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Determination of inorganic cations and anions by ion-exchange chromatography with evaporative light-scattering detection

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Abstract

Evaporative light-scattering detection (ELSD) was investigated for the direct determination of alkali and alkaline-earth cations by cation-exchange chromatography. Successful single run analysis of Na^+ , K^+ , Mg^{2+} and Ca^{2+} was achieved in 11 min on the Hamilton PRP-X200 column using an aqueous solution of ammonium formate as mobile phase under a salt concentration step gradient mode (20 mM and 100 mM). Surprisingly the use of ELSD reveals a weak retention of inorganic anions (Cl^- , NO_3^- , SO_4^{2-}) onto the polymeric cation exchanger, which enables the simultaneous determination of inorganic anions (Cl^- and NO_3^-) associated with the cations analysed (Na^+ and K^+). © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ion-exchange chromatography (IEC) was first introduced about 25 years ago and has become a well-established technique. The simultaneous analysis of common anions such as chloride, nitrate, phosphate or sulfate, is the most important IEC routine application, followed by the determination of ammonium, alkali and alkaline-earth cations. These solutes are usually determined by ion-exchange separation with suppressed conductivity detection [1]. IEC with suppressed conductivity detection permits the use of higher capacity stationary phases

and higher ionic strength eluents than those which use conductivity without chemical suppression detection methods, and provides greater variation in ion-exchange selectivity. The other common means of detection are conductivity without chemical suppression [2] and direct or indirect UV detection [3,4].

Although universal detection by a bulk property detector based on conductivity measurements is still the preferred mode for routine work in ion chromatography (IC), new applications have called for the development of advanced detection techniques that might provide more information about the identity, the structure or the elemental composition of the analytes [5–7].

In our work the detector used an evaporative light-scattering detection (ELSD) system which has already been used for the detection of sodium [8] and

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for the detection of anions [9–13]. The principle of ELSD is based on the nebulization and the evaporation of the mobile phase and on the scattered light intensity resulting from the residual particles remaining after the evaporation step. Any solute having a lower volatility than the corresponding mobile phase used for separation can be detected with ELSD. Thus, the detection of a volatile solute is not possible with ELSD and the detection of a semi-volatile solute depends on the experimental conditions as well as on the performances of the ELSD model. The use of a volatile mobile phase also enables quantisation of low levels of non-volatile ions in the presence of high levels of volatile ion (as ammonium) without any baseline perturbation and without any matrix pretreatment or particular analytical system like crown ether [14].

The purpose of this paper is to show that ELSD can be successfully used for cation detection in IEC with volatile mobile phases containing ammonium formate or ethylene diammonium formate as competitor salts. Moreover, by using this detection mode it has been demonstrated that on a Hamilton PRP-X200 column, which is dedicated classically to cation analysis, an unexpected retention of some inorganic anions is observed.

2. Experimental

2.1. Apparatus

The pump of the chromatographic system used was a P200 from ThermoQuest (Les Ulis, France). It was connected to the A100 autosampler from TSQ. A Sedex 75 ELSD system from Sedere (Alfortville, France) was used for detection. The usual ELSD settings were as follows: drift tube temperature 60°C, nebulizer gas pressure 3.6 bar, photomultiplier 9. Data were collected with EZChrom Elite software from Scientific Software (Pleasanton, CA, USA) running under Windows NT 4.0.

All experiments were carried out with a Hamilton (Reno, NV, USA) PRP-X200 column (150×4.1 mm) packed with a sulfonated polystyrene–divinylbenzene copolymer resin with a low cation-exchange capacity (35 $\mu\text{equiv. g}^{-1}$) and particle size of 10 μm . Void volume was calculated from the elution

volume of glucose in the studied mobile phases and was equal to 1.70 ml.

2.2. Reagents

All solutions were prepared from analytical-reagent grade chemicals in 18 M Ω water obtained from an Elgastat UHQ II System from ELGA (Antony, France). Potassium chloride, sodium chloride, calcium chloride and magnesium chloride were purchased from Sigma (St. Louis, MO, USA); ammonium chloride, ammonium nitrate, ammonium sulfate, ammonium hydroxide and ethylenediamine from Fluka (St. Quentin Fallavier, France); potassium carbonate and potassium sulfate from Merck (Darmstadt, Germany); and formic acid from J.T. Baker (Noisy Le Sec, France).

