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## **Practical applications of solid-phase reagent conductivity detection in ion chromatography**

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### ABSTRACT

The post-column addition of a solid-phase reagent (SPR) is a new detection technique which permits enhanced conductivity detection in ion chromatography. The SPR for anion analysis is a high-capacity, hydrogen-form cation-exchange resin which is added as a suspension to the column effluent. This results in reducing the background conductivity of the eluent while producing an enhanced analyte signal. SPR conductivity detection has the advantages of linear calibration curves, is relatively matrix-independent and, most importantly, it enables conductivity detection to be used in conjunction with relatively broad ionic strength gradients. The parameters which influence SPR detection are discussed and a variety of eluents were investigated for gradient use. The practical utility of gradient separations with SPR conductivity detection is demonstrated for a number of complex anion analyses.

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### INTRODUCTION

The technique of ion chromatography (IC) has broadened significantly to encompass many separation and detection modes since its introduction in 1975 [1]. Of the detection modes available, conductivity is the most universal and still most widely used [2]. One disadvantage of conductivity detection is that it has historically been difficult to use in conjunction with gradient separations. Gradient IC separations offer the potential to give good resolution of short-chain carboxylic acids from weakly retained anions such as fluoride and chloride, while maintaining reasonable chromatographic run times and peak shapes for later-eluting anions such as sulfate, thio-sulfate and chromate. Varying approaches which enable the use of gradient separation and conductivity detection in IC have been reported. Micromembrane suppressor devices permit the use of ionic strength gradients with conductivity detection by minimizing the background conductivity of the eluent before it reaches the detector [3]. Alternatively, isoconductive gradients use two counter cations of different conductivity to balance the conductance of two solutions of different eluting

strength [4]. Isoconductive gradients are somewhat limited as they can only be operated over a relatively narrow ionic strength range and membrane devices have also been shown to have eluent ionic strength restrictions [5].

It has recently been demonstrated that the post-column addition of a solid-phase reagent (SPR) permits sensitive conductivity detection in IC [6]. The SPR is typically a high-capacity cation-exchange resin in the hydrogen form which is added as a colloidal suspension to the column effluent prior to the conductivity detector. The SPR reacts with the eluent and solute anions producing an eluent stream of low background conductivity and an enhanced analyte response. The SPR itself is minimally conductive and only slightly contributes to the background signal. This technique has the advantages that it allows gradient separations to be used with conductivity detection and it also offers a relatively high degree of matrix independence making it ideal for the analysis of 'difficult' samples. In this paper the parameters which influence SPR detection are discussed and the practical utility of gradient separations with SPR conductivity detection is demonstrated for a number of complex anion analyses.

## EXPERIMENTAL

### *Instrumentation*

The liquid chromatography consisted of a Waters Chromatography Division of Millipore (Milford, MA, U.S.A.) gradient 600 Series pump, a Model 431 conductivity detector, a Model 441 fixed-wavelength UV detector, a pneumatic reagent delivery module (RDM), a Rheodyne 7010 injector and either a Waters 820 or a Waters 840 data station. The analytical column used was either a Waters IC-Pak Anion HR (75 × 4.6 mm I.D.) or a Waters IC-Pak Anion (50 × 4.6 mm I.D.) methacrylate-based anion exchanger.

### *Reagents*

Water (18 M $\Omega$ ) purified using a Millipore Milli-Q water-purification system (Bedford, MA, U.S.A.) was used for all solutions. Analytical-grade boric acid and potassium hydroxide were obtained from Sigma and analytical-grade sodium hydrogencarbonate and sodium carbonate were obtained from Aldrich. Acetonitrile (HPLC grade) was obtained from J. T. Baker, as were the analytical-grade sodium salts used for the preparation of all the anion standards. Eluents were prepared daily, filtered and degassed with a Waters solvent clarification kit.

### *Solid-phase reagent*

The SPR is manufactured by Sarasep (Santa Clara, CA, U.S.A.) and distributed by Waters. The SPR is obtained as a concentrate of about 12% solids with a cation-exchange capacity of not less than 500 mequiv./l. Typically, the concentrate is diluted 1:8 for post-column use with gradients. The conductivity of the diluted suspension ideally should not exceed 20  $\mu$ S/cm. To eliminate any contaminants, a Waters SPR polisher column is inserted between the RDM and the mixing tee. The SPR polisher column is a strong anion exchanger in the hydroxide form which requires regeneration after about 50 h of continuous use. Regeneration is simply a matter of flushing the column with 10 ml of 100 mM potassium hydroxide, then 15 ml of deionized water.

