase was published in 1968 by Saunders and Pecsok.[24] There are also recent literatures dealing with ion separation (using water as the mobile phase) using different types of stationary phases.[25,26] However, no literature has been found regarding the analysis of samples containing very low levels of analyte ions. Furthermore, the separation abilities of these methods were found to be poor compared to conventional IC. In our experience, when aqueous samples containing inorganic ions at ultra-low levels (low-μM) are injected into either a tightly cross linked polyacrylamide gel packed column[24] or a crown ether immobilized stationary phase,[25] analytes are retained in the column, i.e. are unable to elute out from the column.

Figure 2. **(left)** Chromatograms of an aqueous solution conatining 1.0 mM $Ba(NO_3)_2$ obtained using a conductivity detection (A); a photodiode array UV-vis detection (B) and an ICP-AES detection (C). Column: ODS-packed column (250 x 4.6 I.D. mm) coated with Zwittergent-3-14; mobile phase: water; flow rate: 1.0 mL/min. The main peak (1) is corresponding to the ion pair Ba^{2+}-$2NO_3^-$ eluted from diffuse layer; the secondary peak (1') is corresponding to the identical ion pair eluted from the Stern layer.

This is probably due to the residual silanols and/or the hydrophobic properties of the stationary phase. The inability of the analyte ions to elute from the column, as experienced in trace analysis, is not observed with the EIC technique. Therefore, the separation abilities obtained using EIC are comparable to those of conventional IC, furthermore, a superior detection ability using conductometry with EIC over conventional IC is expected.

Four aqueous stock solutions containing (i) 0.02 μM each of NaCl, $NaNO_2$, NaBr $NaNO_3$ and NaI, (ii) 0.1 μM each of NaCl, $NaNO_2$, NaBr, $NaNO_3$ and NaI, (iii) 0.01 μM each of $CaCl_2$, $CaBr_2$, $Ca(NO_3)_2$, CaI_2 and (iv) 0.1 μM each of $CaCl_2$, $CaBr_2$, $Ca(NO_3)_2$ and CaI_2 were prepared and a sample volume of 100 mL was injected into the EIC system. Identical experimental conditions were used throughout. To test for reproducibility and stability of EIC during trace analysis, each of these solution mixtures were analyzed ten times. The sensitivity for conductometric detection was 0.1 μS/cm. Typical chromatograms for the stock solutions (ii and iv) are shown in Figures 3 and 4, respectively.

For stock solution (i), the standard deviation (%) was found to be 1.8 and 5.2 for the concentration (peak area) of the ion pairs Na^+ - Br^- and Na^+ - NO_3^-; and 0.8, 0.6, 0.6 in the retention time for ion pairs of Na^+ - Cl^-, Na^+ - Br^- and Na^+ - NO_3^-, respectively. A peak, corresponding to the Na^+ - I^- ion pair, is not observed probably because the conductance of the Na^+ - I^- involved in this elution is ultra-low, and beyond the detection limit. The NaCl has been contaminated during preparation and previous experience has shown that uncontaminated aqueous solutions containing ultra-low NaCl or $CaCl_2$ are very difficult to obtain. The analytes Na^+ - NO_2^-, were obscured by the negative "water-dip" and the peak corresponding to the ion pair Na^+ - NO_3^- was partly overlapped by a peak caused by dissolved carbon dioxide (CO_2). The "water-dip" observed in conventional IC, including the suppression type and non-suppression type),[27,28] is also observed in EIC. To further investigate the occurrence of the water-dip and the CO_2 peak, the water used as the mobile phase was injected into the EIC system. The chromatogram shown in Figure 5 shows a negative peak caused by the water-dip and a positive peak caused by dissolved CO_2. It is proposed that the dissolved CO_2 in the sample is separated from the water and that the negative peak corresponds to water without CO_2. The positive peak is thought to be caused by the presence of carbonate and these assumptions were confirmed when an aqueous solution containing $NaHCO_3/Na_2CO_3$ was injected into the EIC system. A peak, corresponding to carbonate, appeared with an identical retention time as CO_2.

