Use of factorial experimental design for the rapid evaluation of main and interactive factors affecting linearity in calibration curves for sulfate analysis by ion chromatography

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Abstract

Analytical results of anion determination by suppressed ion chromatography are significantly affected by calibration curve calculation. In this paper, as expected, eluent pK_a is shown to influence calibration linearity in the range 1–20 mg/l sulfate, with A carbonate–hydrogencarbonate mixture producing a larger non-linearity than NaOH. Evidence is given for very large errors (about 30–40%) in estimating sample sulfate concentration when linear regression is used instead of a quadratic calibration curve. This study was performed following a 2^4 run full factorial experimental design, including eluent pK_a, counterion type, solution composition and current level for background suppression as main variables.

Keywords: Factorial design; Calibration; Sulfate; Inorganic anions

1. Introduction

The theory of conductivity response in ion chromatography shows that the change in conductance during elution of an anionic solute S is [1]:

$$\Delta C = C_S - C_B = \frac{(\lambda_S - \lambda_E)c_S I_S}{10^{-3}K}$$

where C_S is the elution conductance and C_B the background conductance, λ_S and λ_E are, respectively, the limiting equivalent ionic conductance for anion solute and eluent, being measured in S cm equiv.$^{-1}$; c_S is anion concentration (equiv. l^{-1}) and I_S is the degree of ionization, being equal to 1 for fully ionized species such as chloride and sulfate.

It appears that a direct proportionality exists between ΔC and solute concentration, giving origin to linear response as a function of concentration. However, when using common suppression systems

it is not unusual to find deviations from linearity, both positive and negative [2]. For sulfate and other common anions a positive deviation can be experienced in a medium concentration range (1–20 mg/l).

One reason for this behaviour can be found by considering the response equation for suppressed conductivity detection [1,3]:

$$C = \frac{(\lambda_H + \lambda_S)c_S}{10^{-3}K}$$

in the ideal case of quantitative suppression (the explanation of the symbols is as before and λ_H is the limiting equivalent ionic conductance of hydrogen ion).

When the capacity of the ion-exchange column is not overloaded, a linear relationship exists between C and c_S.

In real conditions suppression is not quantitative and eluent conjugate weak acid can be dissociated to

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a varying extent, depending also on the concentration of hydrogen ions deriving from the dissociated sample (H^+ and S^- solute anion) in the suppressed eluent. During sample elution the dissociation equilibrium of the eluent is forced to the left and the background conductance is decreased compared to the value when eluent alone passes through the detector. At increasing sample concentration the decrease in background value is more pronounced, thus giving rise to a positive deviation from linearity of calibration curves [4,5]. The mathematical description of this behaviour is given in specialized literature [6].

In the present paper this effect was studied as a function of eluent type (pK_a), counterion type, solution composition and current level for background conductivity suppression. A full factorial experimental design and columns of contrast coefficients calculation [7] were used for rapid estimation of the effects of the four factors. Eluents were selected on the basis of common laboratory practice, including two of the most classical eluent solutions

used in routine analysis whilst allowing sulfate elution at reasonable time and with good resolution from other common anions.

The aim of this work is to show the large errors occurring in anion analysis when using unsuitable linear regression of calibration data, instead of a quadratic curve, thus significantly affecting the accuracy of analytical data.

2. Experimental

2.1. Reagents

All chemicals were analytical grade reagents. Na_2SO_4, $NaNO_3$, NaCl, K_2SO_4, KNO_3 and KCl (Fluka) were used for anion stock standard solutions (1000 mg/l) preparation. Anhydrous sodium carbonate and sodium hydrogencarbonate (Merck) were used for eluent 1 (4 mM carbonate–2.4 mM hydrogencarbonate) preparation. Eluent 2 (20 mM NaOH) was prepared from sodium hydroxide (Carlo Erba).