### *Instrumental configuration for SPR*

A basic instrumental configuration for gradient separations with SPR conductivity detection was as previously described [7,8]. The RDM is a standard pneumatic post-column delivery device. The flow-rate of SPR into the eluent stream is determined by the pressure from the air supply to the pneumatic delivery module: typically a flow-rate of 0.5 ml/min is used for SPR addition. The RDM contains a check-valve to prevent accidental introduction of mobile phase into the SPR supply in the pressurized vessel. Careful attention was paid to minimizing tubing lengths in the set-up of the delivery module in order to prevent unnecessary band broadening. Concerns about pumping a 1% solid suspension of submicron particles through the RDM and conductivity detector proved unfounded as no blockage problems occurred provided that the RDM and detector were briefly flushed out with water at the conclusion of each days work.

## RESULTS AND DISCUSSION

### *Eluents for use with gradient SPR conductivity detection*

The eluents most commonly used for gradient separations with conductivity detection are carbonate–bicarbonate, hydroxide and borate buffers. Two reactions occur on contact of the SPR with the eluent stream. Considering the case of a bicarbonate eluent, firstly, the eluent co-cations (usually sodium) are exchanged for  $H^+$  from the SPR forming the protonated acid of the eluent anion, *i.e.*  $H_2CO_3$ . Secondly, the analyte co-cations are exchanged for  $H^+$  from the SPR producing an enhanced analyte signal. The more basic the eluent anion, the less conductive is the background after reacting with the SPR, *e.g.* the reaction product from SPR with hydroxide is water which has the lowest conductivity, borate is converted to  $H_3BO_3$  while carbonate–bicarbonate is converted to  $H_2CO_3$  as discussed above. This is illustrated in Fig. 1 which shows the effect of the rate of post-column addition of SPR on the peak height of chloride using hydroxide, borate and carbonate–bicarbonate eluents of similar eluting strength. The flow-rate of the eluent was kept constant at 1.0 ml/min. Hydroxide produces the greatest signal, followed by borate, then carbonate as would be expected from the  $pK_a$  values of water,  $H_3BO_3$  and  $H_2CO_3$ . Fig. 1 also shows that the optimal rate of addition of SPR is between 0.4 and 0.7 ml/min. The net peak height obtained is the sum of three contributions: the total cation-exchange capacity of the SPR added, the dilution of the column effluent resulting from the addition of the SPR and the fact that peak heights are reduced as a result of increasing total flow through the detector.

The three eluents listed above were investigated for use with gradient separations and SPR conductivity detection. Each of the three eluents proved to have their relative advantages and disadvantages. Carbonate–bicarbonate is a mobile phase with good eluting strength which allowed the separation of a wide range of anions. The major drawback was that the SPR reaction product,  $H_2CO_3$ , is too strong an acid, therefore a significant baseline shift occurred during the course of a gradient run. This behavior has been noted previously and for this reason, carbonate–bicarbonate eluents are not generally favored for use with gradients in IC [2]. A very interesting feature of SPR conductivity detection with carbonate–bicarbonate eluents was the fact that linear calibration curves were obtained for both strong and weak acid

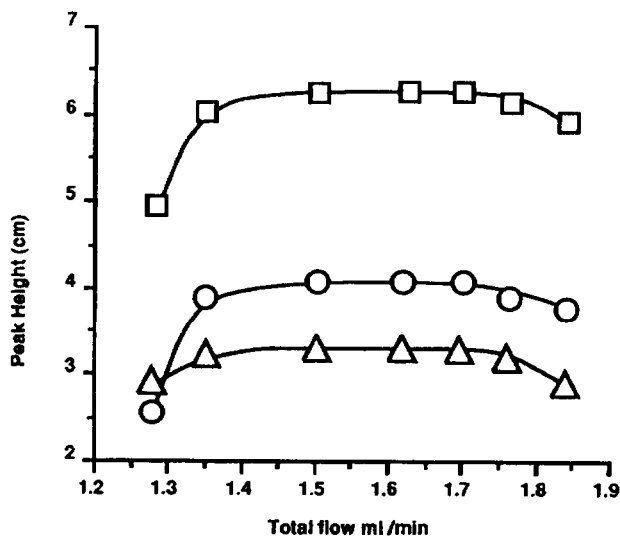


Fig. 1. Dependency of chloride peak height upon the rate of post-column addition of SPR with hydroxide, borate and carbonate-bicarbonate eluents. The ion-exchange capacity of the SPR was constant at 70 mequiv./l. The eluent flow-rate was constant at 1.0 ml/min and the SPR flow-rate was varied between 0.25 and 0.85 ml/min. □ = 15 mM KOH; ○ = 50 mM H<sub>3</sub>BO<sub>4</sub>, pH 8.35; △ = 2.8 mM NaHCO<sub>3</sub>-2.2 mM Na<sub>2</sub>CO<sub>3</sub>.

