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Elution mechanism in electrostatic ion chromatography with histidine as an isoelectric ampholytic mobile phase

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Abstract

Electrostatic ion chromatography (EIC) using a zwitterionic stationary phase (formed by coating a C_{18} material with a hydrophobic zwitterionic surfactant) was studied with a mobile phase comprising an aqueous solution of histidine at the pH of its isoelectric point, together with non-suppressed conductometric detection. Anions and cations were found to be eluted as separate peaks, unlike the elution behaviour observed on the same system when pure water was used as mobile phase. An explanation was suggested in terms of protonation equilibria of the overall uncharged histidine to form small amounts of histidine cations and anions in the mobile phase which could act as counterions for analyte anions and cations. This suggestion was supported by measured pH changes occurring in the bands of eluted analyte anions (a decreased pH compared to the mobile phase) and cations (an increased pH compared to the mobile phase). The analytical potential of this type of EIC is discussed.

Keywords: Electrostatic ion chromatography; Stationary phases, LC; Ampholytes; Mobile phase composition; Inorganic anions; Inorganic cations

1. Introduction

In conventional ion-exchange chromatography, the presence of co-ions (usually referred to as competing ions) in the mobile phase is necessary to displace the analyte ions at the ion-exchange sites of the stationary phase. On the other hand, when the stationary phase comprises a zwitterionic site in which the positive and negative charges are located in close proximity, a non-ionic mobile phase such as pure water can be used. This type of chromatography, developed by Hu and co-workers and termed ''electrostatic ion chromatography (EIC)'' [1–9], has the advantage that a relatively weak interaction occurs between the charged sites on the stationary phase and analyte cations and anions, allowing the use of very weak eluents [1–3]. The elution mechanism is such that the analytes are eluted as ion-pairs (throughout this paper the term ion-pair implies a pair of ions eluted simultaneously), so that when water is used as mobile phase, a peak is usually observed for each combination of the anions and cations in the sample. This can often lead to the complication of the same analyte anion appearing as several different peaks in the final chromatogram, being paired with a different cation on each occasion.

A significant drawback of using pure water as mobile phase in EIC is the lack of buffering and this can limit the application of the technique to acidic or alkaline samples, where sufficient buffering capacity

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is essential [10,11]. At the same time, when using non-suppressed conductometric detection, the conductivity of the mobile phase should be kept as low as possible in order to enhance detection sensitivity [12]. Although the requirements for buffering and low conductivity background seem contradictory, these two properties can be combined if isoelectric ampholytic buffers are used as the mobile phase. These buffers are zwitterionic substances in which the two pK_a values bracketing the isoelectric point (pI) are sufficiently close (within 1 pH unit from the pI) to produce some degree of buffering at the pI. Amino acids, such as glutamic acid (pI=3.3), histidine (pI=7.7), or lysine (pI=9.7) [13] are some examples of ampholytic species which show buffering capacity.

Isoelectric buffers used at a pH value corresponding to their pI have been used in capillary electrophoresis (CE) first by Hjerten et al. [13] and later by Righetti and co-workers [14–23] to minimise the separation current, and in this laboratory [24–27] to prevent competitive displacement when applying indirect detection. Although zwitterionic buffers such as morpholinoethanesulfonic acid (MES) have been used in suppressed ion chromatography to separate anions [28] and acidic mobile phases containing cationic bases such as diaminopropionic acid or histidine have been used in cation-exchange chromatography [29], isoelectric buffers operated at their pI have not been used as chromatographic eluents. To the best of our knowledge only one account of the use of an isoelectric mobile phase has appeared: isoelectric histidine has been used to elute metal ions from a chelating stationary phase [30], but in this case the histidine was at its isoelectric point (pI 7.6) only at the beginning of the elution, after which a pH gradient to pH 3.4 was applied, so that histidine became increasingly cationic over the course of the run.

