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NON-LINEARITY OF CALIBRATIONS IN THE ANALYSIS OF ANIONS BY ION-CHROMATOGRAPHY WITH SUPRESSED CONDUCTIVITY DETECTION

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ABSTRACT

Non-linearity of calibration in the determination of anions by ion-chromatography with supressed conductivity detection is re-examined. With a carbonate eluent, the curvature is demonstrated for NO_3^-, Br^- and SO_4^{2-} calibrations using plots of the logarithm of the normalized peak area against the logarithm of the anion concentration. These graphs were also evaluated theoretically assuming the peak shape is Gaussian. It is demonstrated that for sufficiently low concentrations linear calibrations are obtained and that deviations are to be found in the middle of the concentration range. Several procedures are discussed to avoid having large errors in analysing samples over a wide range of concentrations.

INTRODUCTION

The term "ion-chromatography", introduced by Small et al in 1975 [1], originally was used to describe a system of ion-exchange chromatography using a low capacity ion-exchange column, a suppressor column and a conductivity detector. More recently, the definition has been broadened so that "ion-chromatography" is now generally applied to any modern chromatographic method for determining inorganic and organic ions. The word "modern" implies that the separation is high performance (or rapid) and that automatic detection is employed.

Suppressed anion and cation chromatography employ post-column chemistry to reduce the background signal and allow conductometric detection of sample ions [2]. Ion determinations by single column ion-chromatography are performed with the separation column connected directly to the detector [3].

Ion chromatography is a useful and sensitive method for the determination of ions, specially for inorganic anions and has found widespread application. When a

conductometric cell is used as an universal detector, the use of a suppressor device improves sensitivity and detection limit. In these techniques, the eluate containing the solute anions such as Cl^-, NO_3^-, Br^- or SO_4^{2-} is a solution of a strong acid in a weaker acid, eg. H_2CO_3, which forms from $NaHCO_3$ and Na_2CO_3 in the ion-exchange processes that occur in the supressor device. It is relatively simple to calculate the conductance of such a solution and it may be shown [2] that at moderately low concentrations of the strong acid HX the conductance is not linear with HX concentration but becomes so at high concentrations (eg. ≥ 0.004 M). The reason for this is that the H^+ of the strong acid supresses the ionization of the weak acid. Consequently, the contribution of the weak acid to conductivity is not constant but decreases with increasing strong acid concentration.

Putting this effect in chromatographic terms, the base-line conductance of the carbonic acid is analogous to a "*spongy platform*" (using the same term as Small [2]) on which the HX peak rests. The HX compresses this platform to a degree that depends on the concentration of the HX in the peak. Eventually, if the eluite peak is concentrated enough, the platform is totally compressed and further increases in concentration produce no further compression. As a result calibration graphs, whether based on peak heights or peak areas, are not linear, leading to errors of up to 100% if linearity is assumed. At high concentrations of HX, assuming that the electroforetic and relaxation effects would not be operating, one would expect linear calibration graphs. However, these high concentrations are certainly not the typical situations where one uses ion-chromagraphy for analysis.

These deviations from linearity in calibration graphs were discussed by Van Os et al [4], who "linearized" the calibration by calculating the instantaneous anion concentration in the eluate at a series of points on the chromatographic peak and then integrating to give the quantity injected. Midgley and Parker [5] made further theoretical investigations of non-linear calibrations and discuss ways of dealing with them.

In this paper we re-investigate the approach of Midgley and Parker [5] and suggest further ways of dealing with non-linear calibrations.

EXPERIMENTAL

The chromatograms were obtained by using a Dionex 4000i ion-chromatograph with an AG4A guard column, an AS4A separator column and a Dionex micro-

membrane anion supressor. The injection loops were of 50.6 μl, 150 μl and 368 μl capacity, the eluent was 0.75 mM sodium hydrogen carbonate/ 2.20 mM sodium carbonate solution and the flow rate was 2.0 ml/min. A Dionex helium sparging system was used and the flow of helium was maintained throughout all experiments. The conductivity was measured on the 10 μS, 3μS, 1μS and 0.3μS ranges.

