New conductivity detection response equation for anions eluted with fully and partially ionised eluents in non-suppressed ion chromatography

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Abstract

A response equation for conductivity detection in ion chromatography has been derived. This equation is applicable to non-suppressed ion chromatography using both fully ionised and partially ionised eluents. A prime assumption of this equation is that when partially ionised eluents are used (such as benzoic acid), both the ionised and neutral components of the eluent species contribute to the detector response of anionic analytes. Experimental evidence is provided to support this assumption in that pH changes accompanying the elution of an analyte have been measured. These pH changes are proportional to the concentration of analyte injected onto the column, in accordance with predictions from the response equation. Furthermore, it is shown that protonated eluents (such as benzoic acid) give more sensitive detection than equivalent ionised eluents (such as potassium benzoate) and the signal enhancement achieved using a protonated eluent species is in accordance with theory.   © 2002 Published by Elsevier Science B.V.

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1. Introduction

Conductivity is the most common method for detecting inorganic anions in ion chromatography (IC), and since all ions are electrically conducting, this mode of detection is universal in response. Furthermore, the detectors themselves are relatively simple to manufacture and operate. The development of detector response equations which allow the conductance signal and its relationship to various eluent and analyte parameters to be calculated is therefore an important aspect of conductivity detection.

A number of conductivity detection response equations have been derived. In the case where the eluent competing species is completely ionised, these different derivations have yielded response equations which are generally consistent in form. Partially ionised eluents are also used commonly in non-suppressed IC, especially benzenecarboxylic acids such as benzoic, phthalic and trimesic acids which typically find use for the separation of monovalent and polyvalent analyte anions [1–4]. The degree of ionisation of these eluents is dependent on the eluent pH and this parameter is often used as a convenient means to manipulate retention times. A recent review of the determination of anions in complex matrices by IC demonstrates the continuing applicability of using these eluents to analyse various samples [5].

There have been several previous approaches to

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the derivation of a conductivity response equation for partially ionised eluents. Close scrutiny of these equations reveals major inconsistencies, such that there is general ambiguity regarding the accuracy of each equation and the conditions under which it can be applied. The purpose of the present work has been to provide a detailed derivation of a conductivity detection response equation which is applicable to both fully and partially ionised eluents and which has all assumptions stated clearly. The particular focus of this study is to clarify the manner in which analyte elution is accomplished when partially ionised eluents are used and to examine the effects of this elution process on the conductivity detection response. Further, experimental evidence is provided to support the major assumptions made.

2. Theory

2.1. Fully ionised eluents

Gjerde et al. [6,7] were the first to derive a theoretical response equation for the change in conductance arising from the elution of an analyte anion in IC, using a fully ionised eluent. The detection response equation can be derived from the difference in the detector response between the background eluent and the eluted sample band, and is given by:

$$G_{\text{Background}} = \frac{(\lambda_{\text{E}^+} + \lambda_{\text{E}^-})C_{\text{E}}}{10^{-3} K} \qquad (1)$$

$$G_{\text{Elution}} = \frac{(\lambda_{\text{E}^+} + \lambda_{\text{E}^-})(C_{\text{E}} - C_{\text{S}})}{10^{-3} K} + \frac{(\lambda_{\text{E}^+} + \lambda_{\text{S}^-})C_{\text{S}}}{10^{-3} K} \qquad (2)$$

$$\Delta G = G_{\text{Elution}} - G_{\text{Background}} = \frac{(\lambda_{\text{S}^-} - \lambda_{\text{E}^-})C_{\text{S}}}{10^{-3} K} \qquad (3)$$

Here, the eluent comprises the electrolyte E^+E^- and the analyte anion is represented by S^-. G_{Elution} is the conductance of the analyte band, $G_{\text{Background}}$ is the conductance of the background, and ΔG is the change in conductance during elution of the analyte. λ_{S^-}, λ_{E^+} and λ_{E^-} are the limiting ionic conductances of the analyte ion and eluent ions, respectively, C_{S} and C_{E} are the total concentrations of the analyte and

eluent ions (i.e. their "formal" concentration), and K is the conductivity cell constant, expressed in cm^{-1}.

