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## QUANTITATIVE INTERPRETATION OF THE INJECTION PEAK IN ION CHROMATOGRAPHY

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### SUMMARY

The injection peak in ion chromatography has been found to be a measure of the total ionic content of the injected sample. A model calculation of all factors contributing to the conductivity of this peak is described. The calculated results are shown to be in good agreement with experimental findings.

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### INTRODUCTION

Anionic and cationic species in aqueous solutions can be quantitatively determined by either of two versions of ion chromatography. In the first, developed by Small *et al.*<sup>1</sup>, so-called suppressor columns are required, while the second, later version, is characterized by the use of low-conductivity eluents<sup>2,3</sup> and dispenses with suppressor columns.

Whenever the suppressor column is absent<sup>4,5</sup>, the first peak to appear on the chromatogram is an "injection peak", which is analogous to the "void volume peak" encountered in other chromatographic methods. Each of the subsequent peaks represents one individual ion, the positions of the peaks being determined by the retention times of the specific ions on the separating ion-exchange column. If a conductivity detector is used, the signals of the normal chromatographic peaks are a measure of the difference between the conductivities of the particular ions and that of the eluent (the "background conductivity"). The conductivity of the injection peak, on the other hand, is related to the total ionic content of the injected sample.

The injection peak is sharp, and its size is very sensitive to changes in concentration. It can therefore usefully serve rapidly to provide quantitative information on ions with long retention times. The chromatogram in Fig. 1, for example, shows well defined peaks for  $\text{Cl}^-$ ,  $\text{Br}^-$  and  $\text{NO}_3^-$ , while the peak for  $\text{SO}_4^{2-}$  is diffuse. If all the contributions to the injection peak, apart from that of  $\text{SO}_4^{2-}$ , are added up, it should be possible mathematically to derive the concentration of  $\text{SO}_4^{2-}$  from the difference between the conductivity of the injection peak and that of the other peaks, provided that the calibration has been appropriately adjusted.

In the following all of the factors that contribute to the conductivity of the injection peak are analysed, and the mode of its calculation is discussed. The agree-

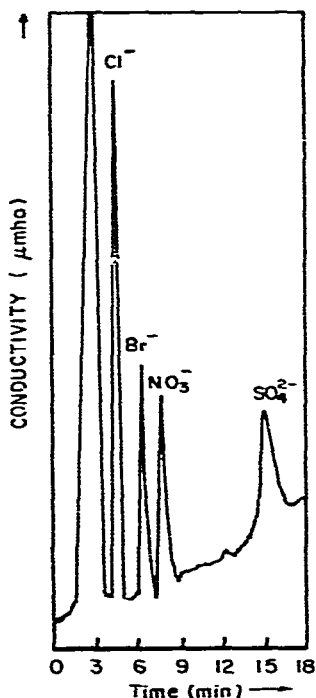


Fig. 1. Separation of  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  (50 ppm each) on a Vydac SC anion-exchange column ( $250 \times 4.6$  mm I.D.). Mobile phase: potassium biphthalate,  $5 \cdot 10^{-3}$  M, at pH 4.50.

ment between experimental results and those of the calculations referred to will be demonstrated.

#### THEORETICAL

##### *Calculations involving the injection peak*

In a chromatographic system in which the stationary phase consists of a pellicular anion exchanger (Vydac SC) and the mobile phase is a mixed aqueous solution of a weak organic acid and its salt, *e.g.*, benzoic acid and sodium benzoate, the background conductivity can be determined as follows. The known data are the eluent concentration  $[\text{HB}] + [\text{NaB}]$ , the pH and the  $K_a$  of the weak organic acid. As the equilibrium

$$K_a = \frac{[\text{H}^+][\text{B}^-]}{[\text{HB}]}$$

always exists, the concentrations of  $[\text{H}^+]$ ,  $[\text{B}^-]$  and  $[\text{Na}^+]$  can be calculated, and their contribution to the background conductivity will be

$$KG_1 = \lambda_{\text{H}^+} [\text{H}^+]_1 + \lambda_{\text{B}^-} [\text{B}^-]_1 + \lambda_{\text{Na}^+} + [\text{Na}^+]_1$$

where  $G$  is the measured conductivity in  $\mu\text{mho}$ ,  $K$  is the cell constant, which is  $33.23 \text{ cm}^{-1}$  at  $18^\circ\text{C}$  and  $\lambda$  is the equivalent conductance of the ions at  $18^\circ\text{C}$ .