. Sodium nitrate (Merck), KBr (M&B) and Na_2SO_4 (M&B) were used for preparing solutions of known anion concentrations. At least six injections were made for each solution. The high purity water used to prepare all solutions was obtained from a Milipore – Q Water Purification System equipment.

RESULTS AND DISCUSSION

Linear fits of the data usually give correlation coefficients > 0.99 (eg fig. 1) which may lead to false confidence. A clearer indication of non-linearity is given by graphs [6] in which the logaritm of the normalized peak area is plotted against the logarithm of the total anion concentration (C_{An}). The normalized peak area represents the relative difference between the observed area (A) and that expected from the linear regression equation and is given by $(A - a)/bC_{An}$ where a and b are the intercept and the slope, respectively, of the linear regression fit of the data.
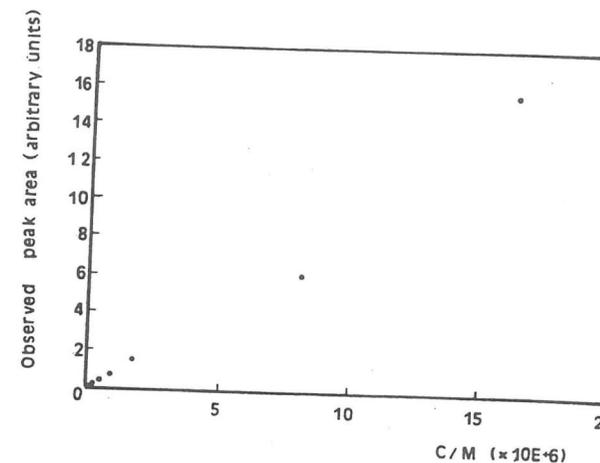

Fig. 1 – Calibration graph for nitrate (usual form) with an injection loop of 368 μl.
A total of 8 points were included in the regression equation. Data for C= 4.05×10^{-5}M was not included in the figure. The calibration appears to be linear and the correlation coefficient is 0.995(2).
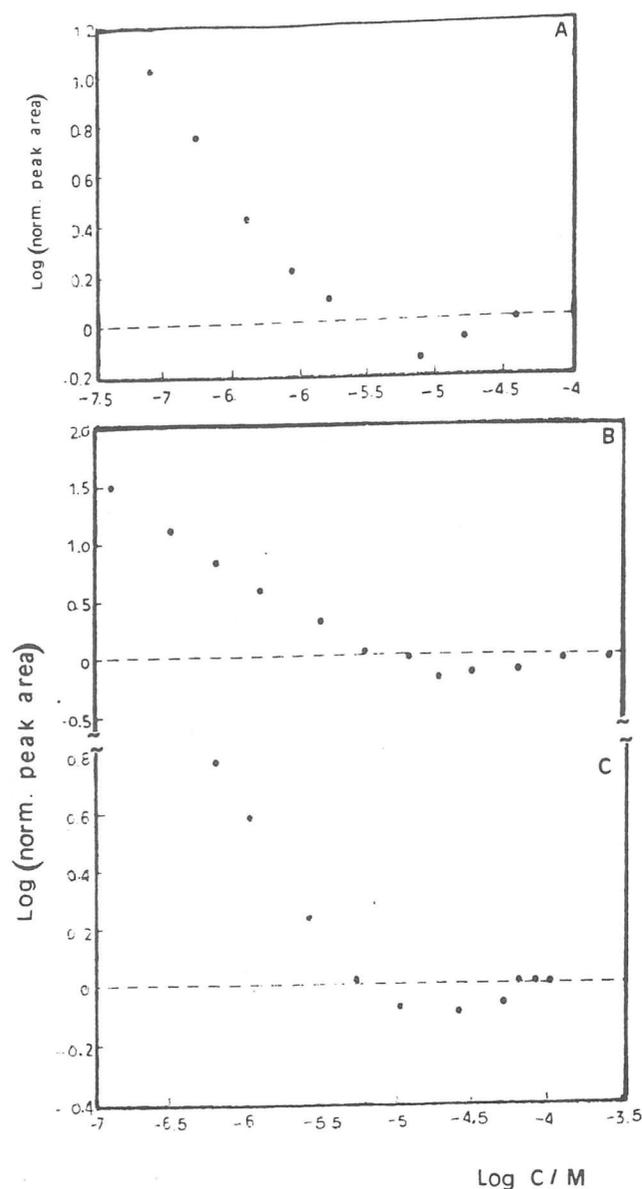
Fig. 2 — Log-log plots of normalized **observed** peak area vs. concentration.
 (A) — for nitrate for calibration in fig 1.
 (B) — for bromide with an injection loop of 368 μl.
 (C) — for sulphate with an injection loop of 368 μl.