In this study we have investigated the use of isoelectric ampholytes as mobile phases in EIC. Specific aims were to determine whether elution of analytes would occur with an isoelectric ampholytic mobile phase, whether the analyte ions would be eluted as ion-pairs formed between the analyte anions and analyte cations, or as anions and cations separately with counterions being provided by the eluent, what was the separation selectivity, and whether non-suppressed conductivity detection could be used.

2. Experimental

2.1. Instrumental

The chromatographic hardware comprised a Waters Model 590 Pump (Waters, Milford, MA, USA), a UK6 injector valve (Waters) and a Waters Model 430 conductivity detector interfaced to a Waters Maxima 820 data station. The column was an L-Column ODS (250×4.6 mm I.D.), packed with 5 μm particles having 120 Å pore size, 17% C, 340 m^2/g (CIT, Tokyo, Japan), and operated at 40°C.

A pH meter (Activon Model 210, Activon, Pennant Hills, Australia) fitted with a semi-micro combined glass–calomel electrode (AEP336) was used for the pH detection studies in which the outlet of the conductivity detector was allowed to run over the side at the sensing bulb of the electrode held in a holder at an angle of approx. 45° to the horizontal.

2.2. Reagents

3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) (98% purity, Aldrich, Milwaukee, WI, USA), 3-(*N*,*N*-dimethyldodecylammonio)propane sulfonate (>97% purity, Fluka, Buchs, Switzerland), MES (>99% purity, Fluka) and histidine (98% purity, Aldrich) were used. Z1-Methyl [*N*,*N*,*N*-trimethylammonium butanesulfonate, $^{-}O_3S–(CH_3)_4–N(CH_3)_3^{+}$] is a proprietary reagent of Waters. All other chemicals were analytical-reagent grade. Water was treated with a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus.

2.3. Procedures

The zwitterionic stationary phase was prepared using a two-step coating method [31]. In brief, the ODS column was flushed with 100 ml acetonitrile–water (70:30) followed by 100 ml water (both at 1 ml/min). After this, CHAPS (30 m*M* in propanol–

water, 5:95) was pumped through the column (50 ml at 1 ml/min), followed by 3-(*N*,*N*-dimethyldodecylammonio)propane sulfonate solution (40 m*M* in propanol–water, 5:95, 50 ml at 1 ml/min), and finally water was pumped through at 1 ml/min until the baseline on the conductivity detector was stable.

Mobile phases were filtered through a 0.45-μm membrane filter and degassed prior to use.

3. Results and discussion

3.1. Elution mechanism

Elution with water as the mobile phase in EIC causes the analyte ions to be eluted as ion-pairs formed between the analyte anions and analyte cations, as shown in Fig. 1. This occurs as a result of the lack of counterions in the mobile phase [12] and because the electroneutrality of the analyte band must be preserved. When using an isoelectric ampholyte as mobile phase, the first question which arises is whether analytes will be eluted as ion-pairs comprising those species present originally in the sample (as in EIC with water as mobile phase) or as separate peaks for each anion and cation with the mobile phase ions acting as counterions (as in conventional ion-exchange chromatography). Separations of standard mixtures of up to seven anions and three cations are shown in Figs. 2a and 3a and reveal that the analyte anions and cations were eluted separately. This implies that the isoelectric ampholyte histidine (which has an overall zero charge at the pH used) produced cations and anions to act as