Table 1
Factorial design and second order quadratic coefficients and estimates of factor effects (Blocks: 1; Factors: 4; Runs: 16)

Run	a	b	c	d	Response[a]	Factor effects	
14	−	−	−	−	276/63	a	39.25
5	+	−	−	−	246/70	b	**−218.25**
11	−	+	−	−	57/66	ab	−13
15	+	+	−	−	100/62	c	**54**
4	−	−	+	−	292/64	ac	7.25
3	+	−	+	−	525/20	bc	−29.75
16	−	+	+	−	146/85	abc	−48
12	+	+	+	−	116/49	d	−31.75
13	−	−	−	+	269/75	ad	−14.5
7	+	−	−	+	294/67	bd	11.5
2	−	+	−	+	41/66	abd	34.25
10	+	+	−	+	132/61	cd	−45.75
1	−	−	+	+	310/67	acd	−40
6	+	−	+	+	293/51	bcd	17.5
9	−	+	+	+	82/63	abcd	35.75
8	+	+	+	+	83/47		

	+	−
a: counterion type	K^+	Na^+
b: eluent type	OH^-	$CO_3^=$/HCO_3^-
c: composition of solution	Multi	Single
d: supressor current level	3	1

[a] Response is calculated as the second order coefficient of quadratic regression/standard error (95% confidence level).

All reagents, eluents and working solutions were prepared using Milli-Q water.

2.2. Instrumentation

A Dionex 2000i ion chromatograph with a Dionex gradient pump, eluent degassing module and conductivity detector was used for the experimental work. Anions were separated on a AS4A-SC (4 mm) ion-exchange column, with AG4A-SC guard column, and detected after suppression with ASRS1 anion electrical self regenerating suppressor. Acquisition of data and chromatograms and instrument remote control was performed with Dionex AI450 software.

2.3. Experimental conditions

Elution with eluent 1 was obtained with a gradient program as follows: (1) 4 mM Na₂CO₃ + 2.4 mM NaHCO₃; (2) Milli-Q water.

Time (min)	Flow (ml/min)	%1	%2	Curve
0.00	1	40	60	Linear
12.0	1	100	0	Linear

Sulfate eluted at 9.89 min.

Elution with eluent 2 was obtained isocratically (flow-rate 1 ml/min), with sulfate eluting at 8.12 min.

In both cases injection volume was 100 μl and run time was set to 12 min. Conductivity detector full scale was 30 μS.

2.4. Full factorial design

The effects of four variables on non-linearity of calibration curves was studied following the factorial design reported in Table 1.

Counterion type was selected on the basis of ionic radius of hydrated ion in solution, with potassium showing a larger value than sodium.

Two eluents (OH⁻, pK = 14; CO₃²⁻/HCO₃⁻, pK = 10.33) with different pK values were selected to investigate the effect on calibration characteristics.

Variation of solution composition included the addition of chloride and nitrate to sulfate as the sodium salts at 10 mg/l concentration level ("multi" level) or the use of single component sulfate standard solutions ("single" level).

Finally, two different current levels were selected for background suppression, corresponding to 3 mA and 1 mA.

A total of 16 runs was obtained and the order of execution was randomly selected as reported in Table 1.

After performing all runs as described in experimental design, a commercial software was used for linear ($y = bx + c$) and non-linear quadratic regression ($y = ax^2 + bx + c$). The second order coefficients of the quadratic regressions with standard error (95% confidence level) were calculated for each run and used as the responses to estimate main and interactive effects of the four factors, by columns of contrast coefficients method.
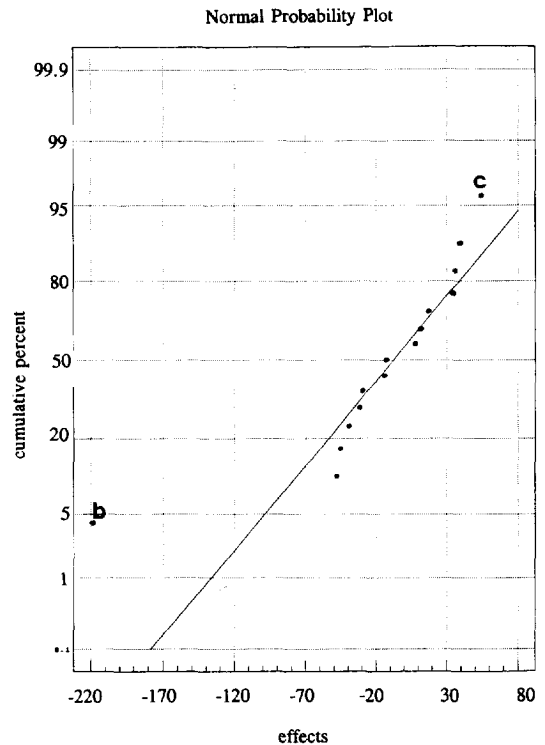