The values of **a** and **b** depend on how the regression was carried out, *i.e.*, on whether weighted or unweigted data were used and on the range and spacing of the concentrations tested. With unweigted data, the higher concentrations tend to dominate both the regression equation and the conventional graphical representation, as in fig. 1.

Midgley and Parker [5] replotted the data as log (normalized peak area) against log (C_{An}) and showed how in this way the different deviations from linearity at low concentrations for the anions Cl^-, NO_3^- and SO_4^{2-} can be clearly seen. Fig. 2 shows this type of plots for NO_3^-, Br^- and SO_4^{2-} obtained in this work.

Plots as those of fig. 2 can be predicted theoretically, assuming the bands to be of Gaussian shape, after calculation of the peak areas. In order to make these calculations one assumes that the chemical suppression is complete and therefore the conductivity (k_{An}) for each point of the chromatographic band is given by:

$$k_{An} = \lambda_H [H^+] + \lambda_{HCO_3} [HCO_3^-] + n \lambda_{An} [An^{n-}]$$
$$= n (\lambda_H + \lambda_{An}) [An^{n-}] + (\lambda_H + \lambda_{HCO_3}) [HCO_3^-] \qquad (1)$$

and the baseline conductivity:

$$k_E = (\lambda_H + \lambda_{HCO_3}) (-1 + \sqrt{1 + 4 Ka\, C_{H_2CO_3}}) \big/ 2\, Ka \qquad (2)$$

where λ_H, λ_{An}, and λ_{HCO_3} are the ionic conductivities of H^+, An^{n-} and HCO_3^-, respectively, Ka the first dissociation constant of H_2CO_3 and $C_{H_2CO_3}$ the total carbonate concentration in the eluent. The area of the band corresponds to the integration of ($k_{An} - k_E$) during the time the solute band passes through the cell and it can be shown that:

$$\text{Peak area} = 2 \int_{t_i}^{t_R} (k_{An} - k_E) \, dt =$$

$$= n (\lambda_H + \lambda_{An}) C_{An} V / F -$$

$$- (\lambda_H + \lambda_{HCO_3}) \sqrt{1 + 4 \, Ka \, C_{H_2CO_3}} \; (t_R - t_i) \big/ Ka -$$

$$- n (\lambda_H + \lambda_{HCO_3}) C_{An} V / 2 F +$$

$$+ n (\lambda_H + \lambda_{HCO_3}) \times \int_{t_i}^{t_R} \sqrt{[An^{n-}]^2 + (1 + 4 \, Ka \, C_{H_2CO_3}) / n^2 \, Ka^2} \; dt \qquad (3)$$

where F is the flow (in ml/min), V is the sample volume injected, C_{An} is the total anion concentration in the sample, t_i is the break-through time and t_R the retention time.

The first term in eq. (3) (A1) gives the "ideal" net response for the band of the strong acid H_nAn and the second (A2) the "ideal" contribution of the eluent. The two remaining terms (A3 and A4) allow for the true contributions of the eluent to the peak conductivity, allowing for the carbonate lost by ion-exchange and the suppression of dissociation of H_2CO_3 because of the presence of the strong acid H_nAn. The final integral in eq. (3) cannot be evaluated directly and was determined numerically by Simpson's rule [9].