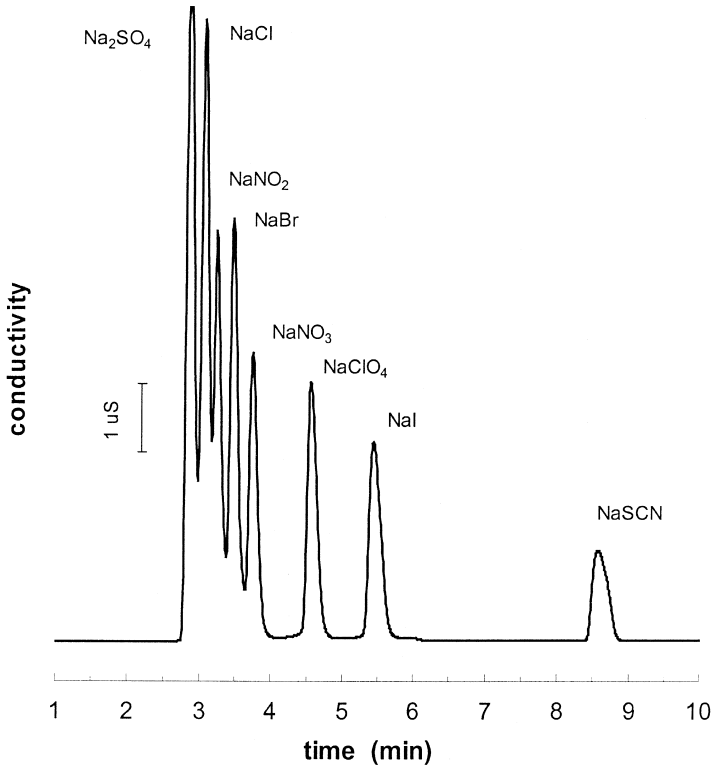

Fig. 1. Chromatogram of a mixture of sodium salts of eight anions using water as the mobile phase and conductometric detection. Mobile phase, water; flow-rate 1.0 ml/min; sample, 50 μl of a solution containing 1.0 m*M* of each analyte.

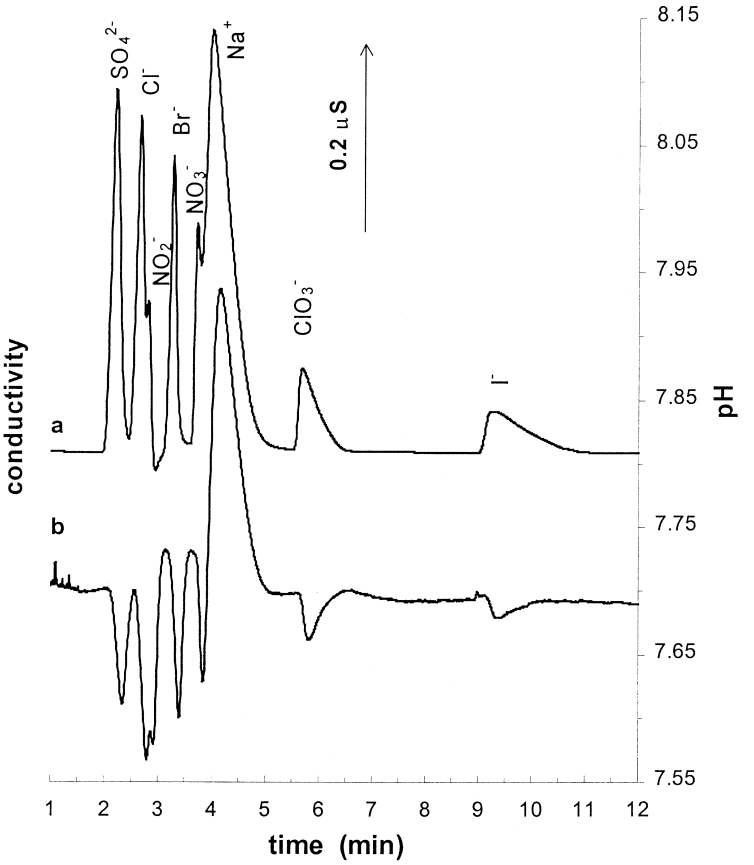

Fig. 2. Chromatogram of a mixture of sodium salts of seven anions using aqueous histidine as the mobile phase and (a) conductometric detection or (b) pH-sensitive detection. Mobile phase, 50 m*M* histidine, pH 7.7; sample, 50 μl of a solution containing 0.1 m*M* of each analyte; other conditions as in Fig. 1.

