# Determination of inorganic anions on porous graphitic carbon using evaporative light scattering detection
## Use of carboxylic acids as electronic competitors

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## Abstract

Different volatile carboxylic acids have been investigated as electronic competitors to optimize the separation of 12 common anions ($F^-$, $H_2PO_4^-$, $Cl^-$, $IO_3^-$, $IO_4^-$, $Br^-$, $BrO_3^-$, $SO_4^{2-}$, $ClO_3^-$, $NO_3^-$, $I^-$, $ClO_4^-$) on a porous graphitic carbon (PGC) column with evaporative light scattering detection. The addition of pyridine in the aqueous acidic mobile phase improves the efficiency of the separation and the detector response. The retention of inorganic anions on non-modified PGC is dominated by electronic interactions based purely on donor–acceptor (charge transfer) interaction between the lone pair electrons of the solute and the π-electrons of the PGC. © 1998 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Good separations of four anions (iodate, nitrite, bromide and nitrate) have been achieved by Knox and Wan [1] on a new graphite-based ion exchanger obtained by adsorption of polyethylene imine from aqueous solution on porous graphitic carbon. The performance of this new ion exchanger compares favourably with that of columns packed with chemically bonded silica gel-based ion-exchangers. Nevertheless, the separation of inorganic anions on commercial reversed stationary phases and conventional high-performance liquid chromatography instrumentations offer, with respect to ion chromatography, the advantages of lower cost and can be advantageously employed in laboratories where only conventional HPLC systems are available. Recently, a review [2] was devoted to the more recent papers (published between 1989 and 1997) concerning the ion-interaction chromatographic determination of inorganic anions. The performances obtained in ion-interaction chromatography by the use of different polymeric and octadecyl silica (ODS) packing materials were compared but no evaluation of porous graphitic carbon (PGC) as an alternative stationary phase was undertaken. Nevertheless more recently (in 1998), a method was published for the determination of different inorganic anions [3] on a graphitized carbon packing (carbon BI-01), with suppressed conductivity detection, using tetrabutylammonium hydroxide as ion-pairing reagent and sodium carbonate, hydroxide or tetraborate as eluting agents.

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PGC is regarded primarily as a strong reversed-phase packing material but it has proved to have a number of unsuspected properties. PGC is possessed of a rigid, planar surface and sites capable of dispersion and charge transfer interactions. It is now well established that PGC is particularly useful for the separation of highly polar compounds which would be difficult to retain on ODS phases and for the separation of solutes with closely related structures including stereoisomers [4,5]. Using the separation of oxo anions of technetium and rhenium as an example, Lim [6] has demonstrated that electronic interaction chromatography (EIC), based totally on electronic interaction is also possible on a PGC column. Various cationic and anionic [7] complexes of technetium and rhenium were separated using trifluoroacetic acid (TFA) solutions containing 1% acetonitrile. The separation of small ionizable compounds of biomedical interest [8] or natural anionic glucosinolates [9] has been also successfully achieved with this chromatographic technique.

The analysis of inorganic anions has traditionally employed a conductivity detection but recently, the use of evaporative light scattering detection (ELSD) has been proposed as an effective alternative for the determination of sodium in pharmaceutical compounds [10] or other ions such as chloride [11]. Indeed, ELSD is commonly referred to as a sensitive universal detector in liquid chromatography (LC) for solutes which are less volatile than the eluting solvents [12].

In this paper, a non-modified PGC column (Hypercarb S column) is evaluated under isocratic elution conditions for the analysis of inorganic anions with ELSD. Different carboxylic anions derived from volatile acids compatible with ELSD volatility requirements have been investigated as electronic competitors in order to manage the retention of inorganic anions.

## 2. Experimental

### 2.1. Apparatus

The liquid chromatographic apparatus consisted of a Gilson (Villiers-le-Bel, France) Model 302 isocratic pump, a Rheodyne (Berkeley, CA, USA) Model 7125 injector with a 20-µl sample loop and an ELSD system (Sedere, Alfortville, France) Model Sedex 45. The usual ELSD settings were as follows: photomultiplier, 9; evaporative temperature, 40°C; air pressure, 2.2 bar. The porous graphitic carbon column was Hypercarb S (100×4.6 mm I.D., particle size 7 µm) from Hypersil (Runcorn, UK). Data were processed using a Shimadzu (Kyoto, Japan) Model CR 5A integrator.