Fig. 3 shows plots of these theoretical normalized peak areas against solute concentrations and fig. 4 shows the contributions of terms A1 – A4 in eq. 3. It may be noted that for low concentrations the contribution of the A1 term is very small.
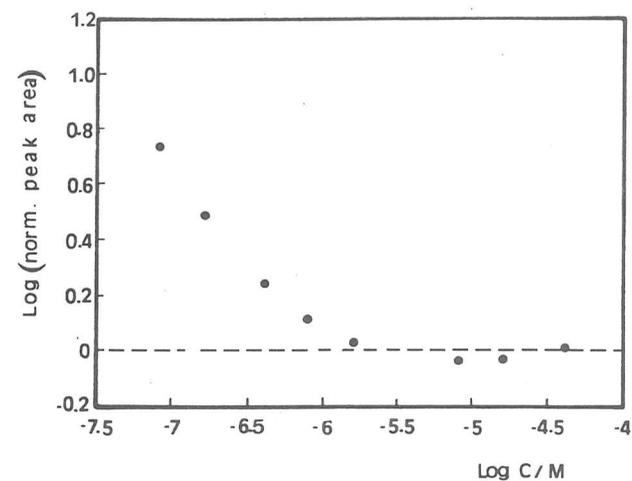
Fig. 3 — Log-log plots of **calculated** normalized peak area vs concentration for calibration in fig. 1.
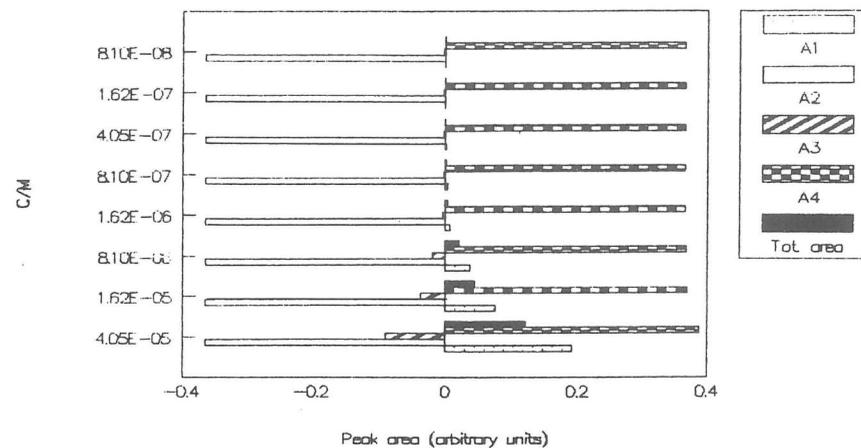

Fig. 4 — Contributions of terms A1 — A4 in eq. 3 to the total area of the peak.

The change in the conductometric signal (ΔG) when the anion (An^-) zone passes through the cell, for a HCO_3^-/CO_3^{2-} eluent, assuming that the chemical suppression is complete is [7,8] :

$$\Delta G = G_{An} - G_{H_2CO_3} = \frac{\lambda_H\left\{\sqrt{Ka(C_{H_2CO_3} - C_{An})} - \sqrt{Ka\,[H_2CO_3] + C_{An}}\right\}}{1000Q} +$$

$$+ \frac{\lambda_{HCO_3}\left\{\sqrt{Ka(C_{H_2CO_3} - C_{An})} - \sqrt{Ka\,[H_2CO_3]}\right\} + \lambda_{An}C_{An}}{1000Q} \qquad (4)$$

where Q is the cell constant and $[H_2CO_3]$ is the concentration of species H_2CO_3, in the solution that passes through the cell.