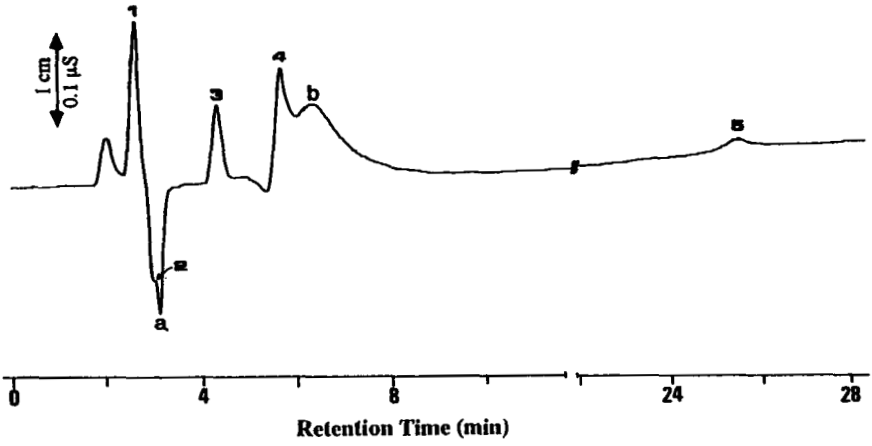


Figure 3. Chromatogram of an aqueous solution containing 0.1 μM each of NaCl, $NaNO_2$, NaBr, $NaNO_3$, and NaI obtained using EIC. Detection: conductivity. Other conditions are the same as described in Fig. 2. Peaks: 1, Na^+-Cl^-; 2, Na^+-NO_2^- (partly obscured by the water-dip); 3, Na^+-Br^-; 4, Na^+-NO_3^-; 5, Na^+-I^-; a, water-dip; b, CO_2.

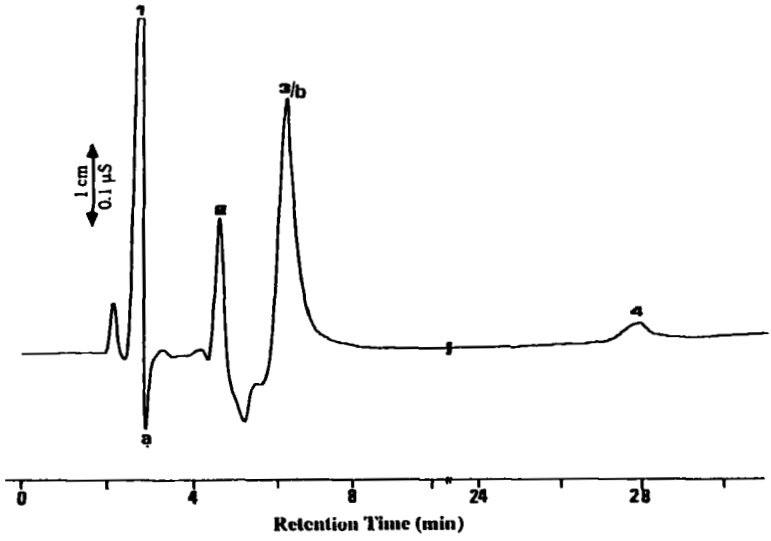


Figure 4. Chromatogram of an aqueous solution containing 0.1 μM each of $CaCl_2$*, $CaBr_2$, $Ca(NO_3)_2$ and CaI_2 obtained using EIC. Detection: conductivity. Other conditions are the same as described in Fig. 2. Peaks: 1, Ca^{2+}-$2Cl^-$; 2, Ca^{2+}-$2Br^-$; 3, Ca^{2+}-$2NO_3^-$; 4, Ca^{2+}-$2I^-$; a, water-dip; b, CO_2. *$CaCl_2$ has been contaminated.

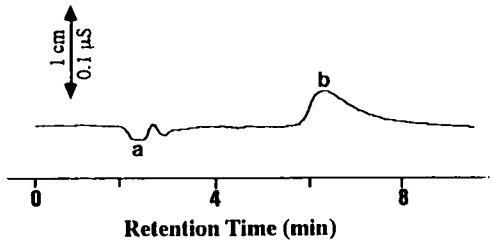


Figure 5. Chromatogram of 100 μL water used as the mobile phase. Other conditions are the same as described in Fig. 3. Peaks: a, water-dip; b, CO_2.

For solution (ii), the standard deviation (%) is 6.8, 0.6, 4.2 and 4.3 for the peak area and 0.8, 0.6, 0.6 and 0.7 for the retention times for ion pairs, Na^+-Cl^-, Na^+ - Br^-, Na^+ - NO_3^- and Na^+ - I^-, respectively. For solution (iii), the standard deviation (%) was found to be 3.4 and 8.5 for the peak area for ion pairs, Ca^{2+} - $2Br^-$ and Ca^{2+} -$2NO_3^-$. For the retention times, 0.8, 0.5 and 1.2 for ion pairs of Ca^{2+} - $2Cl^-$, Ca^{2+} -$2Br^-$ and Ca^{2+} - $2NO_3^-$, respectively. A peak corresponding to Ca^{2+} - $2I^-$ was not observed. The peak corresponding to Ca^{2+} - $2NO_3^-$ was completely overlapped by the CO_2 peak. Therefore, the peak area for Ca^{2+} - $2NO_3^-$ was calculated as the difference between the area of the peak due to Ca^{2+} - $2NO_3^-$/CO_2 and the area of the CO_2 peak. However, the ion pair Ca^{2+} - $2NO_3^-$ was completely separated from CO_2 when an ammonium sulfobetaine-3 stationary phase was used. For solution (iv), the standard deviation (%) for the peak areas were 2.1 and 5.6 and 3.5 for ion pairs of Ca^{2+} - $2Br^-$, Ca^{2+} - $2NO_3^-$ and Ca^{2+} - $2I^-$ respectively. The retention times for ion pairs Ca^{2+} -$2Cl^-$, Ca^{2+} - $2Br^-$, Ca^{2+} -$2NO_3^-$ and Ca^{2+} - $2I^-$, are 0.8, 0.5, 0.9 and 0.7 respectively.