anions. The non-linearity of calibration curves with carbonate-bicarbonate eluents and conventional chemically suppressed conductivity detection has been well documented and it is generally agreed that the non-linearity occurs as a result of variations in the extent of ionization of the suppressed eluent acid, *e.g.* H<sub>2</sub>CO<sub>3</sub> [9,10].

Linearity of calibration using SPR conductivity detection was subjected to a test described by Scott [11]. According to that procedure, calibration curves can be considered as linear, if the value of the slope of a logarithmic plot (*i.e.* log peak area *versus* log concentration) remains within the range 0.98–1.02. Chloride and formate were chosen for the linearity evaluation to represent both, weakly and strongly acidic analyte anions. For eight evenly spaced concentrations between 0.05 and 50 ppm the respective slopes of logarithmic plots (logarithmic response factors) for formate and chloride were found to be 0.99394 and 0.99320, respectively. This result, indicating linearity over three orders of magnitude, was somewhat unexpected, and further investigations into the linearity of SPR conductivity detection are currently underway in our laboratory.

Hydroxide is a weak ion-exchange eluent and is particularly suited to the separation of weakly retained anions, however, this requires high eluent concentrations to elute strongly retained anions such as citrate and thiocyanate. This fact has led to problems in the past when using hydroxide gradients with chemically suppressed conductivity detection as suppressor breakthrough may occur before the peaks of interest elute from the column, although the addition of modifiers such as *p*-cyanophenol can reduce the required hydroxide concentration in the eluent [5]. As the ion-exchange reaction for the SPR enhancement is carried out directly in the bulk

liquid phase rather than across a membrane as is the case with suppressor devices, higher ion-exchange capacities can be applied by simply increasing the rate of addition of the SPR or increasing the concentration of the applied post-column reagent. Other problems with hydroxide gradients are that the eluent will readily absorb  $\text{CO}_2$  from the atmosphere resulting in baseline drift unless precautions such as helium sparging and the use of ascarite tubes are taken; and very-high-purity hydroxide must be used to minimize accumulation of anionic impurities on the column when operating low-ionic-strength steps in the gradient [2]. Despite the difficulties involved with hydroxide gradients, they permit the most sensitive detection in SPR conductivity, have a rapid re-equilibration time and, as will be shown later, give useful separations, particularly for oxyhalide speciation.

Of the three eluents investigated for use with gradient separations and SPR conductivity detection, borate proved to be the most versatile. It has intermediate eluting strength with a high buffering capacity and can be used for the separation of a wide range of anions. As the SPR reaction product is a weak acid with a  $\text{p}K_a$  of 9.24, only a relatively small baseline rise is observed during the course of a gradient run. Its use does not require any special precautions and it also has a rapid re-equilibration time between successive gradient runs. Examples of SPR gradient chromatograms are given in the next section of the paper.

#### *Practical applications of gradient SPR conductivity detection*

Fig. 2 shows a chromatogram of fluoride through to citrate using a borate gradient with SPR conductivity detection. The early-eluting peaks (fluoride, acetate, formate and chloride) are well resolved with this eluent while strongly retained analytes such as chromate and citrate are eluted within 25 min. The gradient program for this separation is given in Table I. Considering that the gradient is from 10 to 150 mM borate, pH 8.3, the baseline rise with this eluent is quite insignificant. Fig. 3 shows a chromatogram of anionic impurities in a diluted synthetic peptide obtained using the same conditions as for Fig. 2. The separation of acetate, chloride and trifluoroacetate is a difficult isocratic separation and the quantitation of these impurities (especially trifluoroacetate) is of importance in the pharmaceutical use of synthetic peptides.