counterions for the eluted analyte ions. Histidine exhibits the following protonation equilibria:

$$[\overline{NH^+\text{-CH-NH-CH}=C}\text{-CH}_2\text{-CH}(NH_3)^+\text{-COO}^-]^+ \Leftrightarrow$$

$$\Leftrightarrow [\overline{N=\text{CH-NH-CH}=C}\text{-CH}_2\text{-CH}(NH_3)^+\text{-COO}^-]^0 \Leftrightarrow$$

$$\Leftrightarrow [\overline{N=\text{CH-NH-CH}=C}\text{-CH}_2\text{-CH}(NH_2)\text{-COO}^-]^-$$

The corresponding species distribution diagram is given in Fig. 4 and shows that at the isoelectric point histidine exists not only as a neutral molecule, but as both a cation and an anion, with the latter species present at a level of approx. 3%. These cations and anions of histidine can act as counterions for both analyte anions and cations. Fig. 5 shows the effective charge and buffering capacity of histidine at various pH values.

However, an experimental confirmation of this hypothesis was necessary and attention was therefore directed towards the pH of the eluted sample bands. If a sample band of an eluted analyte anion also contains protonated histidine as its countercation, this band should have a lower pH than that of the isoelectric mobile phase. Conversely, if a sample band of an eluted analyte cation contains deprotonated histidine as the counteranion, this band should have a higher pH than the isoelectric mobile phase. When pH-sensitive detection was applied (in addi-

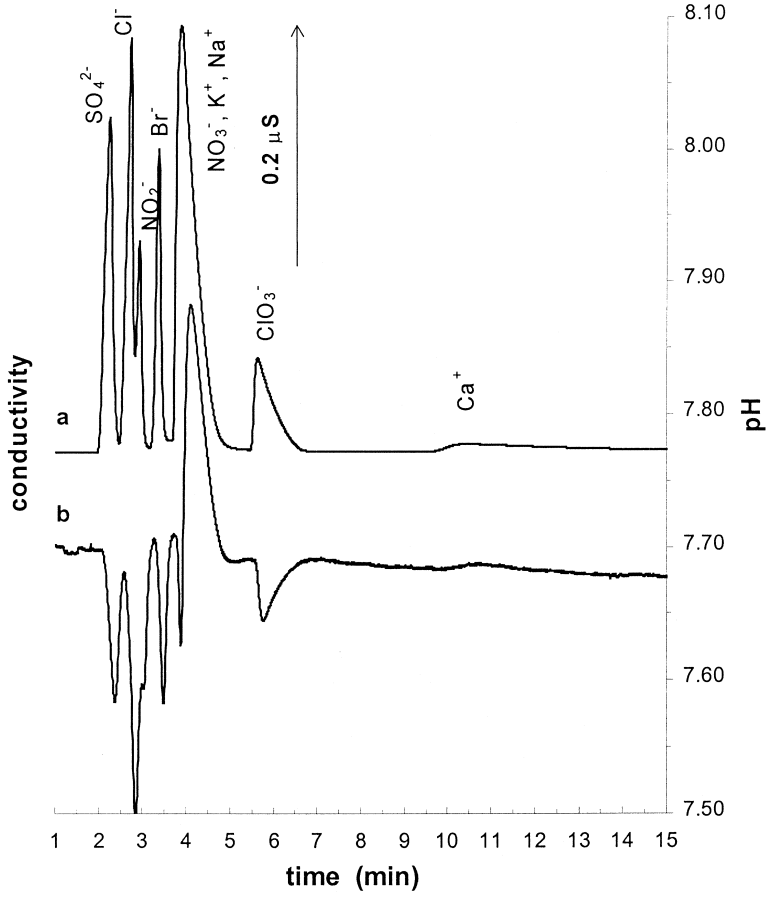

Fig. 3. Chromatogram of a mixture of six anions and three cations using aqueous histidine as the mobile phase and (a) conductometric detection or (b) pH-sensitive detection. Conditions as in Fig. 2.

tion to conductometric detection) the chromatograms shown in Figs. 2b and 3b were obtained and the observed pH changes were consistent with the hypothesis proposed above.