Both the eluent electrolyte and the analyte ion are assumed to be fully ionised and the analyte anion is assumed to undergo stoichiometric exchange with E^- at the ion-exchange sites on the stationary phase. That is:

$$[\text{E}^-]_{\text{At sample elution}} = C_{\text{E}} - C_{\text{S}} \qquad (4)$$

Similar conductivity detection response equations have appeared in various monographs on ion chromatography (e.g. Ref. [8]) and have been found to provide an accurate description of experimental behaviour when fully ionised eluents and analytes are involved.

2.2. Partially ionised eluents

We now consider the case where both the eluent and the analyte are not completely dissociated as a result of the operating pH of the eluent system. It is convenient to define I_{E} as the fraction of the total eluent concentration (C_{E}) that is dissociated, such that:

$$[\text{E}^-] = C_{\text{E}}I_{\text{E}} \qquad (5)$$

$$[\text{HE}] = C_{\text{E}}(1 - I_{\text{E}}) \qquad (6)$$

Similarly, we can define I_{S} as the fraction of the total analyte concentration (C_{S}) that is dissociated (note that I is equivalent to α, which is a commonly used term to denote the fraction of a species existing in a particular form), such that:

$$[\text{S}^-] = C_{\text{S}}I_{\text{S}} \qquad (7)$$

$$[\text{HS}] = C_{\text{S}}(1 - I_{\text{S}}) \qquad (8)$$

Eq. (1) needs to be modified to account for the fact that the eluent is only partially dissociated, to give:

$$G_{\text{Background}} = \frac{(\lambda_{\text{E}^+} + \lambda_{\text{E}^-})C_{\text{E}}I_{\text{E}}}{10^{-3} K} \qquad (9)$$

Eq. (2) also needs to be modified to reflect the fact that both the neutral and ionised forms of the eluent and the analyte are present. The following assumptions are made:

(i) The analyte binds to the resin only as S^-.

(ii) Even when analyte is injected as a mixture of its protonated (HS) and deprotonated (S⁻) forms, all of the analyte becomes bound to the stationary phase as S⁻. However, it should be remembered that most of the analytes used in IC are anions of strong acids and therefore are normally present only in the fully ionised form.

(iii) Both the protonated (HE) and deprotonated (E⁻) forms of the eluent participate in the elution process, as depicted schematically in Fig. 1.

On the basis of these assumptions, the concentration of eluent present in the analyte band is given by:

$$[\text{Eluent}]_{\text{At sample elution}} = C_E - C_S \tag{10}$$

Fig. 1 shows that when eluents containing HE are used, some H^+ is produced in the analyte band. However, it can be assumed that I_E for the bulk eluent will remain the same as I_E for the analyte band because the pH change accompanying sample elution will be small and C_E is generally much greater than C_S. [E⁻] in the analyte band is therefore given by:

$$[E-]_{\text{At sample elution}} = (C_E - C_S)I_E \tag{11}$$

and if allowance is made for the fact that the analyte can become protonated in the eluent phase, the total conductance of the eluted analyte band is:

$$G_{\text{Elution}} = \frac{(\lambda_{E^+} + \lambda_{E^-})(C_E - C_S)I_E}{10^{-3}\,K}$$
$$+ \frac{(\lambda_{E^+} + \lambda_{S^-})C_S I_S}{10^{-3}\,K} \tag{12}$$

As before, we can now determine the conductance change occurring when the analyte is eluted by subtracting the conductance of the background (Eq. (9)) from that of the analyte band (Eq. (12)). This gives:
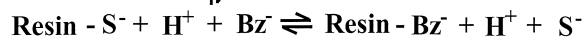
HBz
⇅
Resin - S⁻ + H⁺ + Bz⁻ ⇌ Resin - Bz⁻ + H⁺ + S⁻

Fig. 1. Schematic showing the role of the neutral, protonated form of the eluent (HE) in the elution of an analyte anions A⁻. Modified from Ref. [12].

$$\Delta G = G_{\text{Elution}} - G_{\text{Background}}$$
$$= \left(\frac{(\lambda_{E^+} + \lambda_{S^-})I_S - (\lambda_{E^+} + \lambda_{E^-})I_E I_S}{10^{-3}\,K} \right) \cdot C_S \tag{13}$$

Some clarification is necessary on the nature of the term E^+ in Eq. (13). For an eluent comprising an ionic salt, such as potassium benzoate, then E^+ will be the potassium ion. In the case of an eluent comprising a weak acid, HE, assumption (iii) above and Fig. 1 show that the cation accompanying the sample anion when it reaches the detector will be H^+. The higher value of the limiting equivalent ionic conductance of H^+ compared to K^+ leads to the detection signal for the weak acid eluent (e.g. benzoic acid) being higher than that for a potassium salt of the same species (potassium benzoate). Eluents comprising a mixture of protonated and deprotonated species (e.g. benzoic acid and benzoate) will give a conductivity detector response intermediate between the values calculated from Eq. (13) using either the ionic salt or the weak acid as eluent.