PhoeBus, an application program guide for buffer studies (Sedere, Franklin, MA, USA) was used for the mobile phase preparation. The mobile phases were mixtures of ammonia or ethylenediamine with formic acid. They were prepared by imposing a pH value of 4 and ionic strength values of 2, 5, 10, 20, 30 or 50 mM. The ammonium, ethylene diammonium or formate concentrations were, respectively, calculated by the software package. The pH value of each mobile phase was checked immediately after preparation with a Beckman pH meter (Model Φ 200; Gagny, France).

3. Results and discussion

3.1. Cation analysis under an isocratic elution mode

Due to high differences in retention observed between monovalent and divalent cations on the PRP-X200 stationary phase, the use of two different mobile phases – nitric acid in water–methanol mixture for the analysis of alkali metal ions and ethylene diammonium nitrate in aqueous solution for the analysis of alkaline-earth metal ions – is recommended by the supplier for the suitable isocratic separation and quantitation of these ions with conductivity detection.

In this work, ELSD was chosen as the universal detection mode instead of the usual conductivity

detection for ion analysis. Hence, the mobile phase addition of a common competitor agent for cation-exchange chromatography should be compatible with ELSD volatility requirements. To accomplish this, it was first necessary to replace the nitrate salt in the mobile phase with a more volatile salt such as a formate salt. Generally in IEC, ammonium is considered as a better competitor cation than proton; so ammonium formate and ethylene diammonium formate were tested as mobile phase modifiers for the elution of alkali and alkaline-earth cations using ELSD. Moreover, ammonium formate has the advantage of being less corrosive than nitric acid for the evaporative drift tube of the ELSD system.

Table 1 reports the retention factors (k) for monocations (Na^+ , K^+) and dications (Ca^{2+} , Mg^{2+}) under isocratic elution conditions using ammonium or ethylene diammonium as competitor ion at different concentrations in aqueous mobile phase. As usually observed in IEC, at a given pH, a decrease in the competitor ion concentration or in the ionic strength of the mobile phase results in an increase in the cation retention factor. Whatever the nature of the eluent competitor ion, considerable differences in retention were observed between the monovalent cations and the divalent cations. A low ammonium concentration (≤ 10 mM) was sufficient to obtain satisfactory retention and separation for Na^+ and K^+ , but the interactions between the dications and the stationary phase were so strong that dication elution was prevented under these elution conditions.

A high ammonium concentration (≥ 50 mM) was necessary to elute Mg^{2+} and Ca^{2+} but Na^+ and K^+ elute in the void volume. While such high salt concentrations (50 mM) in mobile phase with conductivity detection without chemical suppression would not be possible, there is no problem with ELSD if the salt is volatile. A satisfactory ELSD of ions at the ppm level has been previously demonstrated in a paper devoted to ion analysis in natural water using high salt concentration (100 mM ammonium formate) in mobile phase [13].

Table 1 demonstrates that ethylene diammonium as cation competitor has a higher elution strength than ammonium as expected in IEC. A low ethylene diammonium concentration (0.6 mM) allows Mg^{2+} elution with a retention factor of 7.0 while a high concentration of ammonium (49.9 mM) is required to obtain an equivalent retention factor. With ethylene diammonium in mobile phase, a suitable selectivity is observed for dication whereas there is no retention for monocation. This result is in good agreement with the supplier's recommendation, which recommends a mobile phase containing ethylene diammonium nitrate for alkaline-earth cation analysis.