Fig. 1. Normal probability plot of calculated estimates of factor effects. Estimates for factors b and c are shown.

3. Results and discussion

3.1. Main and interactive effects

In Table 1 the second order coefficients of the quadratic regression are reported together with standard errors at 95% confidence level. These values were used to obtain the estimates of factor effects, as reported in Table 1. As expected factor b (eluent type) is the largest one and multiple factor interactive effects are generally lower than main effects. Using carbonate eluent a large positive second order coefficient is obtained, thus showing a positive curvature of calibration at increasing concentration values.

From Table 1 it is evident that among all other estimates only factor c (composition of solution) is of some importance, with multi-component standard solutions producing more evident quadratic effects with positive curvature of the calibration curve.

Table 2
Linear and quadratic regressions and correlation coefficients with carbonate or hydroxide eluent

Eluent type	Linear: $bx + c$	Quadratic: $ax^2 + bx + c$
Carbonate	$40\ 633x - 21\ 311$ ($R^2 = 0.9951$)	$524x^2 + 30\ 159x - 1311$ ($R^2 = 0.9996$)
Hydroxide	$41\ 328x - 8891$ ($R^2 = 0.9996$)	$116x^2 + 39\ 020x - 4483$ ($R^2 = 0.9998$)

The other factors are less significant, as can be seen from the very small estimates reported in Table 1 and from the normal probability plot in Fig. 1, reporting the frequency of calculated estimates of factors against the values which have certain probabilities of occurring. Factors which exhibit a negligible effect fall on a straight line, whereas any large effect does not [7].
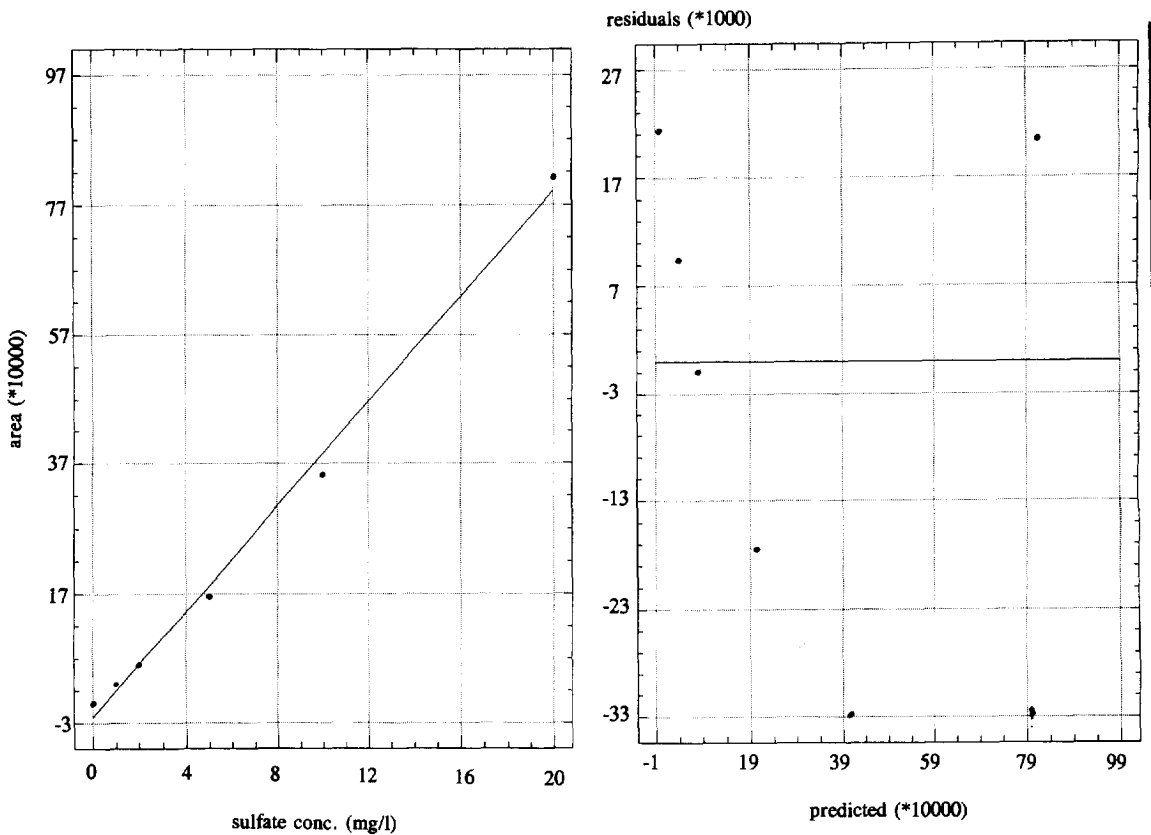
Unexpectedly, current level in the conductivity