A commercial column (Shim-pack IC-A3) was coupled to the HPLC system. An aqueous solution containing 8.0 mM p-hydroxybenzoic acid and 3.2 mM Bis-tris were used for the mobile phase (as recommended by the manufacturer). Stock solutions (ii) and (iv) were then analyzed using conventional IC. The conditions for conductometric detection were identical as used previously for EIC. Chromatograms in Figure 6 and 7 show that the level of base-line noise is very high and is almost the same intensity as the peak signal intensity for 0.1 μM of Br^- and NO_2^-. Furthermore, the stability of the base-line was very poor. In order to analyze all of the samples it was necessary to re-position the base line many times. After injection an inherent systematic peak appeared around a retention time of 14 minutes which was attributed to the CO_2. The peak corresponding to I^- was not observed under these conditions.

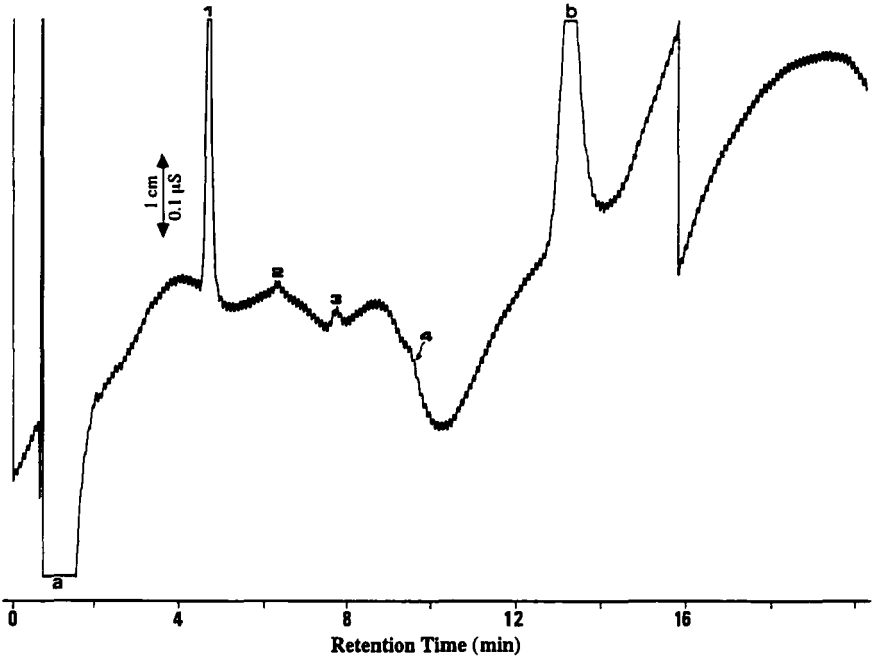


Figure 6. Chromatogram of an aqueous solution containing 0.1 μM each of NaCl*, $NaNO_2$, NaBr, $NaNO_3$ and NaI obtained using a conventional IC. Column: Shim-pack IC A3 (150 x 4.6 I.D mm); mobile phase: an aqueous solution conatining 8.0 mM p-hydroxybenzoic acid and 3.2 mM Bis-tris; flow rate: 1.0 mL/min. Other conditions are the same as described in Fig. 3. Peaks: 1, Cl^-; 2, NO_2^-; 3, Br^-; 4, NO_3^-; a, water-dip; b, CO_2. A peak due to I^- was not observed. *NaCl has been contaminated.

By direct comparison of the detection abilities of both EIC and conventional IC methodologies, the advantages of a water mobile phase over a mobile phase containing replacing ions have been demonstrated. Furthermore, while using water as the mobile phase, the detection ability of a UV-visible detection system is vastly improved.

Ultra-low levels of inorganic ions which have strong UV-absorption, for example, NO_2^-, Br^-, NO_3^- etc., is more easily detected. More so, even inorganic ions with much lower UV-absorption such as Cl^- could also be detected with a UV-visible detection when water was used as the mobile phase for IC.