3.2. Cation analysis under an eluent gradient mode

In the light of these results, it appeared that the use of Hamilton PRP-X200 column with ammonium

Table 1

Retention factors, k , of inorganic cations on a Hamilton PRP-X200 column (150×4.1 mm I.D.) with different mobile phases constituted of aqueous solutions of formic acid and ammonia or ethylene diamine in variable amounts

Mobile phase	Ionic strength (mM)	Ammonium concentration (mM)	Formate concentration (mM)	Retention factor (k)			
				Na^+	K^+	Mg^{2+}	Ca^{2+}
Ammonium formate, pH 4	50	49.9	50	0.2	0.3	5.9	10
	20	19.9	20	0.7	1.0	35.4	– ^a
	10	9.9	10	1.6	2.1	–	–
	5	4.9	5	3.1	4.2	–	–
	2	1.9	2	5.4	7.2	–	–
Ethylene diammonium formate, pH 4	30	10.0	20.0	0.0	0.0	0.1	0.2
	20	6.6	13.4	0.0	0.0	0.3	0.7
	10	3.3	6.7	0.0	0.0	0.8	1.3
	5	1.6	3.4	0.1	0.0	1.8	2.7
	2	0.6	1.4	0.4	0.3	7.0	11.1

^a –, Not eluted.

formate gradient elution should provide an efficient means of analysing, in a single run, monovalent and divalent cations. Unlike conductivity detection, ELSD is compatible with a gradient elution mode since its detection principle is linked to mobile phase volatility. Thus, Fig. 1 depicts a chromatogram illustrating a satisfactory resolution, in 11 min, of a standard mixture of four chloride salts: NaCl, KCl, MgCl₂ and CaCl₂. These compounds are detected by ELSD, following separation on the cation exchanger with a salt gradient elution which consisted of an aqueous solution of 20 mM ammonium formate for 4 min then a step gradient to aqueous solution of 100 mM ammonium formate for 10 min for a total run time of 14 min. A good selectivity between the last eluted monocation (K⁺) and the first eluted dication (Mg²⁺) is observed. So this is the first time that alkali and alkaline-earth cations have been simultaneously analysed on a PRP-X200 material packing with good selectivities.

In Fig. 1, an elution peak additional to those corresponding to the four cations analysed is observed. Because it was previously described that inorganic anions can be detected by ELSD [11,12], this further peak was assigned to chloride elution.

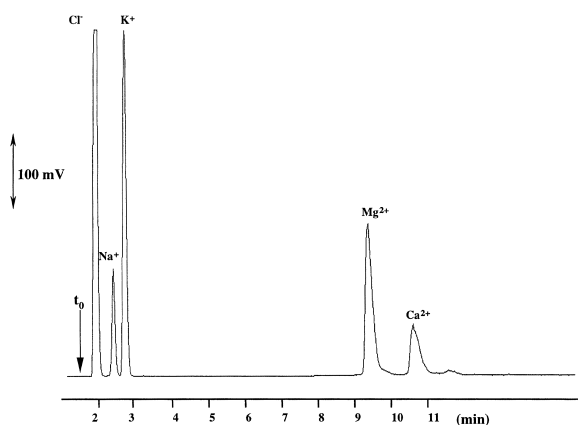


Fig. 1. Single run analysis of four chloride salts: NaCl, KCl, MgCl₂ and CaCl₂. Column Hamilton PRP-X200 (150×4.1 mm I.D.); step gradient elution: aqueous solution of 20 mM ammonium formate during 4 min then step gradient to aqueous solution of 100 mM ammonium formate during 10 min; flow-rate: 1.0 ml min⁻¹; injected volume, 100 μl; solute concentration: 50 mg l⁻¹ each cation. Detection: ELSD Sedex 75, drift tube temperature 60°C, nebulizer gas pressure 3.6 bar, photomultiplier 9.

The analysis of ammonium chloride under the same chromatographic conditions confirms this assignment. Indeed, when chloride is associated with NH₄⁺, a volatile cation which cannot be detected by ELSD, the retention time value of the single chromatographic peak observed, is in good agreement with the one of the further peak in Fig. 1. Surprisingly, the chloride retention time (1.95 min) is not exactly the hold up time (1.70 min), suggesting a weak retention of chloride onto the polymeric cation exchanger. So, to obtain more information about this behaviour of the PRP-X200 packing material, a study on anion retention onto this support was performed with the mobile phases used in Table 1.