### 2.2. Reagents

Eluent constituents were purchased as follows: acetonitrile (ACN) RS for LC from Carlo Erba (Milan, Italy); methanol (MeOH) Hypersolv grade from BDH (Poole, UK); water from an Elgastat UHQ II System from Elga (Antony, France); formic acid (HCOOH); acetic acid ($CH_3COOH$); trifluoroacetic acid (TFA); heptafluorobutyric acid (HFBA) and pyridine from Merck (Darmstadt, Germany).

Solutes were purchased as follows: sodium fluoride, sodium chloride, sodium bromide, potassium iodide, sodium nitrite, sodium nitrate, ammonium sulfate, sodium chlorate, potassium bromate, potassium iodate, sodium perchlorate and potassium periodate from Prolabo (Paris, France); ammonium fluoride and phosphoric acid from Merck (Darmstadt, Germany). All reagents were of analytical grade.

The concentration of each standard in the different injected samples was about 100 to 500 mg $l^{-1}$ in aqueous solution.

The theoretical values of pH and ionic strength of each eluent were calculated from PHoEBuS, an application program help for buffer studies (Sedere, Alfortville, France) and then for each mobile phase, the pH value was checked with a Beckman pH meter (Model Φ10, Gagny, France).

## 3. Results and discussion

Common inorganic anions ($F^-$, $Cl^-$, $Br^-$, $I^-$, $NO_3^-$, $H_2PO_4^-$, $SO_4^{2-}$, $ClO_3^-$, $BrO_3^-$, $IO_3^-$, $ClO_4^-$, $IO_4^-$) which could not be retained and separated without ion-pairing when using conventional silica-based reversed-phase materials, were, however, totally retained on PGC with deionized water as mobile

phase. Such results have already been reported previously for $TcO_4^-$ and $ReO_4^-$ anionic complexes [6] and for anionic glucosinolates [9]. For these compounds, the retention mechanism could be a combination of interactions involving the size, the polarizability and the hydrophobicity in addition to the negative charge. In the case of the studied inorganic anions, the retention on PGC cannot be simply due to hydrophobic interaction between the solutes and the hydrophobic carbon surface since none of these anions possess hydrophobic functions and the addition of an organic modifier (5 to 10%) such as acetonitrile or methanol did not affect the retention. Although there are no ion-exchange sites on PGC, there appeared to be strong attractive interactions between the charged centers in the analyte and the graphitic surface, leading to the observed retention from an aqueous eluent.

In contrast to these results, monocharged cations as $Na^+$, $K^+$ or $NH_4^+$ were eluted without separation near the void volume on PGC support with deionized water as mobile phase.

Hence, the addition in the aqueous mobile phase of different common competing agents for EIC [6,8,9] was investigated. The choice of these electronic modifiers should be compatible with ELSD volatility requirements.

In Table 1, the retention factors, $k$, are reported for ten inorganic anions using different carboxylic anions in aqueous solution as mobile phase. The electronic modifier strength decreased in the order: HFBA>TFA>HCOOH>CH$_3$COOH.

CH$_3$COOH, even at high concentration (100 m$M$), did not provide an adequate carboxylate anion concentration to elute all inorganic anions by competitive electronic interaction. The use of two strong acids, TFA or HFBA, which are totally dissociated at 5 m$M$, brings about variations in the retention factors although similar pH and ionic strength values were noticed for these two mobile phases. As observed in ion-exchange chromatography, the nature of the carboxylate anion and its charge distribution were as significant as its acidic strength to control the elution strength. Moreover, the elution strength of a given eluent anion increases by increasing its concentration. It was clear that the elution and separation of inorganic anions from PGC by carboxylic acid is due to the successful competition of the carboxylate anion with the solutes for electronic interaction with the $\pi$-electrons of PGC. For a better understanding, it would be interesting to evaluate the elution strength of some aromatic acids but unfortunately, their low volatility is not compatible with the choice of ELSD.