Eq. (13) differs markedly from most previously derived response equations, but is very similar to the response equations presented recently by Fritz and Gjerde [9] and Yu [10]. However, C_E has been defined differently in the present derivation. In Refs. [9,10], C_E was defined as the concentration of ionised eluent (i.e. [E⁻]), whereas in the above derivation C_E is the *total* eluent concentration (i.e. [HE]+[E⁻]), based on the key assumption that both HE and E⁻ contribute to elution of analyte anions. This assumption is investigated in the present work.

A number of important observations arise from Eq. (13). First, the conductance signal is proportional to the analyte concentration, C_S, and increases as the degree of ionization of the analyte in the eluent phase, I_S, is increased. Second, the conductance change predicted by Eq. (13) is zero when the eluent and the analyte are the same, that is when:

$$\lambda_{S^-} = \lambda_{E^-}, \quad I_S = I_E \tag{14}$$

This behaviour is observed in practice and provides a mechanism to detect components of overlapping peaks. This can be achieved using one component of the overlapping peak pair as the eluent, so that no detection signal will be observed for that component,

allowing the second component of the overlapping peak pair to be quantified [10,11]. Third, Eq. (13) shows that in non-suppressed IC, the detection signal increases as I_E decreases, so that eluents which are weakly dissociated give more sensitive detection than those that are strongly dissociated. Finally, when a fully ionised eluent and analyte are used (i.e. I_E and I_S are unity), Eq. (13) simplifies to Eq. (3).

3. Experimental

3.1. Instrumentation

The chromatographic system comprised a Waters Model 510 pump (Milford, MA, USA), a Waters Model 717 auto-sampler set to inject 75 µl of sample, and a Waters Model 430 conductivity detector interfaced to a Waters Maxima 820 data station. The analytical column used was a Dionex (Sunnyvale, CA, USA) Ionpac AS11 together with an AG11 guard column, operated at 35 °C. An Activon Model 210 pH meter (Pennant Hills, Australia) fitted with a semi-micro combined glass–calomel electrode (AEP336) was used for the pH detection studies. The outlet tubing from the conductivity detector was connected to a 0.65-ml microcentrifuge tube via a small hole drilled at the base and sealed, with another small hole on the side to allow the column effluent to run to waste. The tube was maintained in an upright position with a clamp, and the electrode bulb placed inside and held stationary with a clamp.

3.2. Reagents

Benzoic acid (9.45 m*M*; BDH, Kilsyth, Australia) and 0.75 m*M* potassium benzoate (Pfaltz and Bauer, Waterbury, CT, USA) eluents were prepared from analytical grade salts using Ultra-pure Milli-Q water (Millipore, MA, USA). The potassium benzoate eluent, initially pH 9.8, was adjusted to pH 6.2 using Dowex 50W-X2-100 strong cation-exchange beads (Aldrich, WI, USA) and dilute potassium hydroxide (BDH) as required, in order to avoid the addition of further anionic species to the eluent. Both eluents were delivered at 1 ml min^{-1}.

Anion standards were prepared daily by dissolution of analytical grade salts in the sodium form using Milli-Q water. Chlorite was obtained from Aldrich; bromate and chlorate from BDH; iodate, formate, nitrate, nitrite and chloride from Ajax, Auburn, Australia; bromide from Sigma (St Louis, MO, USA) and fluoride from Prolabo (Paris, France).