If, now, a small volume ( $100 \mu\text{l}$ ) of a solution containing a sodium salt is injected into the column, the anion of the salt will be adsorbed on the column and  $[\text{B}^-]$  will be displaced into the mobile phase; the concentrations of  $[\text{H}^+]$ ,  $[\text{HB}]$  and  $[\text{Na}^+]$  will therefore also change. These changes can be calculated in the following way. Let  $N'_\text{B}^-$  be the number of equivalents of  $\text{B}^-$  which have been displaced as a result of injection, and let  $X$  be the equivalent fraction of  $\text{B}^-$  which has combined with  $\text{H}^+$ . The new concentrations will then be as follows:

$$[\text{H}^+]_2 = \frac{N_{\text{H}^+} - XN'_{\text{B}^-}}{V}$$

$$[\text{B}^-]_2 = \frac{N_{\text{B}^-} + (1 - X)N'_{\text{B}^-}}{V}$$

$$[\text{HB}]_2 = \frac{N_{\text{HB}} + XN'_{\text{B}^-}}{V}$$

$$[\text{Na}^+]_2 = [\text{B}^-]_2 - [\text{H}^+]_2$$

where  $V$  is the injection peak volume.

The conductivity will be

$$KG_2 = \lambda_{\text{H}^+} [\text{H}^+]_2 + \lambda_{\text{B}^-} [\text{B}^-]_2 + \lambda_{\text{Na}^+} [\text{Na}^+]_2$$

$N'_{\text{B}^-}$  can be computed from the available chromatographic data:

$$N'_{\text{B}^-} = C_s V_s$$

where  $C_s$  is the concentration of the injected ion and  $V_s$  is the volume of the injected ion.

The conductivity of the injection peak is the difference between  $KG_2$  and  $KG_1$ . If, therefore,  $K_a$ , pH, eluent concentration and sample concentrations are known, the injection peak can be calculated. For this purpose a small computer program has been set up, which is available from the authors on request.

## EXPERIMENTAL

### Apparatus

The liquid chromatography set-up was similar to that described previously<sup>2</sup>. The main parts, such as pump, pressure gauge, sample injection valve and conductivity monitor, were purchased from Wescan.

The separation column ( $250 \times 4.6 \text{ mm I.D.}$ ) consisted of a Vydac SC 302 anion exchanger (Separations Group, Hesperia, CA, U.S.A.), bead size  $30\text{--}44 \mu\text{m}$ , capacity  $0.1 \text{ mequiv./g.}$  The sample injection volume was  $100 \mu\text{l}$ , the flow-rate  $2 \text{ ml/min}$  and the paper speed  $3 \text{ cm/min}$ .

### Sample solutions

Two sets of calibrated solutions of sodium chloride, nitrate and sulphate were prepared from analytical-reagent grade compounds. In one set the salts were dissolved in high-purity water and in the other in high-purity water containing the eluent ( $2 \cdot 10^{-3} M$  sodium benzoate). The concentrations of the salts in both sets were in the range  $10^{-4}$ – $1.5 \cdot 10^{-3} M$ .

### Eluent

The eluent was a  $2 \cdot 10^{-3} M$  solution of benzoic acid in high-purity water, adjusted to pH 4.37 with sodium hydroxide.