Table 1
Retention factors ($k$) of inorganic anions on a Hypercarb S column (100×4.6 mm I.D.) with different carboxylic acids as electronic competitors in an aqueous mobile phase

| Carboxylic acid | pH | Ionic strength (m$M$) | $H_2PO_4^-$ | $Cl^-$ | $IO_3^-$ | $IO_4^-$ | $Br^-$ | $SO_4^{2-}$ | $ClO_3^-$ | $NO_3^-$ | $I^-$ | $ClO_4^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CH$_3$COOH | | | | | | | | | | | | |
| 10 m$M$ | 3.39 | 0.42 | 11.57 | [a] | [a] | [a] | [a] | [a] | [a] | [a] | [a] | [a] |
| 100 m$M$ | 2.88 | 1.37 | 4.63 | 5.40 | 7.35 | 7.50 | 9.76 | [a] | [a] | [a] | [a] | [a] |
| | | | | | | | | | | | | |
| HCOOH | | | | | | | | | | | | |
| 100 m$M$ | 2.39 | 4.45 | 1.32 | 1.86 | 2.35 | 2.43 | 2.62 | 8.62 | 6.01 | 6.09 | 7.34 | 11.10 |
| 300 m$M$ | 2.14 | 7.99 | 0.60 | 0.79 | 1.08 | 1.13 | 1.18 | 2.14 | 2.5 | 2.90 | 3.3 | 4.96 |
| | | | | | | | | | | | | |
| TFA | | | | | | | | | | | | |
| 5 m$M$ | 2.34 | 4.94 | 1.20 | – | 1.96 | 2.05 | – | – | – | – | – | – |
| 10 m$M$ | 2.06 | 9.80 | 0.41 | 0.40 | 0.54 | 0.59 | 0.68 | 0.89 | 1.38 | 1.33 | 1.81 | 2.77 |
| | | | | | | | | | | | | |
| HFBA | | | | | | | | | | | | |
| 5 m$M$ | 2.34 | 5.00 | 0.31 | 0.35 | 0.63 | 0.71 | 0.72 | 1.18 | 1.36 | 1.40 | 1.63 | 2.92 |

TFA: Trifluoroacetic acid; HFBA: heptafluorobutyric acid.
[a] $k_{solute}>13$
– Solute not injected.

Except for $SO_4^{2-}$ in the case where 100 m$M$ HCOOH was used as mobile phase, whatever the nature and the concentration of eluent anion, the same elution order was observed on PGC column: $ClO_4^- > I^- > NO_3^- > ClO_3^- > SO_4^{2-} > Br^- > IO_4^- > IO_3^- > Cl^- > H_2PO_4^-$. $F^-$ was eluted near the void volume as soon as an electronic competitor was added in the aqueous mobile phase. The more polarizable the ion was, the higher the elution time was. For the halides and for $ClO_3^-$ and $ClO_4^-$, the elution order on PGC column was in agreement with that usually observed on a silica anion exchanger [13].

Fig. 1 depicts a typical chromatogram obtained with aqueous carboxylic acid solution as mobile
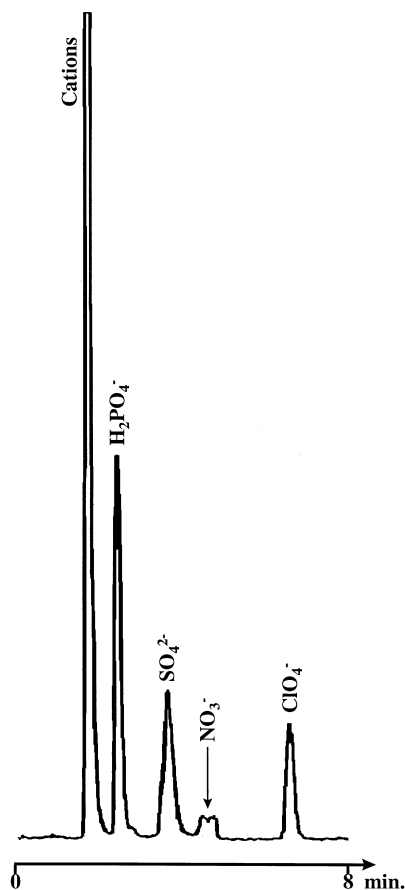


Fig. 1. Separation of $H_2PO_4^-$, $SO_4^{2-}$, $NO_3^-$, $ClO_4^-$ in a standard mixture on a Hypercarb S (100×4.6 mm I.D., 7 μm) column. Eluent: aqueous solution of 300 m$M$ formic acid. Isocratic elution at 1 ml min$^{-1}$. Evaporative light scattering detection.