To confirm further the suggested elution mechanism, a zwitterionic compound with overall zero charge but not capable of undergoing protonation equilibria like histidine was used as a mobile phase. Z1-Methyl is such a zwitterionic compound [^-O_3S–$(CH_3)_4$–$N(CH_3)_3^+$] and when used as mobile phase the elution behaviour (Fig. 6) was almost identical to that obtained with water as mobile phase (Fig. 1). These results suggested that zwitterions which are not capable of protonation equilibria did not produce an ion-exchange elution because they cannot produce ions to act as counterions for the analyte ions in the eluted sample bands. Additionally, the fact that the separation selectivity with aqueous Z1-Methyl as the mobile was the same as that with water alone indicates that there was no stationary phase modification by this zwitterionic compound.

3.2. Retention behaviour

When the histidine isoelectric mobile phase was used, retention times decreased slightly and separation efficiency increased with increasing histidine

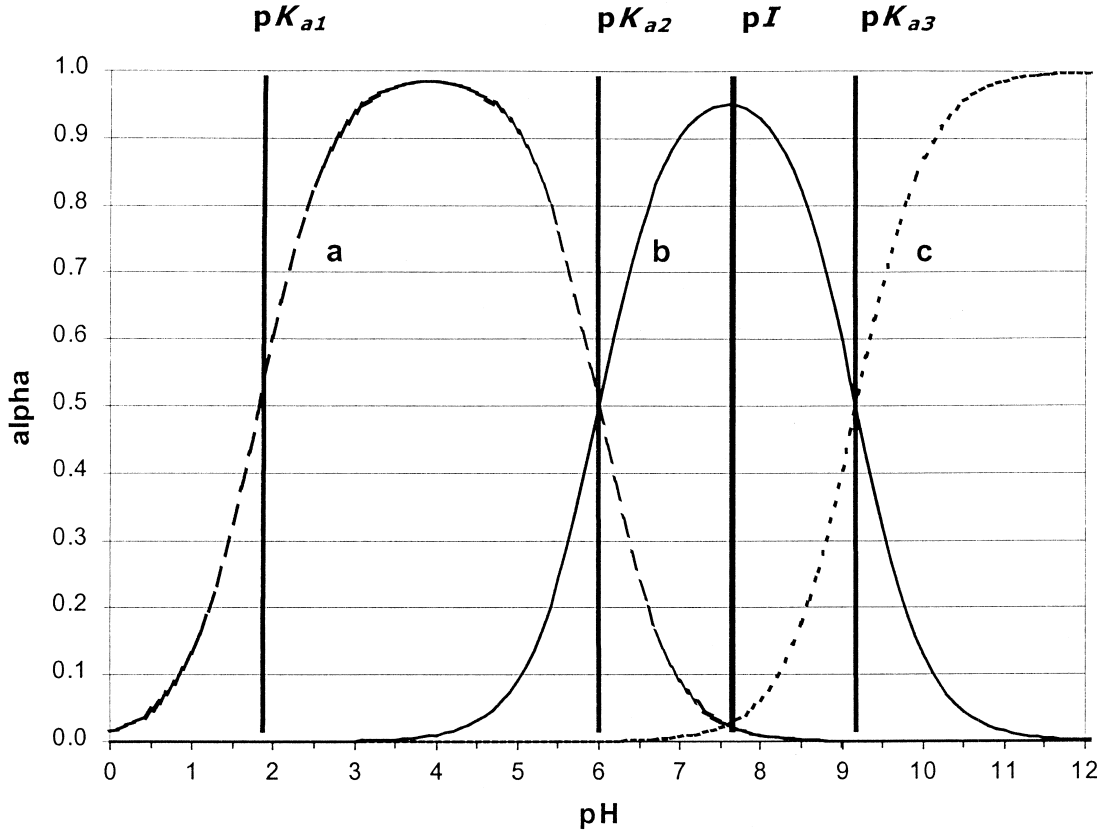

Fig. 4. Species distribution graph showing the fraction of each species (alpha) of histidine as a function of pH. The species are (a) cationic protonated histidine, (b) neutral histidine, and (c) anionic deprotonated histidine.