### Determination of parameters

The peak volume,  $V$ , was determined by measuring the time elapsed from the onset of the peak to its return to the baseline and multiplying this time by the flow-rate.

The cell constant,  $K$ , was found by measuring the conductivity,  $G$ , of  $10^{-3} M$  potassium chloride solution at  $18^\circ C$ . As the specific conductivity,  $k$ , of the potassium chloride solution is known, the cell constant can be determined from the equation  $k = GK$ . The numerical value of  $K$  was found to be  $33.23 \text{ cm}^{-1}$  at  $18^\circ C$ .

## RESULTS AND DISCUSSION

### Benzoate injections

In order to test the assumptions and calculations made, different concentra-

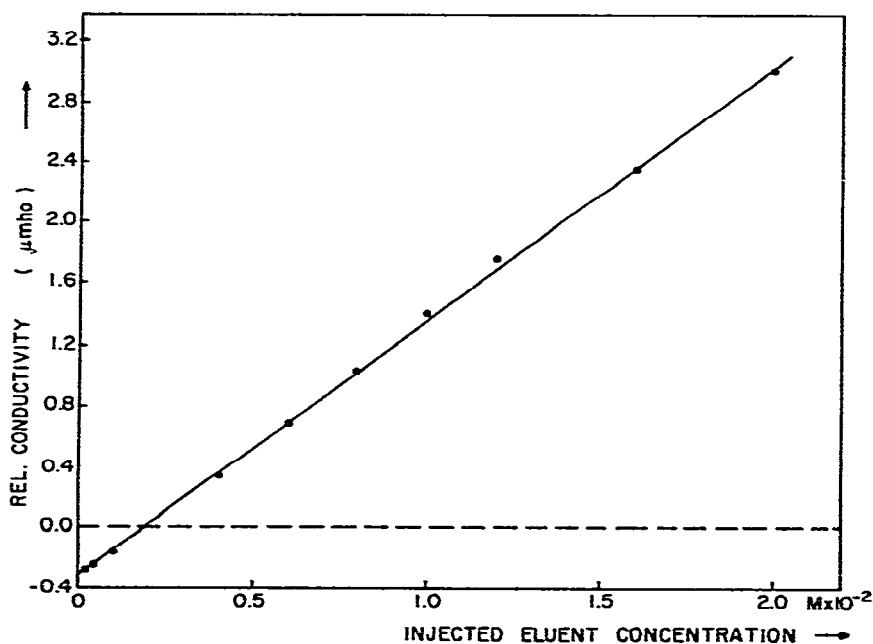


Fig. 2. Calibration graph of the injection peak for benzoic acid at pH 4.15. Eluent: benzoic acid,  $2 \cdot 10^{-3} M$ , at pH 4.15.

tions of the eluent were injected into the chromatographic system. Fig. 2 shows the results obtained with  $2 \cdot 10^{-3} M$  benzoic acid at pH 4.15. It is important to note that the retention time of benzoate is identical with that of all the other injection peaks obtained when injecting mixtures of anions. This would appear to support our assumption that the injection of any anion into the system displaces some adsorbed eluent anions into the mobile phase. The points on the graph show the conductivity of the benzoate anion in the mixture at the above pH. The calibration graph is linear with concentration, indicating that weak electrolytes can be accurately determined by this method. The zero intercept with the abscissa gives the concentration of benzoate that equals the benzoate concentration in the eluent, while the intercept with the ordinate gives the peak conductivity when water only is injected (zero concentration of injected benzoate).