3.3. Anion analysis

Fig. 2a–c and Fig. 3a–c show the chromatograms obtained for three different potassium salts (potassium carbonate, potassium chloride and potassium sulfate) and three ammonium salts (ammonium chloride, ammonium nitrate and ammonium sulfate) analysed on the PRP-X200 column under the same isocratic elution conditions with an aqueous mobile phase constituted of formic acid and ammonia at pH 4 (ionic strength equal to 20 mM).

K⁺ is a non volatile cation which can be detected by ELSD whereas NH₄⁺ is not detected by ELSD (high volatility of this cation). Depending on the nature of the anion associated with K⁺, one or two chromatographic peaks were detected by ELSD for each potassium salt. When potassium was associated to a volatile anion such as carbonate, only K⁺ was detected by ELSD and consequently Fig. 2a shows a chromatogram with a single elution peak assigned to K⁺. When potassium was associated to a semi-volatile anion (chloride in Fig. 2b) or to a non volatile anion (sulfate in Fig. 2c), these anions also gave an ELSD signal.

As ammonium cannot be detected by ELSD, when an ammonium salt was analysed (Fig. 3a–c), the single chromatographic peak observed has to be assigned to the corresponding semi-volatile (Cl⁻, NO₃⁻) or non volatile (SO₄²⁻) eluted anion. Furthermore, the retention time values observed in Fig. 3 for these anions are in good agreement with those observed in Fig. 2 for Cl⁻ and SO₄²⁻ and confirm the elution peak assignments in Fig. 2. Such an ELS

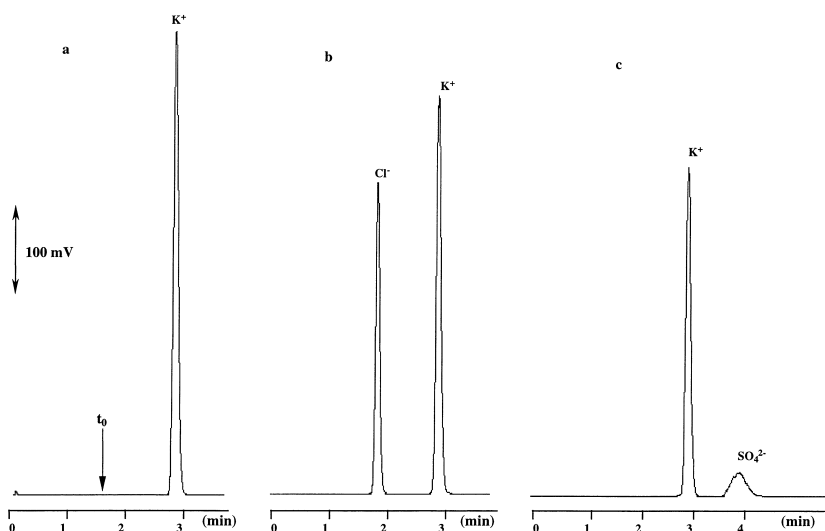


Fig. 2. Isocratic analysis of three potassium salts [(a) carbonate; (b) chloride; (c) sulfate]. Column Hamilton PRP-X200 (150×4.1 mm I.D.). Eluent: ammonium formate at 20 mM ionic strength (pH 4); flow-rate: 1.0 ml min⁻¹; injected volume, 100 μl; solute concentration: 50 mg l⁻¹ each cation. Detection: ELSD Sedex 75, drift tube temperature 60°C, nebulizer gas pressure 3.6 bar, photomultiplier 9.

detection of anion has been yet observed by Peterson and Risley during the determination by IEC–ELSD of Na⁺ in a pharmaceutical compound using a Zorbax 300 SCX cation-exchange column [8]. The use of ELSD reveals not only a weak retention of

inorganic anions on the polymeric cation exchanger but also a retention dependent upon the anion nature: greater retention was observed for sulfate (Figs. 2c and 3c) than for chloride (Figs. 2b and 3a) and the elution order was Cl⁻ then NO₃⁻ and finally SO₄²⁻.