concentration. At 10 m*M* histidine the later eluted peaks exhibited unacceptably broad and tailed shapes but 50 m*M* histidine was found to be a sound compromise between an elevated background conductance and acceptable peak shape and separation efficiency. The separation efficiencies for the histidine mobile phases were somewhat lower than those obtained on the same column operated in the EIC mode using water or dilute electrolytes as mobile phases [4]. This might be caused by the fact that the effective charge of histidine as the analyte countercation or counteranion will be very low so sample dispersion is necessary to achieve electroneutrality. The effective charge can be calculated from the experimental data by noting that the typical pH changes across the eluted bands were about 0.1 pH unit (Figs. 2b and 3b), which at the pH of the mobile phase (7.7) corresponded to a change of $[H^+]$ concentration of $4.8 \cdot 10^{-5}$ *M*, producing a maximum overall charge of approx. ± 0.001 ($+$ charge for the acidic bands of eluted anions and $-$ charge for the alkaline bands of eluted cations) for a 50 m*M* histidine mobile phase. By comparison, a separation run using MES (as anion) and histidine (as cation) at pH 6.1 (Fig. 7) gave the same elution order for the anions as did the above histidine mobile phase, but the peaks were more symmetrical and sharper. This mobile phase, which has been used as an electrolyte for non-suppressed conductometric detection of analytes by CE [32,33], contains histidine with an effective charge of ca. $+0.5$. The presence of anions (MES) and cations (histidine) of a higher effective