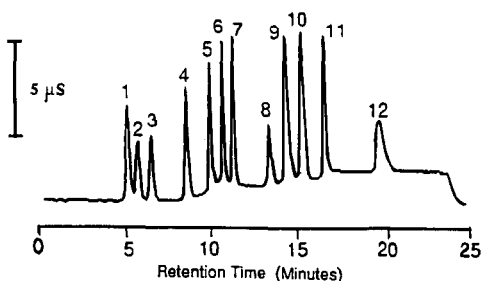


Fig. 2. Gradient separation of anions with borate eluent and SPR conductivity detection. Conditions: column, Waters IC-Pak Anion HR; eluent, 10–150 mM borate gradient, pH 8.3 (see Table I for gradient profile); flow-rates, eluent 1.0 ml/min and SPR 0.7 ml/min; injection, 100  $\mu\text{l}$ . Peaks: 1 = fluoride (1 ppm); 2 = acetate (4 ppm); 3 = formate (2 ppm); 4 = chloride (2 ppm); 5 = nitrite (3 ppm); 6 = bromide (4 ppm); 7 = nitrate (4 ppm); 8 = phosphate (6 ppm); 9 = sulfate (4 ppm); 10 = oxalate (10 ppm); 11 = chromate (10 ppm); 12 = citrate (20 ppm).

TABLE I

## PROGRAM (WATERS 600 SERIES PUMP) FOR BORATE GRADIENT SEPARATION

Gradient: A = Milli-Q water; B = 200 mM sodium borate, pH 8.3.

Time (min)	A (%)	B (%)	Curve <sup>a</sup>
Initial	95	5	—
3.00	95	5	9
9.00	70	30	9
15.00	25	75	10
22.00	95	5	11

<sup>a</sup> Curves 9 and 10 are non-linear (convex) gradient profiles, while curve 11 is a step gradient to return to initial conditions.

Fig. 4A shows a fifteen-anion standard run using a borate gradient with SPR conductivity detection, while Fig. 4B shows the same standard but with UV detection at 214 nm. The UV detector must be placed before the mixing tee when using SPR detection. Such an instrumental configuration is a useful tool, both qualitative and quantitative, for the analysis of samples which contain many analyte peaks at dissimilar levels. This approach was used for the analysis of anions in post-ignition residue from low explosive/pyrotechnic materials. The anion profile in the residue is of interest as it allows different pyrotechnic materials to be distinguished [12]. Fig. 5A shows a chromatogram of a 1:5 dilution of burned Pyrodex Powder with SPR conductivity detection using the same conditions as for Fig. 4, while Fig. 5B shows a chromatogram of a sample with UV detection at 214 nm. The use of a borate gradient in conjunction with a UV and SPR conductivity detection enables the total anion profile of this very complex sample to be characterized in one chromatographic run in under 30 min. Chromatograms obtained for a similar sample, a 1:5 dilution of burned Gopher Gasser, are shown in Fig. 6A and B for SPR conductivity and UV detection,

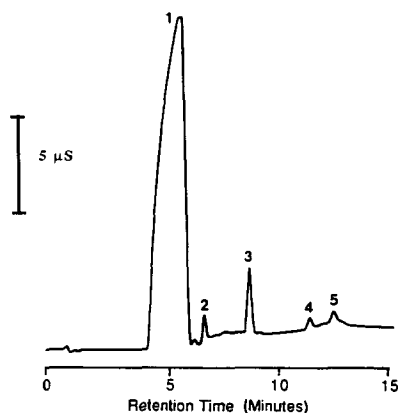


Fig. 3. Gradient separation of anionic impurities in synthetic peptide. Conditions as for Fig. 2. Peaks: 1 = acetate; 2 = chloride; 3 = trifluoroacetate; 4 = phosphate; 5 = sulfate.