phase for a standard mixture of four inorganic anions: $H_2PO_4^-$, $SO_4^{2-}$, $NO_3^-$, $ClO_4^-$. It was possible to elute inorganic anions on the PGC column with only aqueous solution of carboxylic acids as mobile phase but the chromatographic system produced poor chromatographic performance for anion analysis. A lack of sensitivity in the ELSD response was observed for several inorganic anions ($NO_3^-$, $Cl^-$, $Br^-$, $I^-$, $ClO_3^-$, $IO_3^-$, $IO_4^-$) in comparison with the other ones ($H_2PO_4^-$, $SO_4^{2-}$, $ClO_4^-$). Some peaks are badly tailed (as shown for $NO_3^-$ in Fig. 1) and the number of theoretical plates are low even for solutes which are not well retained. This fact should indicate a too slow transfer between the mobile phase and the stationary phase but in the case of solutes with low retention, its remains without explanation. The addition of acetonitrile in the eluent had no significant effect on the chromatographic system consisting of a PGC column and an aqueous carboxylic acid solution as eluent. These results are different from those obtained in ion chromatography as it is well established that in ion chromatography the addition of organic modifier facilitate phase transfer and produce sharper peaks and shorter retention times for the analysis of polarizable anions such as nitrate and bromide [14].

We then investigated the effect on the separation of base addition in the acidic mobile phase. Because pyridine is a basic compound with a planar structure containing aromatic ring electrons and lone-pair electrons (or positively charged in the protonated form), it can interact strongly with the PGC surface and therefore, could play a role in regulating the anion retention.

Table 2 reports the retention factors, $k$, of ten inorganic anions obtained with different aqueous mobile phases containing formic acid and pyridine in variable amounts. Table 2 shows clearly that the addition of pyridine in the eluent reinforces favourably the elution strength of the acidic aqueous mobile phase by increasing the ionic strength. A similar order of magnitude of the retention factor values was observed with 300 m$M$ HCOOH and with mixtures constituted by 20 m$M$ HCOOH and 20 m$M$ pyridine or 30 m$M$ HCOOH and 20 m$M$ pyridine as eluents. It also appears that the elution strength increases by increasing the salt concentration in the mobile phase: the value of the retention

Table 2
Retention factors ($k$) of inorganic anions on a Hypercarb S column obtained with different mobile phases constituted of aqueous solutions of formic acid and pyridine in variable amounts

| Mobile phase | pH | Ionic strength (m$M$) | $H_2PO_4^-$ | $Cl^-$ | $IO_3^-$ | $Br^-$ | $BrO_3^-$ | $SO_4^{2-}$ | $ClO_3^-$ | $NO_3^-$ | $I^-$ | $ClO_4^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 300 m$M$ HCOOH | 2.14 | 7.99 | 0.60 | 0.79 | 1.08 | 1.18 | – | 2.14 | 2.5 | 2.90 | 3.3 | 4.96 |
| 10 m$M$ HCOOH +10 m$M$ pyridine | 4.47 | 8.54 | 1.06 | 1.24 | 1.48 | 2.13 | 2.92 | 4.81 | 5.14 | 4.75 | – | >12 |
| 20 m$M$ HCOOH +20 m$M$ pyridine | 4.47 | 17.13 | 0.48 | 0.49 | 0.61 | 1.03 | 1.37 | 1.76 | 2.57 | 2.20 | 3.60 | 6.51 |
| 30 m$M$ HCOOH +20 m$M$ pyridine | 3.94 | 19.18 | 0.63 | 0.83 | 0.88 | 1.34 | 2.00 | 2.35 | 3.59 | 3.37 | 5.24 | 9.61 |

factor for a given anion was higher with 10 m$M$ formic acid+10 m$M$ pyridine than with 20 m$M$ formic acid+20 m$M$ pyridine as mobile phase. But an excess of formic acid with respect to pyridine in the mobile phase led to a slight increase in the retention time for all the studied anions in spite of the resulting increase in ionic strength. Whatever the mobile phase in Table 2, no separation between $H_2PO_4^-$ and $Cl^-$ was obtained. $H_2PO_4^-$ was always the first anion eluted and $ClO_4^-$ the last one. By comparing the retention factor of these two anions we can notice that it was the mobile phase containing the mixture of 30 m$M$ formic acid and 20 m$M$ pyridine which offers the greatest selectivity area.