### *Effect of pH*

The calculation described in this work can also be applied in predicting the sensitivity of the injection peak to pH changes. In order to investigate this effect a calculation was made on the assumption that the amount of  $\text{Cl}^-$  injected was constant ( $10^{-4} M$ ) and that the pH was varied by adding a mixture of  $10^{-4} M$  benzoic acid and sodium benzoate. The graph of the results calculated (Fig. 3) is very similar to the neutralization curve of a weak acid. Fig. 3 further shows that the height of the peak depends on the degree of ionization of the benzoic acid. At low pH the chloride injection displaces some benzoate ions, which in turn combine with excess of  $\text{H}^+$ , so that the conductivity is relatively low. At pH 4.2, which is also the numerical value of the  $\text{p}K_a$  of benzoic acid, the conductivity change is zero. At lower pH values the injection peak is negative; above pH 4.2 it is positive. Increasing the pH above 5 leads to a plateau, indicating that the ratio between the ionized and the non-ionized forms of benzoic acid is very high and that this type of eluent behaves like any other strong electrolyte. This calculation may therefore be used to predict the optimal pH con-

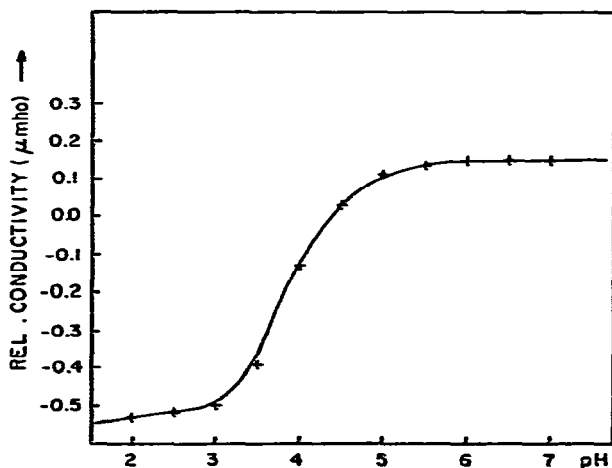


Fig. 3. Calculated results of the dependence of the conductivity of the injection peak on pH for a monovalent anion ( $10^{-4} M$ ). Eluent: benzoic acid,  $10^{-4} M$ .

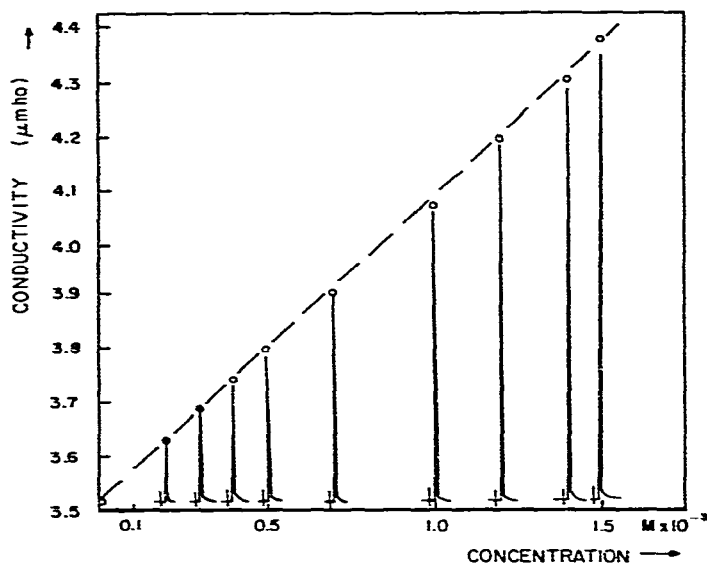


Fig. 4. Dependence of injection peak conductivity on the concentration of an injected monovalent anion. Calculated (O) and experimental values are compared. Eluent: benzoic acid,  $2 \cdot 10^{-3} M$ , at pH 4.37.

ditions for any eluent, *i.e.*, the range of pH that will produce injection peaks of the highest conductivity uninfluenced by transient changes in pH during elution.