4. Results and discussion

A careful evaluation of previous derivations of detector response equations for partially ionised eluents shows that a major point of difference arises from the assumptions made regarding the eluting roles of the neutral and ionised forms of the eluent. Specifically, the important question is whether both the neutral and ionised forms of the eluent participate in the elution of the analyte, or whether elution results solely from the ionised form of the eluent. Intuitively, one would expect that the ionised form of the eluent, E$^-$, would be responsible for analyte elution and the neutral form, HE, would not. However a study using weakly ionised eluent species [12], such as benzoic acid, suggests that both HE and E$^-$ participate in the elution of analytes. The explanation for this behaviour was that neutral benzoic acid became dissociated in the presence of the sample band, providing benzoate ions which then contributed to sample elution. This process is represented schematically in Fig. 1. An outcome of this process is that the sample band should contain H$^+$ ions from the dissociation of the neutral benzoic acid, which should be detectable as pH changes and should also increase the sensitivity of detection.

Gjerde and Fritz [12] have compared the elution strengths of eluents formed from either benzoic acid or potassium benzoate, but containing equal concentrations of benzoate ion. By measuring retention data using a laboratory-fabricated XAD-1 functionalised quaternary ammonium anion-exchanger, they found that the benzoic acid eluent gave stronger elution than the benzoate eluent, in accordance with Fig. 1. We have carried out a similar experiment using 9.45 m*M* benzoic acid at pH 3.1 (which from the pK_a for benzoic acid [4.19] is 8% ionised) or 0.75 m*M* potassium benzoate at pH 6.2 (in order to ensure complete ionisation) as eluent on the Dionex AS11 column. Retention data (Table 1) showed that retention factors were only slightly lower in the

Table 1
Retention of analyte ions on the AS11 column, using benzoic acid and potassium benzoate eluents containing the same concentration of benzoate ions

Solute ion	k' Values	
	Benzoic acid (9.45 mM, pH 3.1)	Potassium benzoate (0.75 mM, pH 6.2)
Fluoride	0.77	1.62
Iodate	1.98	2.04
Chlorite	2.92	3.15
Nitrite	3.03	6.38
Bromate	4.40	4.57
Chloride	5.05	5.40
Chlorate	11.04	11.91
Bromide	11.17	11.87
Nitrate	11.23	11.68

benzoic acid eluent for all ions except fluoride. These data suggest that the protonated benzoic acid acts as a very weak eluent only. In the case of fluoride, the decreased retention observed in the benzoic acid eluent is due to protonation of fluoride in the analyte band. This effect is discussed further below.

The mechanism suggested in Fig. 1 was further investigated by monitoring pH changes when the
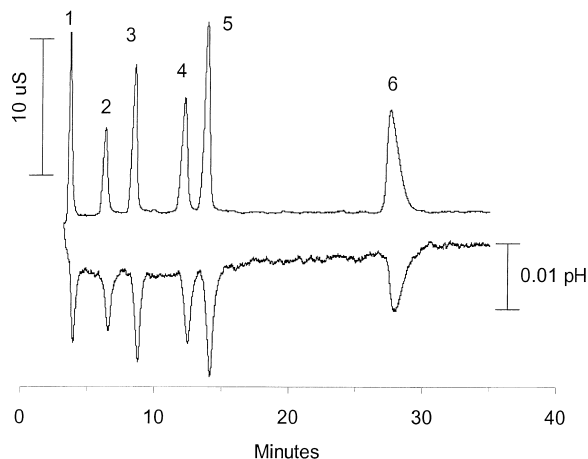

Fig. 2. pH changes accompanying the elution of (1) fluoride, (2) iodate, (3) chlorite, (4) bromate, (5) chloride and (6) bromide on a Dionex AS11 column using a 9.45 mM benzoic acid eluent at pH 3.1 and a flow-rate of 1 ml/min. All solutes were injected at a concentration of 40 mg/l with the exception of fluoride (30 mg/l) and chloride (10 mg/l).

benzoic acid eluent was used. Fig. 2 shows the conductivity detector and pH electrode outputs and demonstrates clearly that the elution of each analyte was accompanied by a measurable decrease in pH, as predicted in Fig. 1. In accordance with the proposed mechanism, the observed pH change for each analyte was found to be directly proportional to the amount of injected sample, as shown in Fig. 3. The different slopes observed for different analytes in Fig. 3 are related to the fact that the data are for the height of the pH peak, rather than peak area, since the latter could not be measured with the experimental set-up and integration software used.