Fig. 2. Linear regression and residuals graph (vs. predicted) of data obtained from run 3: elution with carbonate eluent.

suppressor does not influence the calibration curve: this is likely to be due to the fact that both current levels selected for experiments were above the critical value.

3.2. Comparison between linear and non-linear quadratic regression

In Table 2 linear and quadratic regression for two selected experimental runs (run 3-carbonate eluent and run 12-hydroxide eluent) are reported.

In comparison to the hydroxide eluent, it is clear that with carbonate eluent the type of calibration has significant effects on calibration characteristics, as is shown by the difference in R^2 values. Moreover, it must be stressed that the value of R^2 alone is not sufficient to evaluate the goodness of the fit: the data shown clearly indicate this fact. In most situations, however, only the R^2 value is used to check the

calibration performances, thus leading to possible error in estimating the unknown concentration in a real sample from direct comparison with the calibration graph. This fact is even more important if we consider that carbonate eluent is the classical eluent used in most laboratories for routine analysis.

Using hydroxide eluent the effects on calibration are minimized: in Table 2 the difference between the b and c factors in the two types of regression are less pronounced than in the case of carbonate. R^2 values are more comparable too.

The effect of different regression type using carbonate eluent is evident in Figs. 2 and 3: Fig. 2 clearly shows the positive curvature of the calibration curve at increasing concentrations of sulfate, showing a better fit with a quadratic model. In the case of hydroxide eluent no significant difference exists between the two graphs (Figs. 4 and 5). This positive curvature is not a deviation from linearity at
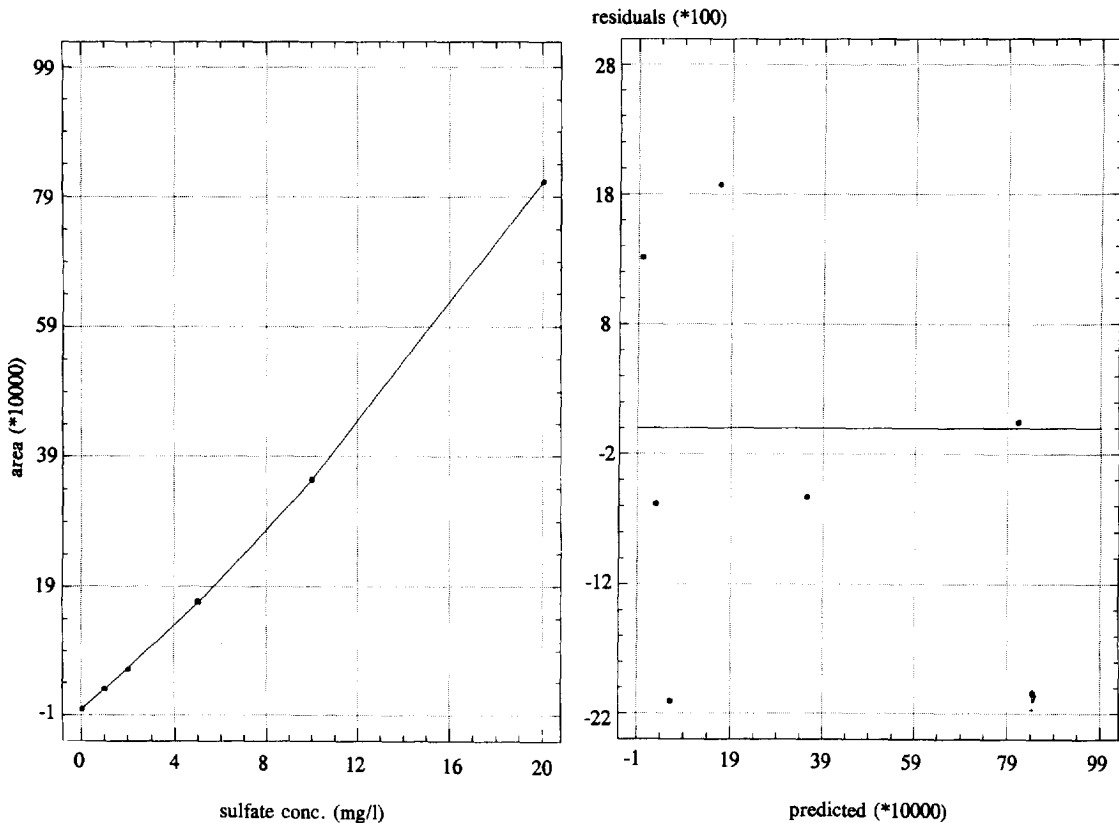

Fig. 3. Quadratic regression and residuals graph (vs. predicted) of data obtained from run 3: elution with carbonate eluent.
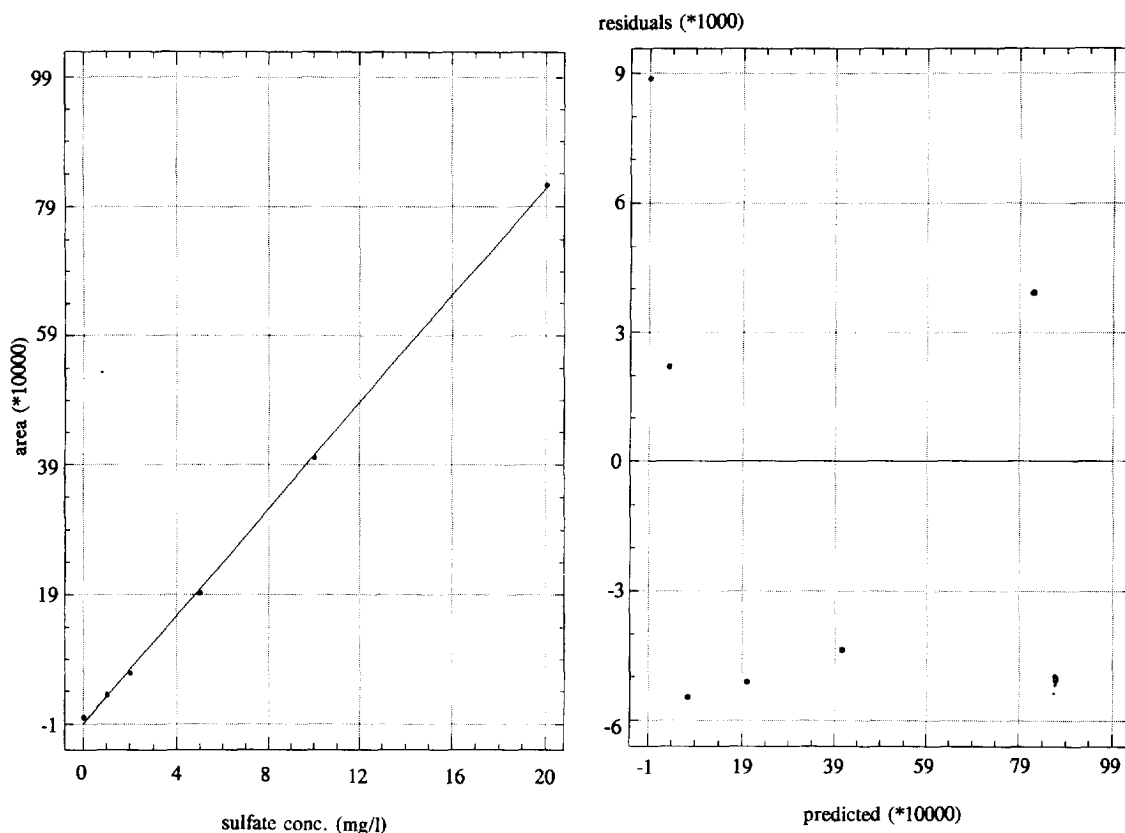
residuals (*1000)