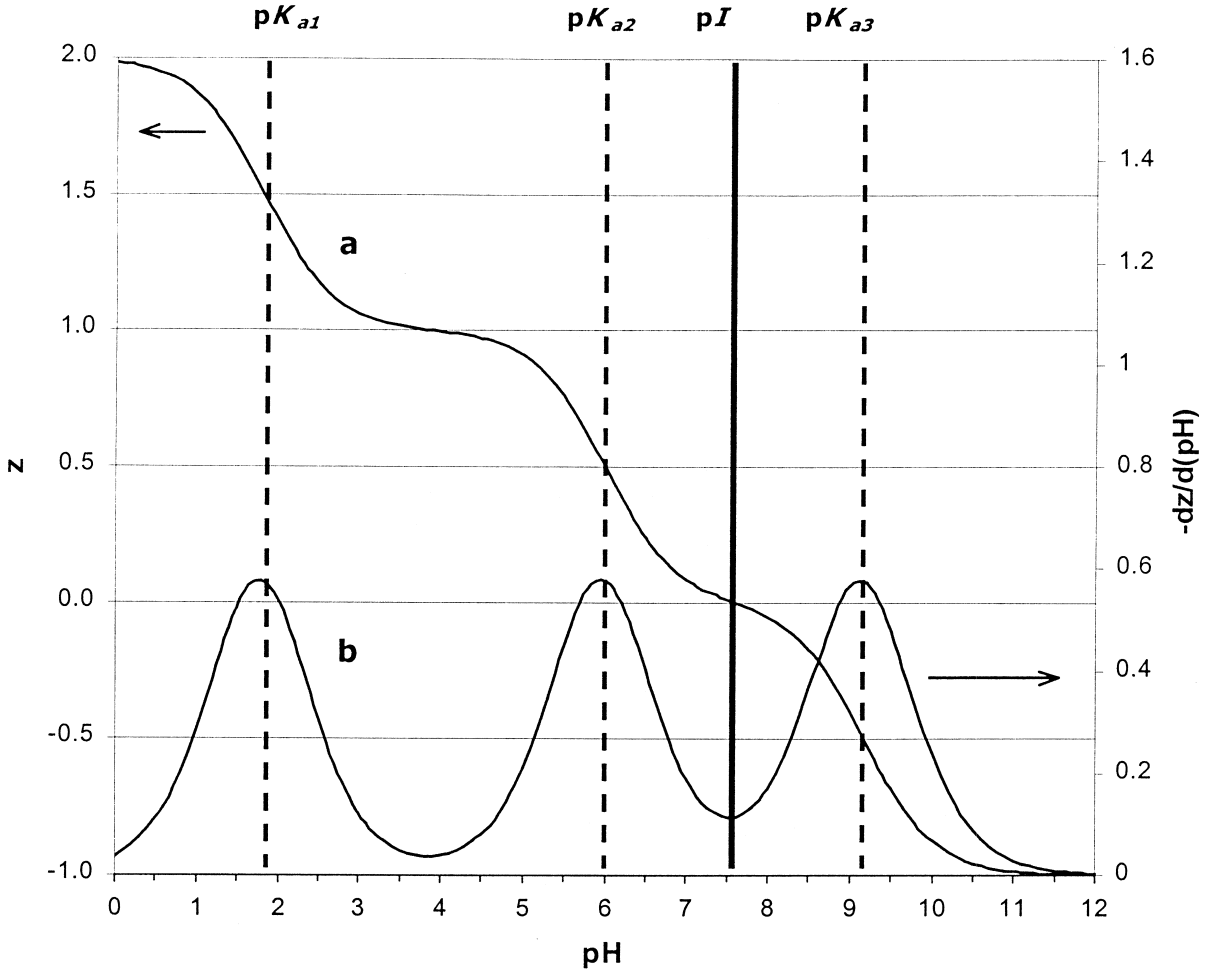

Fig. 5. Plot of the overall charge (z) per molecule of histidine versus (a) pH and (b) first derivative $-dz/dpH$ indicating buffering capacity of histidine as a function of pH.

charge (± 0.5) compared to the isoelectric histidine may be the explanation for the higher efficiency for the MES–histidine mobile phase.

3.3. Separation selectivity and detection sensitivity

Comparison of Fig. 1 with Figs. 2a and 3a shows that the separation selectivity for anions is quite similar in each case. This suggests that the same EIC mechanism is operating for each system, but that in the case of the histidine mobile phase, analyte anions are eluted as ion pairs with histidine counterions. This is consistent with previous studies using EIC with dilute electrolytes as mobile phases [4,5,7]. However, the ability to obtain a single peak for the analyte cation (in this case sodium ion), simultaneously with single peaks for each analyte anion, represents a new development in EIC. It is also noteworthy that the histidine mobile phase does not alter the separation selectivity (judged by the elution order for the anions) which indicates that there was

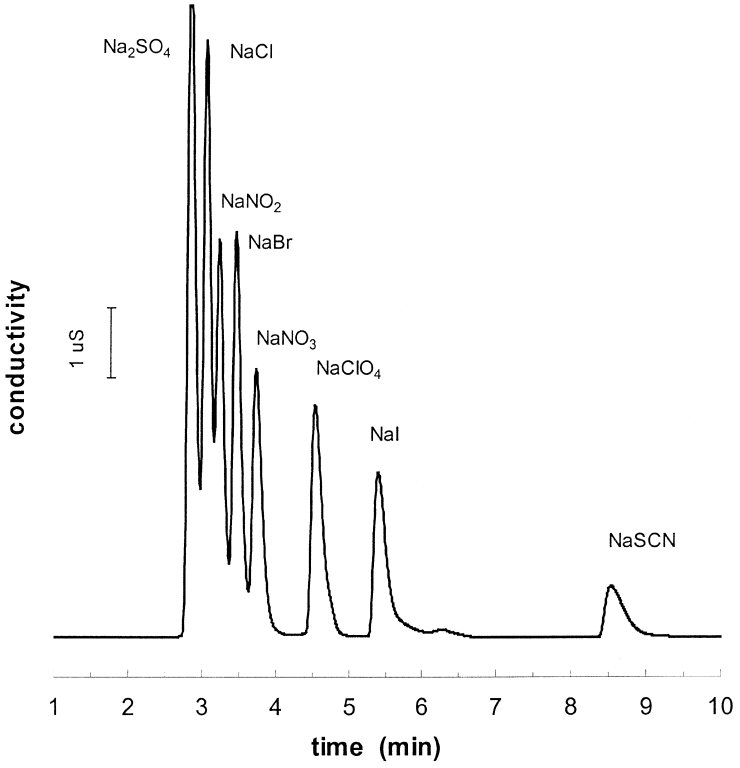

Fig. 6. Chromatogram of a mixture of sodium salts of eight anions using aqueous zwitterionic Z1-Methyl compound as the mobile phase and conductometric detection. Mobile phase, 10 m*M* Z1-Methyl; other conditions as in Fig. 1.