A further outcome of the elution mechanism in Fig. 1 and the response equation given in Eq. (13) is that detection sensitivity should be higher in the weakly ionised benzoic acid eluent than in the benzoate eluent as a result of H^+ ions contributed to the sample band by the dissociation of the eluent. Comparison of peak areas obtained in the two eluents showed that the benzoic acid eluent gave peak areas an average of 4.89 times greater than the benzoate eluent for all analyte anions (except fluoride and nitrite), measured at four different concentrations of each analyte. This enhancement factor is in reasonably close agreement with the expected value of 4.73 which would result from the replacement of potassium ions ($\lambda_+ = 74$ S cm^2 equiv.$^{-1}$) with hydronium ions ($\lambda_+ = 350$ S cm^2 equiv.$^{-1}$) [8]. The enhancement factor for fluoride was only 2.47,
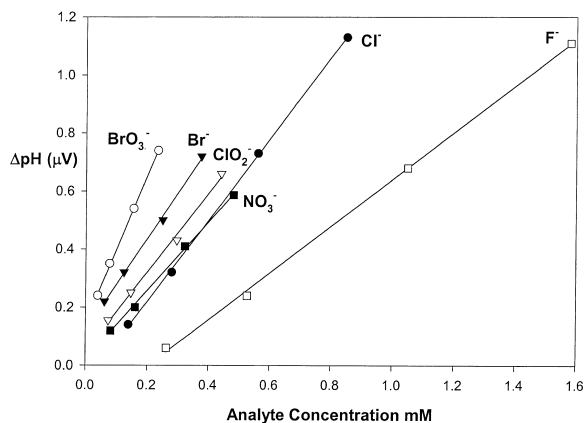

Fig. 3. pH changes accompanying the elution of analyte anions using a benzoic acid eluent. Separation conditions as for Fig. 2.

which can be attributed to protonation of this species by the accompanying hydronium ions due to the weakly acidic nature of hydrofluoric acid (pK_a 3.17).

Eqs. (3) and (13) predict that plots of the conductivity change occurring on analyte elution (ΔG) should be linear for both fully and partially ionised eluents. Fig. 4 shows calibration plots for these conditions and confirms the predicted linear relationship.
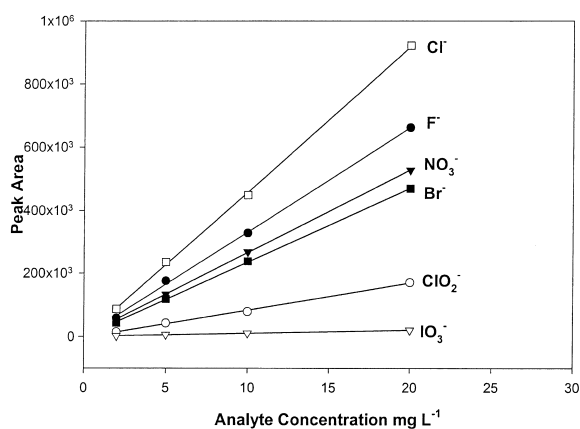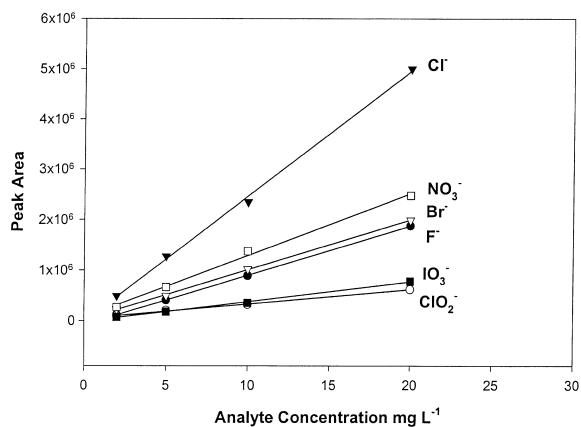
Fig. 4. Calibrations plots obtained using a fully ionised potassium benzoate eluent (a) and a benzoic acid eluent (b). Separation conditions as for Fig. 2.

5. Conclusions

A conductivity detection response equation has been derived with careful definition of terms. This equation applies to both fully and partially ionised eluents and allows for elution of the analyte species by the neutral, protonated form of the eluent. In the case of benzoic acid–benzoate eluents, the results show that whilst use of the protonated form of the eluent leads to a very substantial increase in detection signal, it makes only a minor contribution to the elution of analytes.

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