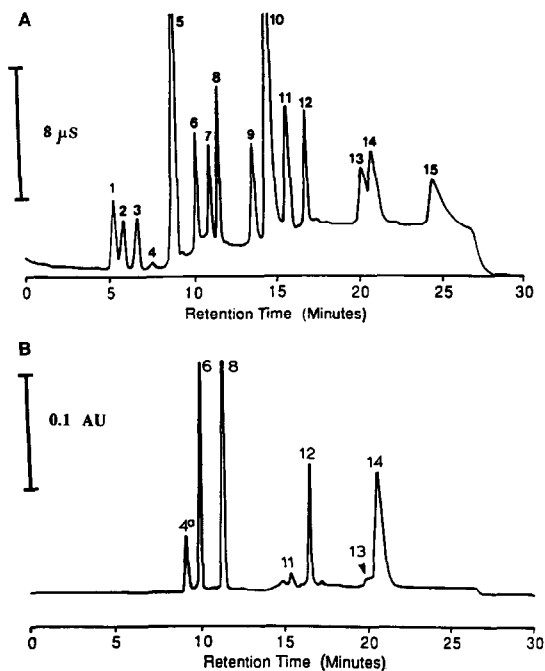


Fig. 4. Gradient separation of a fifteen-anion standard with borate eluent and dual SPR conductivity and UV detection. Conditions as for Fig. 2 except (A) SPR conductivity detection and (B) UV detection at 214 nm. Peaks: 1 = fluoride (1 ppm); 2 = acetate (4 ppm); 3 = formate (2 ppm); 4 = chlorite (4 ppm); 4a = sulfide (4 ppm); 5 = chloride (2 ppm); 6 = nitrite (3 ppm); 7 = chlorate (4 ppm); 8 = nitrate (4 ppm); 9 = phosphate (10 ppm); 10 = sulfate (4 ppm); 11 = oxalate (10 ppm); 12 = thiosulfate (10 ppm); 13 = citrate (20 ppm); 14 = thiocyanate (10 ppm); 15 = perchlorate.

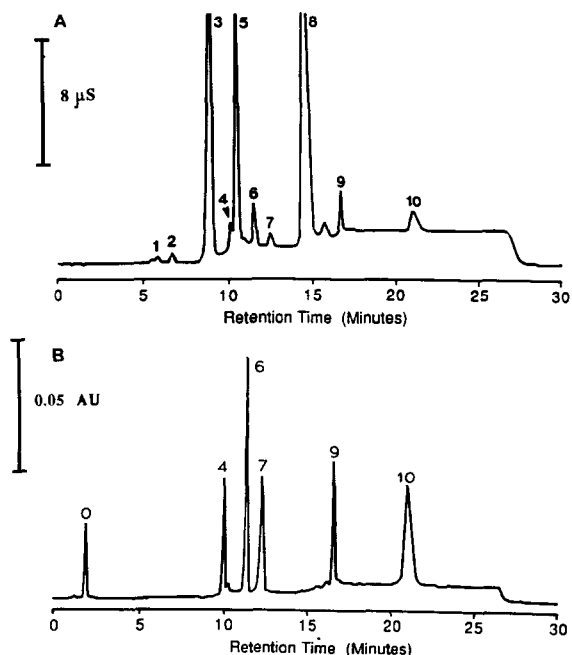


Fig. 5. Determination of anions in burned Pyrodex Powder. Conditions as for Fig. 4 [(A) SPR conductivity detection and (B) UV detection at 214 nm] except sample: 100  $\mu\text{l}$  of a 1:5 dilution of burned Pyrodex Powder. Peaks: 0 = unknown; 1 = acetate; 2 = formate; 3 = chloride; 4 = nitrite; 5 = unknown; 6 =

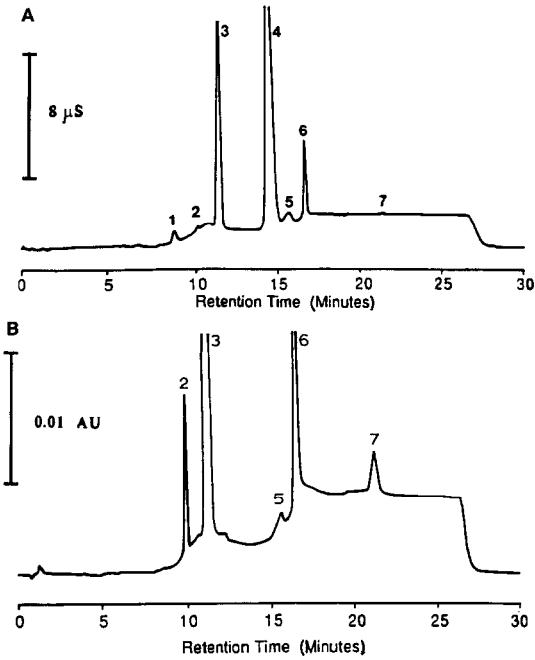


Fig. 6. Determination of anions in burned Gopher Gasser. Conditions as for Fig. 4 [(A) SPR conductivity detection and (B) UV detection at 214 nm] except sample:  $100 \mu\text{l}$  of a 1:5 dilution of burned Gopher Gasser. Peaks: 1 = chloride; 2 = nitrite; 3 = nitrate; 4 = sulfate; 5 = oxalate; 6 = thiosulfate; 7 = thiocyanate.