Equation (4) clearly shows that the relationship between the conductometric signal and the concentration of the analyte anion is not linear. Only when $C_{H_2CO_3} \simeq [H_2CO_3]$ *i.e.*

$$C_{An} \ll C_{H_2CO_3} . \qquad (5)$$

can the conductance be given by:

$$\Delta G = \frac{(\lambda_H + \lambda_{An})C_{An}}{1000Q} \qquad (6)$$

the signal is linearly dependent on the concentration of the anion.

Graphs as those of fig. 2 suggest that data for low concentrations don't obey a linear relation. However equation (6) shows that one should have a linear relationship for very low anion concentrations and deviations from linearity when (5) is not valid. Therefore plots of fig. 2 should be seen in a different perspective, *i. e.* one should check for what values of C_{An} condition (5) holds, calculate new values for the intercept **a** and slope **b** from data where equation (6) is valid, and replot the normalized area vs. concentration. This is shown in fig. 5 and it may be seen that deviations from linearity are only apparent for higher concentrations. This means that **if one calculates concentrations taking these new intercept and slope, errors will be smaller than those obtained from all experimental data.**
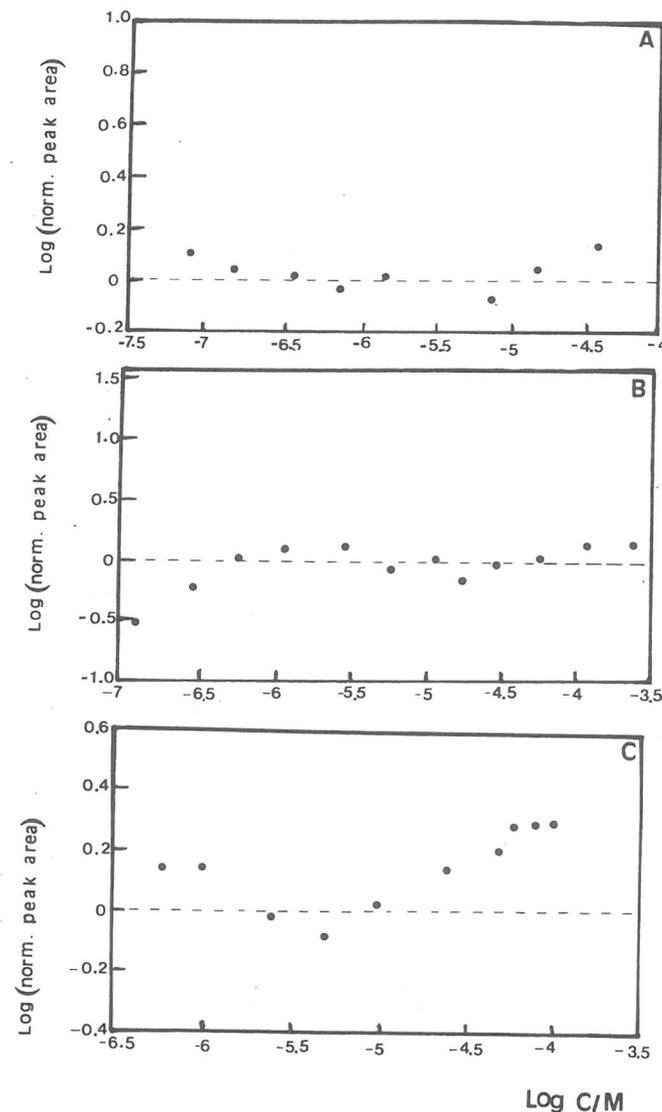
Fig. 5 — Log-log plots or normalized **observed** peak area vs concentration:

(A) — for nitrate for calibration in figs. 1 and 2A, calculating the intercept **a** and slope **b** taking data for $C_{NO_3^-} \leq 1.6 \times 10^{-6}$ M (0.10 ppm) (first five points).

(B) — for bromide for calibration in fig. 2B, calculating the intercept **a** and slope **b** taking data for $C_{Br^-} \leq 1.25 \times 10^{-5}$ M (1.0 ppm) (first seven points).