In Table 3 the values of the separation factor $\alpha$ and of the number of theoretical plates $N$ for different inorganic anions are reported versus the mobile phase composition. The variations observed

for the $\alpha$ values of ($H_2PO_4^-$, $Br^-$) or ($Br^-$, $SO_4^{2-}$) pairs are minor but more significant for the ($SO_4^{2-}$, $NO_3^-$) pair. This can be attributed to the fact that retention of $NO_3^-$ depends more on the mobile phase composition than does the retention of the other anions.

Fig. 2a and b depict, respectively, a satisfactory separation obtained with 20 m$M$ HCOOH and 20 m$M$ pyridine for a standard mixture of $IO_3^-$, $BrO_3^-$ and $ClO_3^-$ and for a standard mixture of $Cl^-$, $SO_4^{2-}$ and $NO_3^-$ whereas Fig. 3 shows the chromatogram obtained with 30 m$M$ HCOOH and 20 m$M$ pyridine for the standard mixture $H_2PO_4^-$, $Br^-$, $SO_4^{2-}$, $ClO_3^-$, $I^-$ and $ClO_4^-$. By comparing these two figures with Fig. 1, it appears that the addition of pyridine in the acidic mobile phase increases the efficiency of the peak for all anions. These observations were confirmed by the values of theoretical plates number, $N$,

Table 3
Separation factors ($\alpha$) and number of theoretical plates ($N$) of some inorganic anions on a Hypercarb S column with different mobile phases constituted of aqueous solutions of formic acid and pyridine in variable amounts

| Mobile phase | Separation factor ($\alpha$) | | | | No. of theoretical plates ($N$) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $H_2PO_4^-$, $ClO_4^-$ | $H_2PO_4^-$, $Br^-$ | $Br^-$, $SO_4^{2-}$ | $SO_4^{2-}$, $NO_3^-$ | $H_2PO_4^-$ | $Br^-$ | $SO_4^{2-}$ | $NO_3^-$ | $ClO_4^-$ |
| 300 m$M$ HCOOH | 8.27 | 1.97 | 1.81 | 1.35 | 550 | [a] | 340 | [a] | 400 |
| 20 m$M$ HCOOH +20 m$M$ pyridine | 13.56 | 2.14 | 1.71 | 1.25 | 880 | 960 | 900 | 400 | 1300 |
| 30 m$M$ HCOOH +20 m$M$ pyridine | 15.25 | 2.13 | 1.75 | 1.43 (1/$\alpha$) | 2200 | 2200 | 2200 | 1050 | 2560 |

[a] Badly tailed peak (as shown in Fig. 1).
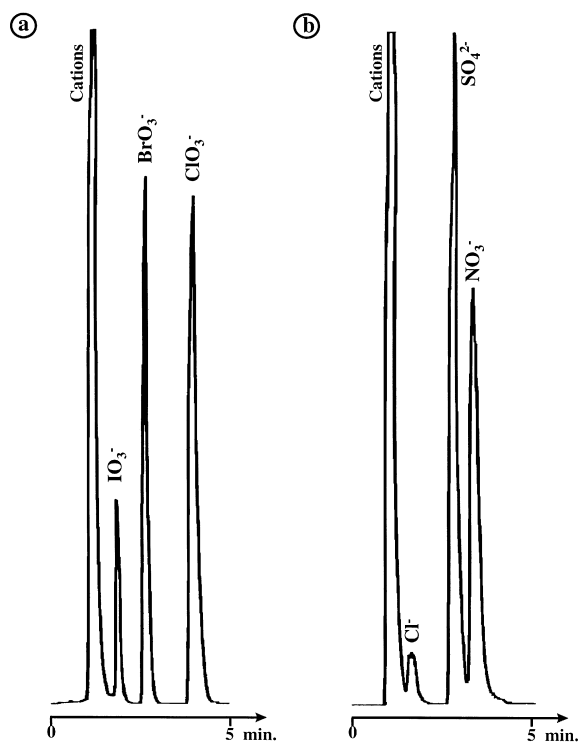$N$ was calculated from the retention time, the peak height and its area.

Fig. 2. Separation of different inorganic anions in a standard mixture on a Hypercarb S (100×4.6 mm I.D., 7 μm) column. Eluent: aqueous mixture of 20 m$M$ formic acid and 20 m$M$ pyridine. Isocratic elution at 1 ml min$^{-1}$. Evaporative light scattering detection. Elution order: (a) 1=K$^+$ and Na$^+$ in void volume; 2=IO$_3^-$; 3=BrO$_3^-$; 4=ClO$_3^-$. (b) 1=Na$^+$ and NH$_4^+$ in void volume; 2=Cl$^-$; 3=SO$_4^{2-}$; 4=NO$_3^-$.