Fig. 4. Linear regression and residuals graph (vs. predicted) of data obtained from run 12: elution with hydroxide eluent.

increasing concentration of sulfate, as some argue, but it is a real non-linearity: in fact, from our experience (data not reported here) it can be demonstrated that reducing the concentration range does not have any influence on the curvature of the calibration, at concentration values normally found in snow samples (20–200 ng/ml).

From the residuals graphs as a function of concentration reported in Fig. 6, it appears that in the former case in addition to the expected larger residual values for linear regression, a larger dispersion of data obtained from the various runs exists in the case of quadratic calibration. Probably linear regression smooths out most of the effects of the other factors at fixed eluent type, while quadratic regression tends to show the difference among runs. In conclusion, quadratic calibration is needed when using carbonate eluent, to accurately take account of

all factor effects; on the contrary, linear calibration would increase inaccuracy in sulfate analysis by ion chromatography.

With hydroxide eluent (and generally high pK eluents), no differences in the residuals graphs exists.

3.3. Errors using the two types of calibration

From the previous discussion it is clear that an accurate selection of regression type for calibration data is very important to reduce errors in estimating unknown concentration in a real sample. Quantitative evaluation of possible errors is best carried out by analysing residual values as a function of predicted ones, as reported in the right portion of Figs. 2 and 5.