no change in the nature of the zwitterionic stationary phase, which could have occurred as a result of adsorption of the histidine.

With regard to the performance of the non-suppressed conductivity detection, the background for the 50 m*M* histidine was approximately an order of magnitude higher compared to water as mobile phase, but the baseline was stable and baseline noise was not significantly higher than the water mobile phase (in both cases approx. $4 \cdot 10^{-5}$ S). While suppressed conductivity detection is known to offer the best sensitivity in ion chromatography [12], the use of isoelectric mobile phases could be an interesting option for non-suppressed conductivity detection.

4. Conclusions

Several conclusions can be drawn with respect to the mechanism, selectivity of the separation, and detection in EIC using isoelectric ampholytes as mobile phases. First, protonation equilibria of the isoelectric ampholyte produces cations and anions which can act as counterions for the eluted analyte anions and cations, which appear as separate bands in the final chromatogram. Second, the separation selectivity for anions is similar to that in EIC with water as mobile phase. Third, low conductivity isoelectric mobile phase such as histidine enables both buffering of mobile phase and the use of sensitive non-suppressed conductivity detection. This approach could be useful as an alternative to suppressed conductivity detection.

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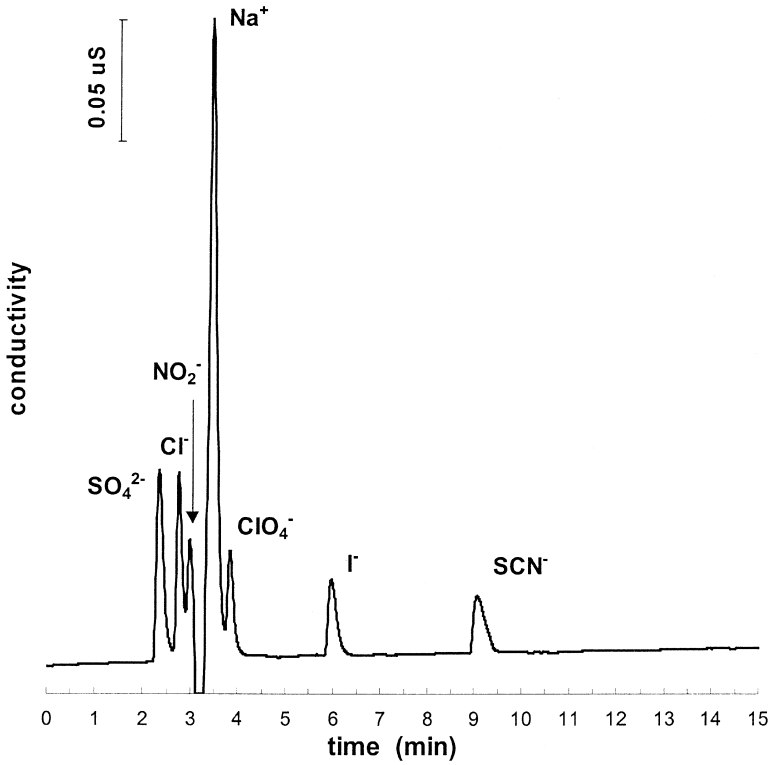

Fig. 7. Chromatogram of a standard mixture of anions using MES–histidine mobile phase. Mobile phase, 10 m*M* MES, 10 m*M* histidine, pH 6.1; sample, 10 μl of a solution containing 1.0 m*M* of each analyte; other conditions as in Fig. 1.

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