Fig. 3. Separation of H$_2$PO$_4^-$, Br$^-$, SO$_4^{2-}$ ClO$_3^-$, I$^-$ and ClO$_4^-$ on a Hypercarb S (100×4.6 mm I.D., 7 μm) column. Eluent: aqueous mixture of 30 m$M$ formic acid and 20 m$M$ pyridine. Isocratic elution at 1 ml min$^{-1}$. Evaporative light scattering detection.

reported in Table 3. For ClO$_4^-$, the most retained anion on these chromatographic systems, the $N$ value, calculated from the retention time, the peak height and its area was in the order of 400 with aqueous formic acid solution as eluent and about 2560 with a mixture of 30 m$M$ formic acid+20 m$M$ pyridine as mobile phase. Moreover, an excess of formic acid with respect to the salt concentration appears to have a favourable effect on the efficiency in spite of the increase in retention time observed under these chromatographic conditions. However, the best observed efficiencies remain lower than those currently observed in ion chromatography.

By comparing Figs. 1 and 2, it appears that the presence of a salt in the mobile phase enhances the
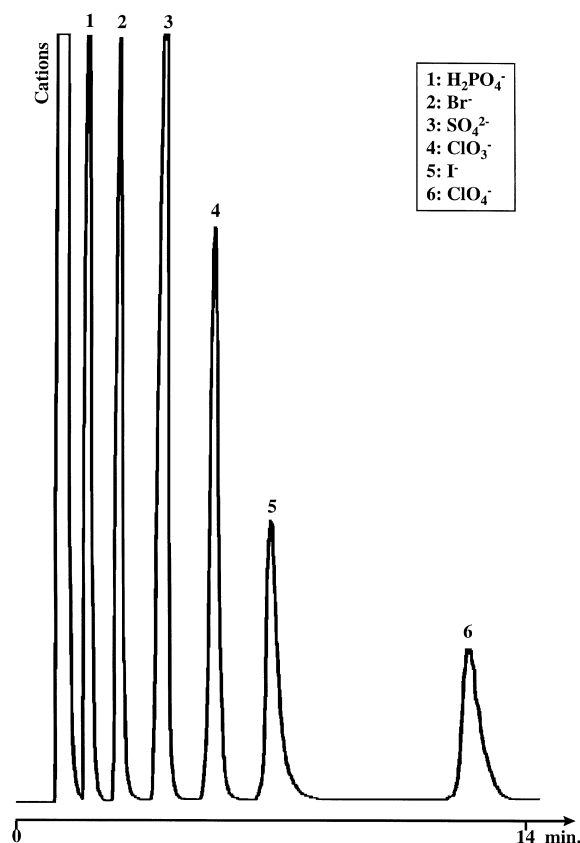
ELSD response of several anions as NO$_3^-$, H$_2$PO$_4^-$, SO$_4^{2-}$ or ClO$_4^-$. The detection limits (expressed as the amount injected that gave a signal that was three-times the background noise) were between 10 and 50 ppm for these anions. Nevertheless the ELSD response of chloride remains too much low. It has already been noticed [10] that the addition of salt such as ammonium acetate in mobile phase enhances the ELSD response of chloride. In this case, LC–MS studies have been conducted indicating the formation of ammonium chloride clusters as the moiety being detected by ELSD. Encouraging results in progress in our laboratory should lead to a better detection sensitivity for all anions by modifying the nature of salts added in the aqueous mobile phase.

## 4. Conclusions

Inorganic anions were successfully separated on a non-modified PGC column with an aqueous eluent containing electronic modifier as carboxylic acid. The addition of organic modifier in the mobile phase was without effect on the retention of inorganic anions but the addition of pyridine improves the efficiency of the separation and the ELSD response. The retention of inorganic anions on non-modified PGC is dominated by electronic interactions based purely on donor–acceptor (charge transfer) interaction between the lone pair electrons of the solute and the $\pi$-electrons of PGC. The use of ELSD enabled volatile eluents to be studied even at high concentration. The studied concentration area could not be investigated with other systems of detection such as direct conductimetry or direct/indirect UV detection due to the high background noise involved by such a high concentration of eluent anion for these detection systems.

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