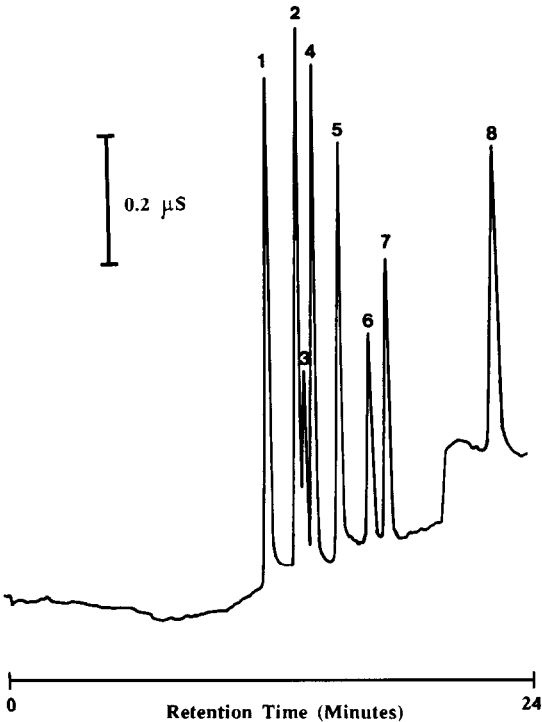


Fig. 7. Gradient separation of oxyhalides and other common anions with hydroxide eluent and SPR conductivity detection. Conditions: column, Waters IC-Pak Anion HR; eluent,  $0.5\text{--}15 \text{ mM}$  potassium hydroxide gradient (5% acetonitrile); flow-rates, eluent  $1.0 \text{ ml/min}$  and SPR  $0.4 \text{ ml/min}$ ; injection,  $100 \mu\text{l}$ . Peaks: 1 = fluoride ( $0.6 \text{ mM}$ ); 2 = chloride ( $10 \text{ mM}$ ); 3 = bromide ( $6 \text{ mM}$ ); 4 = iodide ( $0.5 \text{ mM}$ ); 5 =



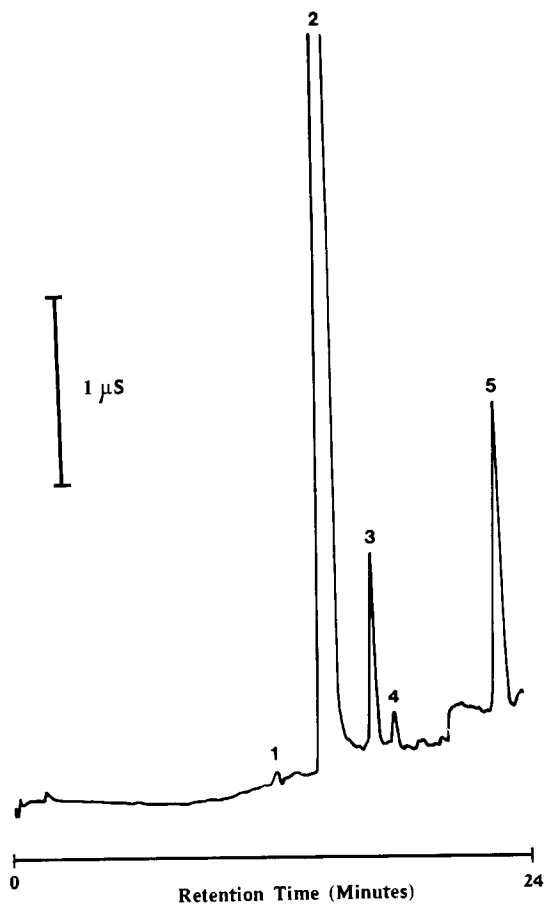


Fig. 8. Determination of disinfection by-products in swimming pool water. Conditions as for Fig. 7 except sample: 100  $\mu$ l of swimming pool water. Peaks: 1 = fluoride (0.2 ppm); 2 = chloride (> 50 ppm); 3 = chlorate (11.3 ppm); 4 = nitrate (1.4 ppm); 5 = sulfate (12.5 ppm).

respectively. This amount of peak information could not be attained using isocratic IC, as was also the case with the previous sample.

Another important application of gradient SPR detection is the analysis of anions formed as by-products from the disinfection of water by chlorination. The levels of these anions, *i.e.* chlorite, bromate, chloride, chlorate, are soon to be regulated by the US Environmental Protection Agency [13]. Fig. 7 shows a chromatogram of some oxyhalides and other common anions using a hydroxide gradient with SPR conductivity detection. Both of the eluents for the gradient were sparged with helium during the chromatographic run. Fig. 8 shows the analysis of chloride and chlorate in swimming pool water chromatographed using the same conditions as for Fig. 7.

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