(C) — for sulphate for calibration in fig. 2C, calculating the intercept **a** and slope **b** taking data for $C_{SO_4^{2-}} \leq 1.05 \times 10^{-5}$ M (1.0 ppm) (first five points).
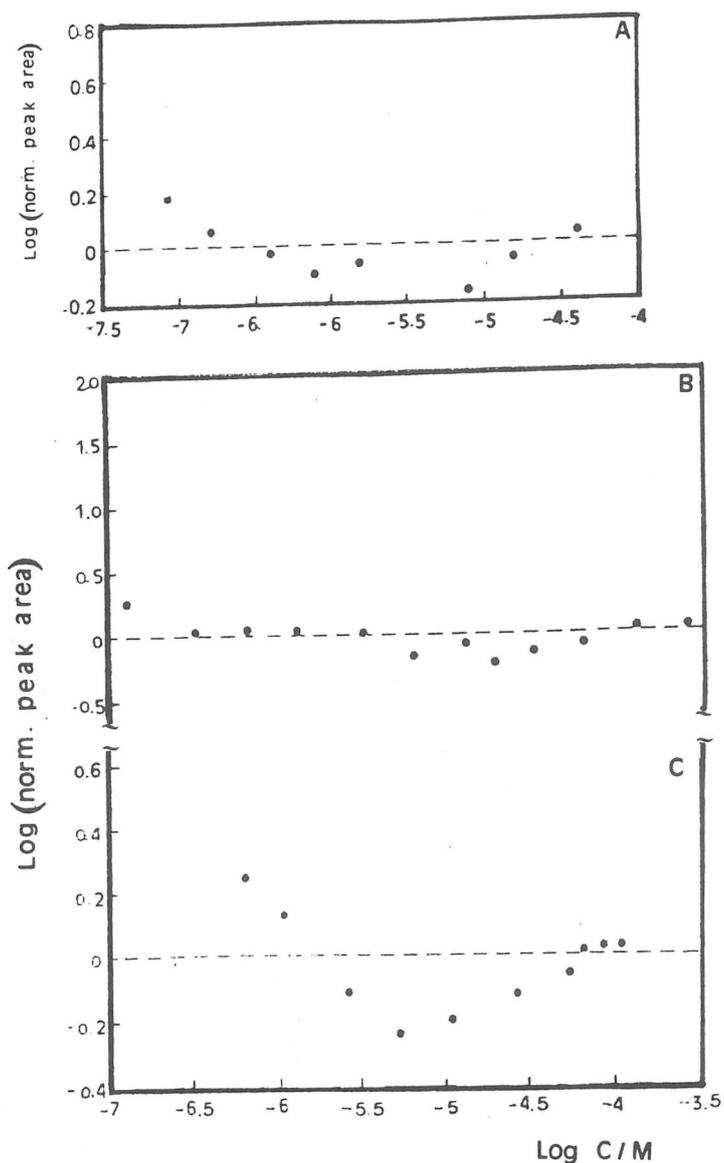
Fig.6 — Log-log plots of normalized **observed** peak area vs. concentration using regression equations including weights proportional to 1/C.
(A) — for nitrate, same calibration as in fig 2A.
(B) — for bromide, same calibration as in fig 2B.
(C) — for sulphate, same calibration as in fig 2C.

In many cases the solute concentration range is such that it is not appropriate to make calculations based on experimental data for low concentrations. One alternative to Midgley and Parker's [5] suggestion of fitting the calibration to a second order equation (of the type: $a + bC + cC^2$) is to take a linear regression using weights in such a way that the contribution of data for higher concentrations is attenuated. One possibility is using weights proportional to 1/C. Using regression equations including these weights [6] we obtained the graphs shown in fig. 6 . Comparing fig. 2 with fig.6 it may be seen that with these type of plots for NO_3 and Br^- errors in obtaining solute concentrations are much lower.