In the case of carbonate eluent (Figs. 2 and 3) errors are 10 times less when using quadratic regres-
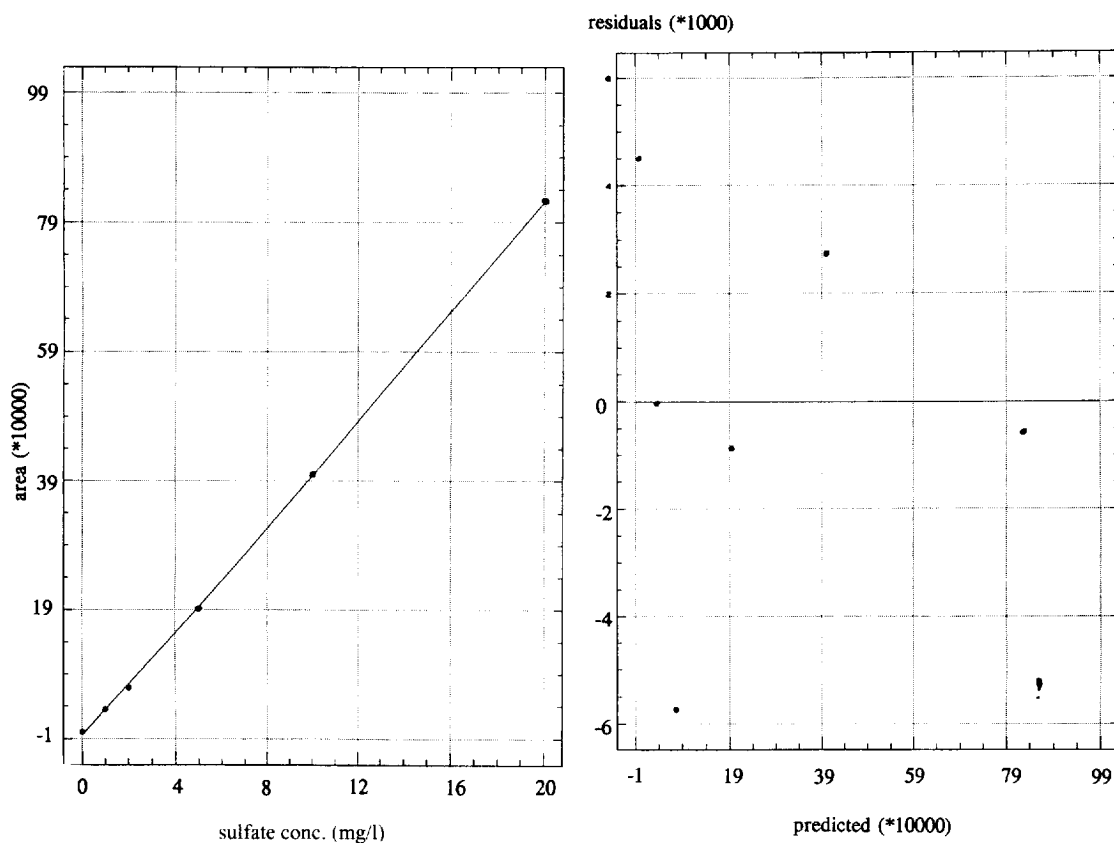
Fig. 5. Quadratic regression and residuals graph (vs. predicted) of data obtained from run 12: elution with hydroxide eluent.

sion in comparison to a linear fit. In this latter case the largest errors are experienced at the extremes of the calibration curve, while quadratic regression gives low residuals values in the whole range. Particularly significant is also the very good fit obtained for the upper part of the concentration range using quadratic regression. In this case errors are always less than 3%, while using a linear regression can give errors high as 40% for extreme values and 10% in the medium concentration range.

Percentage errors are comparable for both types of regression if hydroxide eluent is used (Figs. 4 and 5). Maximum errors do not exceed 6%, but in the case of quadratic regression residuals values are lower in the medium concentration range.

It is important to point out that with both types of eluent a quadratic calibration has the clear advantage of allowing accurate quantitative determination of

low sulfate concentration, because of the very good fit for concentration values approaching zero.

4. Conclusions

From the results of a full factorial experimental design evidence is given of large quadratic effects on calibration curve for sulfate analysis by suppressed ion chromatography, using low pK_a carbonate eluent.

From the experimental data it is clear that we are not in the presence of a deviation from linearity, but we have a real second order calibration curve, well fitted by a non-linear second order regression. For this reason, reducing working concentration range does not affect the shape of the calibration.