#### *Injection of chloride ions*

In order to check the agreement between theory and experiment, a model

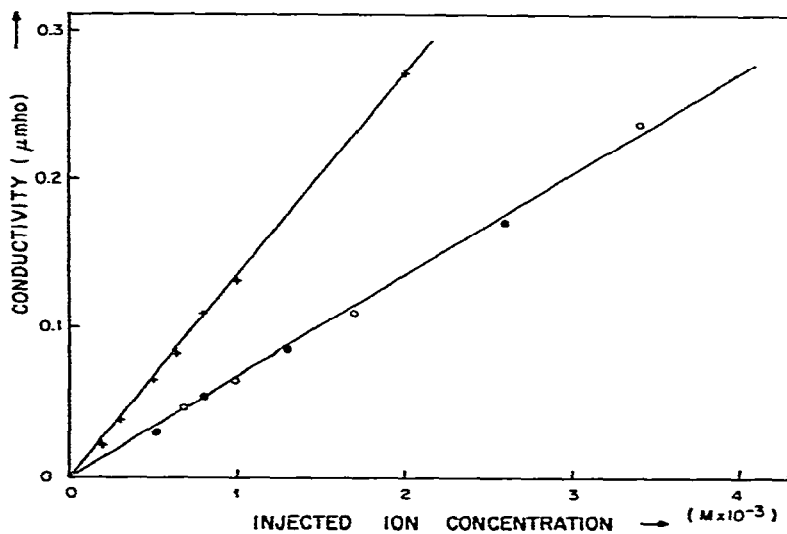


Fig. 5. Injection peak calibration graphs for chloride (O), nitrate (●) and sulphate (+). Eluent as in Fig. 4. Slopes: chloride and nitrate, 6.29; sulphate, 12.34.

calculation was carried out involving different concentrations of injected chloride ions. The eluent was  $2 \cdot 10^{-3} M$  benzoic acid at pH 4.37. The relevant plots of conductivity *versus* concentration are shown in Fig. 4, which also shows the experimental injection peaks of the same chloride solutions. In order to avoid dilution effects caused by excess of water, the chloride samples were prepared in an aqueous solution containing  $2 \cdot 10^{-3} M$  benzoic acid at pH 4.37. Fig. 4 demonstrates the good agreement between the calculated and the experimental results: the edges of the chromatogram touch the calculated points. The zero intercept of the straight line gives the background conductivity of the eluent (for a given cell constant).

### *Injection of other ions*

In Fig. 5 the peak conductivities of three separate ions are plotted. It can be seen that all the points representing monovalent ions, such as  $\text{Cl}^-$  and  $\text{NO}_3^-$ , fall on the same straight line. The divalent  $\text{SO}_4^{2-}$  ions cause a greater displacement of the eluent, as demonstrated by the fact that the slope of the sulphate line is double that of the monovalent ions. The additivity of all the factors contributing to the injection peak is illustrated in Fig. 6. Mixtures of different concentrations of  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  were injected, and conductivity was plotted against concentration of the injection peak (line c). Every point on line c is the sum of the corresponding points on lines a and b, which were plotted with each ion injected separately. The slope of line c is the sum of the slopes of lines a and b.