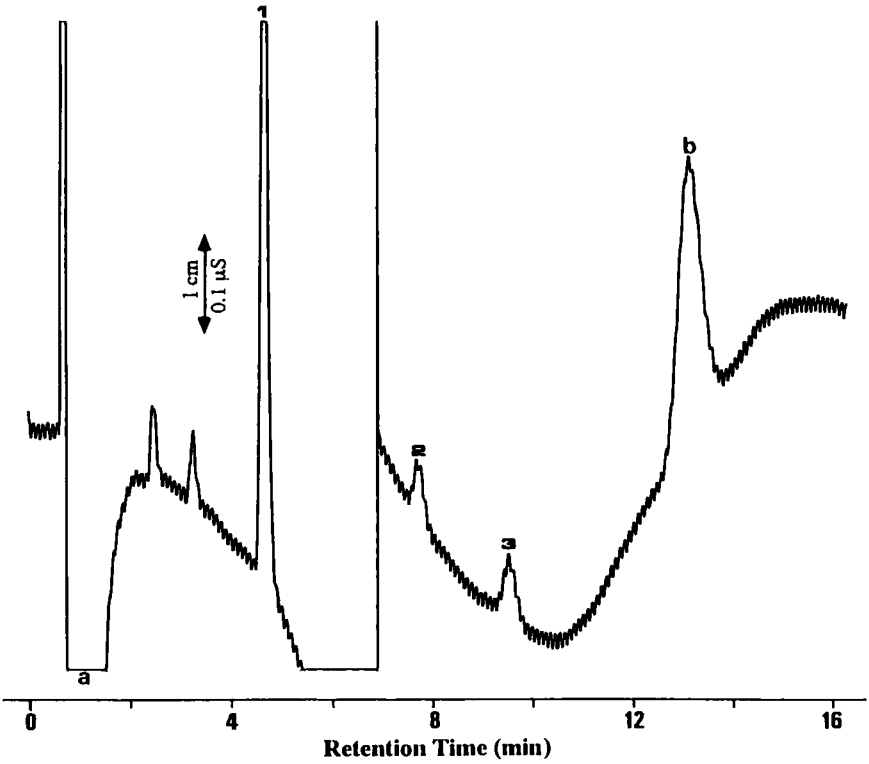


Figure 7. Chromatogram of an aqueous solution containing 0.1 μM each of $CaCl_2$*, $CaBr_2$, $Ca(NO_3)_2$ and CaI_2 obtained using a conventional IC (conditions are the same as described in Fig. 6). Other conditions are the same as described in Fig. 4. Peaks: 1, Cl^-; 2, Br^-; 3, NO_3^-; a, water-dip; b, CO_2. *$CaCl_2$ has been contaminated. A peak due to I^- was not observed.

CONCLUSION

The use of EIC rather than conventional IC offers several advantages. The EIC methodology is particularly useful for the simultaneous detection of inorganic cations and anions and the purification of inorganic salts, etc. However, EIC is to be strongly recommended for the analysis of trace levels of inorganic ions, since the detection limits obtained using EIC are extremely low. Furthermore, for the analysis of trace levels of inorganic ions, the analytes ions eluted from the Stern layer were equal to the sum of all the analytes and appeared as a single sharp peak. When samples containing inorganic ions at relatively high (> low-μM) levels are analyzed, identical analyte species are

involved in both the Stern layer and the diffuse layer. This can cause a secondary peak to be eluted together with the main peak. In this case a 'sacrifice' chemical is suggested which, when introduced into the original sample solution, 'encourages' the release of the analyte ions from the Stern layer to the diffuse layer, resulting in a single elution. The zwitterionic stationary phases, investigated in this and previous research, fail for the base-line separation of inorganic cations when they have the same charge. By designing the zwitterionic stationary phase, such that, the strength of the positive and the negative charges and the distance between the positive and the negative charges are appropriate, it is expected that the resulting zwitterionic stationary phase will result in the base-line separation of inorganic cations having the same charge. Pure water used in this and previous studies was the CO_2 -free one. When the conductivity detector was used with the ultra hight sensitivities, the very small amount of H^+/HCO_3^- due to the dissolved atmospheric CO_2 caused a small water-dip. This problem could be overcome by removing the dissolved CO_2 from the water mobile phase, but this will diminish the simplicity of EIC, which is its most significant advantage. Finally, it should be noted here that the conditions of the supporting column (used to obtain a zwitterionic stationary phase) are very important factors for determining the separation-abilities of EIC. When an ODS-packed column, initially coated with CHAPS, is used as the supporting column (for Zwittergent-3-14 or other zwitterionic surfactant-immobilized stationary phase formation) superior separation properties than those of a newly packed ODS column are obtained.

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