FINAL COMMENTS ON CALIBRATIONS

Truly linear calibration for anions cannot be expected from suppressed ion-chromatography with carbonate eluents. Over a limited range of concentration (eg. 1 decade) a linear treatment will produce only small errors. If samples covering wide ranges of concentration are to be analysed routinely, it is desirable to establish procedures with smaller errors. These complicate the analysis and their practicability depends on the computing facilities available.

Several such procedures were suggested by Midgley and Parker [5] and in this work we propose two new procedures and analyse their relative errors.

(i) The operator may read the concentration off a calibration curve. This should include standards with concentrations similar to those of the samples. If many samples need to be analysed, each with several peaks, this is tedious and time consuming.

(ii) The calibration may be treated as consisting of two linear segments, one for low concentrations and another for relatively high concentrations. The maximum error is likely to occur in the middle of the concentration range. As emphasized in this work it is advisable to use standards with sufficiently low concentrations so that condition (5) holds. In order to minimize errors one can use weights, eg. proportional to 1/C. As analysed in this work, in case the analyst wants to avoid to prepare standards with very low concentrations, weights may be used. In this way errors tend to smooth (but do not disappear). Automation of this processes depends on the computing facilities available.

(iii) As an alternative to option (ii) the calibration may be fitted empirically to a second order equation. As shown by Midgley and Parker [5] this process gives better

fits than the linear calibrations but more data is required to solve the quadratic equation. Practical routine analysis again depends on the concentration range of the solute in the samples and on the computing power available.

(iv) If samples fall within a range where bias are expected, the volume required to produce a signal in the linear part of the calibration can be calculated and injected [5]. This is time consuming and requires further sophistication of the instrumentation. In practice it presents no advantage to option (i) although formally it can be considered as a more correct strategy.

(v) The eluent may be changed. As dissociation constant decreases, terms A3 and A4 in eq. (3) tend to cancel and the "ideal" linear calibration should be approached. Sodium tetraborate eluent ($Ka = 10^{-9.33}$ compared with $10^{-6.35}$ for carbonate) will give a very slight curvature but it has lower eluting ability, so that it needs to be 3-4 times more concentrated to give reasonable elution times, which leads in turn to several problems in the use of the suppressor. This was discussed by Midgley and Parker [5] who concluded that practical chromatograms can be obtained. An alternative is the use of a strong base as eluent. This reduces the curvature of the calibration to negligible proportions but may produce long retention times for sulphate. Gradient elution with NaOH or KOH solutions will produce more convenient chromatograms, if the chromatograph has facilities for it. However these procedures show poor between batch reproducibility because their elution properties are strongly affected by the level of carbonate impurities produced by absorption of atmospheric CO_2 and other impurities that may be introduced with the water used in preparing solutions, even if it is a very high purity water as used in this work. Gradient elution has the further drawback of the time required to re-equilibrate the column before a new injection can be made.

CONCLUSIONS

Non-linearity of calibration in the determination of anions by ion-chromatography with supressed conductivity detection is re-examined.

With a carbonate eluent, the curvature is demonstrated for NO_3^-, Br^- and SO_4^{2-} calibrations using plots of the logaritm of the normalized peak area against the logarithm of the anion concentration. These can be evaluated theoretically assuming the peak shape to be Gaussian. This and other non-specified approximations indicate that the calculations developed above should not be taken as exact predictions of peak

areas, but they should indicate the trends in ion-chromatography with suppressed conductimetric detection.

Over a tenfold range of concentration the errors when assuming the calibration is linear will be small. If the analysis over a wide range of concentrations is undertaken, several procedures were discussed to avoid having errors of 50-100% in analysing samples.

Graphs as those of figs. 1 and 2 suggest that deviations from linearity occur at the lower end of the concentration range. However, formally this is not the correct perspective. As emphasized in this work, for sufficiently low concentrations linear calibrations are expected and deviations are to be found in the middle of the concentration range. Smaller errors in the analysis may also be achieved using weights in such a way that the contribution of data for higher concentrations is attenuated.

In elution with borate or sodium hydroxide eluents deviations from linearity may be negligible but these procedures may be inconvenient in many circumstances.

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