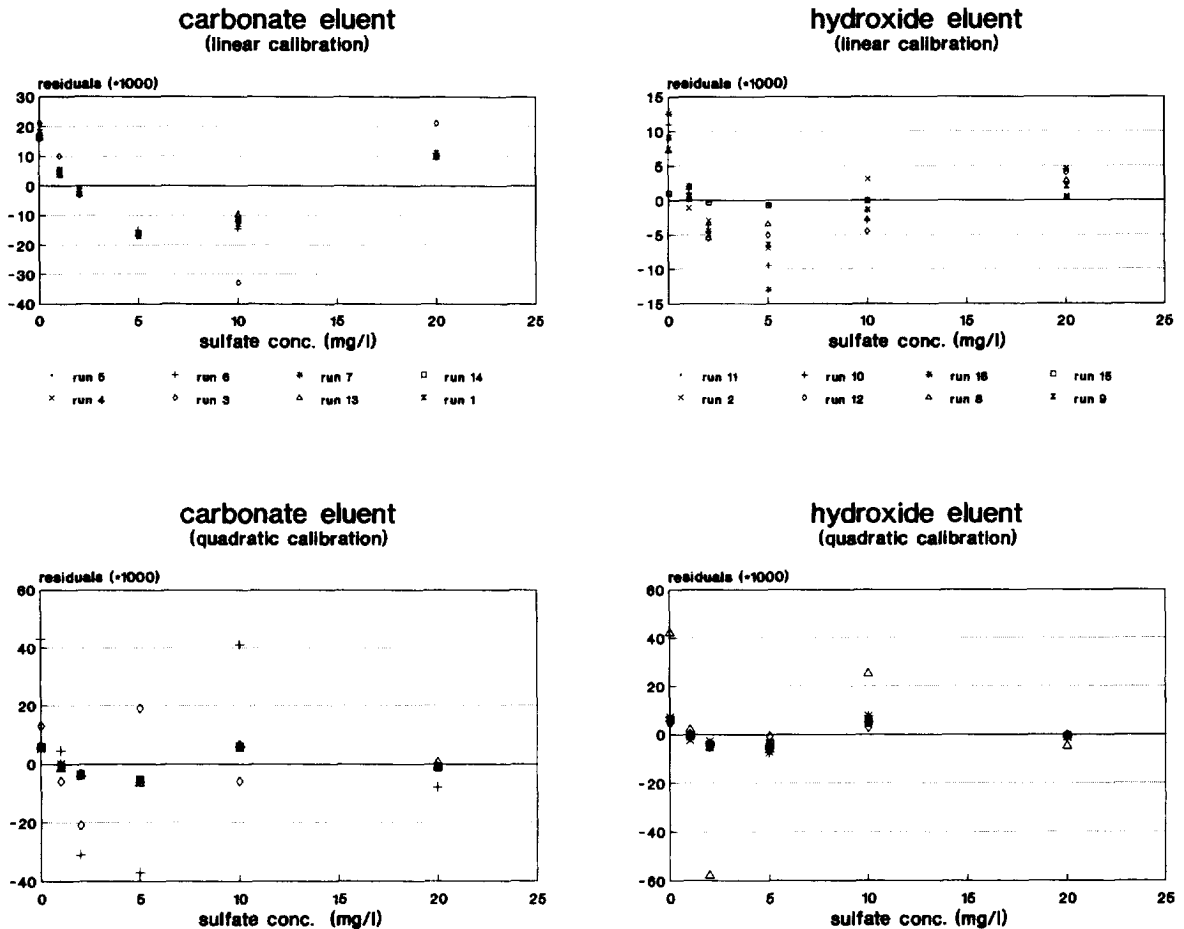Estimation of goodness of fit cannot be done on the basis of the R^2 coefficients alone, because both in

Fig. 6. Residuals graphs (vs. sulfate concentration) relative to linear and quadratic calibration of data from runs performed with carbonate and hydroxide eluent. The symbols for the runs are the same for linear and quadratic calibration. Description of the runs is reported in Table 1.

the case of linear and quadratic regression values as high as 0.99 can easily be obtained. Using carbonate eluent, linear regression can lead to very large percentage errors (45%) in the analysis of unknown samples at low concentration, while quadratic regression gives more accurate results (error less than 6%). With hydroxide eluent no significant differences exist between linear and quadratic calibration.

Though in the present work sulfate calibration was investigated, from a qualitative point of view the same considerations hold also for other common anions like chloride and nitrate, as supported also by other authors [8,9]. Thus use of quadratic type regression for calibration is highly recommended to

improve the accuracy of analytical results, even if the International Standards Organization (ISO) suggests "to reject calibration if not linear".

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