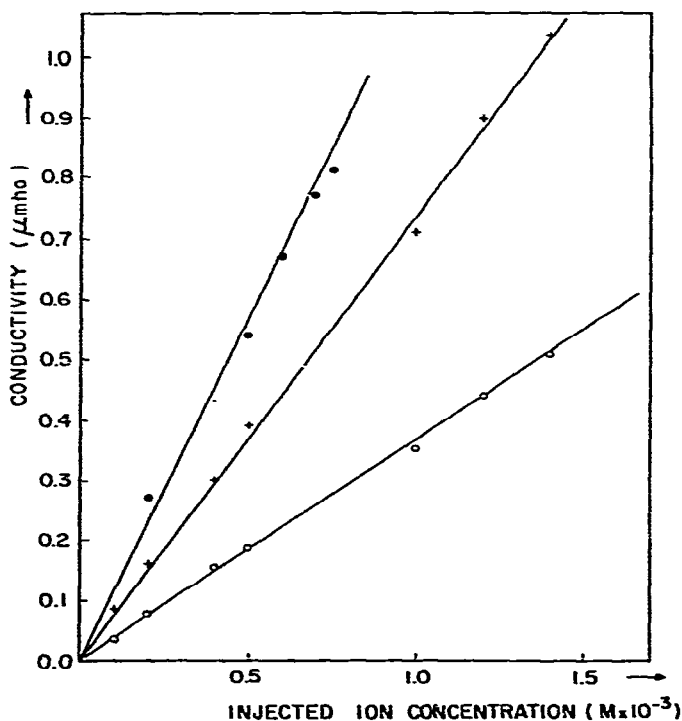


Fig. 6. Calibration graphs for mixtures of ions. Eluent as in Fig. 4. (a) chloride, slope 0.369; +, sulphate, slope 0.73; ●, sum of chloride and sulphate, slope 1.01.

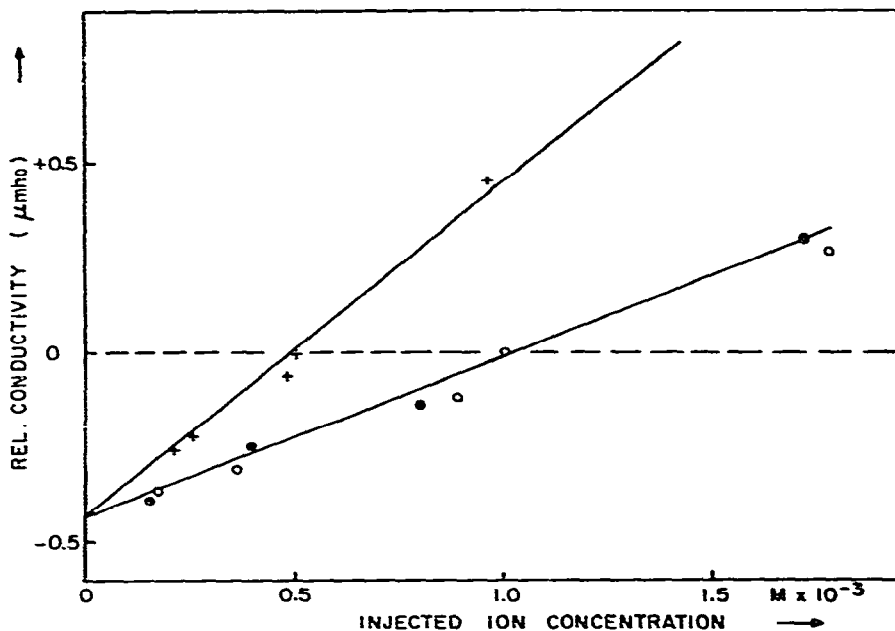


Fig. 7. Calibration graphs for chloride (O), nitrate (●) and sulphate (+) ions injected in water. Eluent as in Fig. 4.

The calculations described so far were tested experimentally on samples that also contained the eluent, in order to understand the model, but it is not really necessary in practical applications. If different concentrations of ionic solutes are injected, none of which contain the eluent ions, straight lines are obtained when the peak conductivity is plotted against concentration. The slopes of these lines are identical with those of the lines shown in Fig. 5. In Fig. 7 the lines represent sulphate, nitrate and chloride ions, and they differ from Fig. 5 only in their intercepts. The intercept with the ordinate gives the negative peak conductivity if only water is injected (zero ion concentration), while the intercept with the abscissa gives the concentration of the injected ion, which is equal to the concentration of the eluent.

Quantitation of the injection peak can be very useful when ions with long retention times are determined. The following procedure is recommended for a chloride-sulphate mixture: construct a calibration graph for various concentrations of chloride alone and plot conductivity data of both the injection peak and the normal elution peak of chloride against concentration. If mixtures of both ions are then injected, the sulphate concentration can be derived from the injection peak and the chloride concentration from its elution peak. This concept may, of course, also be applied to mixtures containing more than two ions.

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