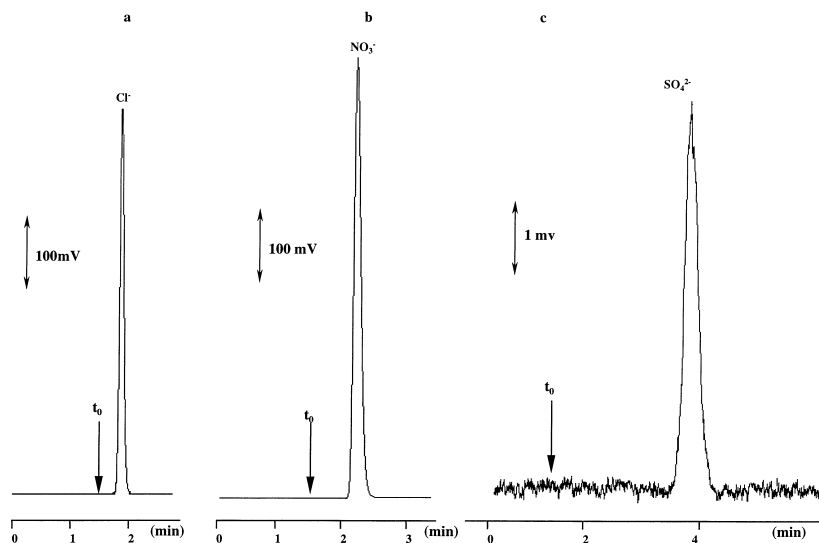


Fig. 3. Isocratic analysis of ammonium salts [(a) chloride; (b) nitrate; (c) sulfate]. Column Hamilton PRP-X200 (150×4.1 mm I.D.). Eluent: ammonium formate at 20 mM ionic strength (pH 4); flow-rate: 1.0 ml min⁻¹; injected volume, 100 μl; solute concentration: 50 mg l⁻¹ each cation. Detection: ELSD Sedex 75, drift tube temperature 60°C, nebulizer gas pressure 3.6 bar, photomultiplier 9.

Table 2

Retention factors, k , of inorganic anions on a Hamilton PRP-X200 column (150×4.1 mm I.D.) with the mobile phases used in Table 1

Mobile phase	Ionic strength (mM)	Formate concentration (mM)	Retention factor (k)		
			Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻
Ammonium	50	50	0.0	0.2	0.2
formate,	20	20	0.3	0.6	1.6
pH 4	10	10	0.7	1.3	6.0
	5	5	1.3	2.5	– ^a
	2	2	2.4	4.2	–
Ethylene	30	20.0	0.2	0.5	0.5
diammonium	20	13.4	0.4	0.6	1.5
formate,	10	6.7	0.8	1.5	5.0
pH 4	5	3.4	1.5	2.8	–
	2	1.4	6.0	9.4	–

^a –, Not eluted.

Table 2 reports the retention factors (k) observed for Cl⁻, NO₃⁻, SO₄²⁻ under the isocratic elution conditions used in Table 1 for the inorganic cation analyses. For these anions, the formate concentration in mobile phase appears to be the main factor which regulates the retention on the stationary phase. As expected on an anion exchanger, the lower the formate concentration in mobile phase, the higher the anion retention. For a given formate concentration (20 mM) in mobile phase, the anion retention also weakly depends on the cation concentration in mobile phase: the SO₄²⁻ retention factor is equal to 1.6 with ammonium formate and 0.5 with ethylene diammonium formate. Nevertheless, for a 20 mM formate concentration, the weaker the ionic strength of the mobile phase, the higher the SO₄²⁻ retention factor.

The unexpected retention of inorganic anions could be explained by the formation of some residual anionic exchange sites on the cationic polymer-based exchanger during its manufacturing however, no information from the vendor confirms that this chemistry may be happening.

Several reasons can explain why this very weak capacity of anion exchanger is not observed when one uses the methodology proposed by the manufacturer for cation analysis (nitrate salts as mobile phase and conductivity meter detection). First, as expected in IEC, NO₃⁻ is a stronger competitor anion than formate. Consequently Cl⁻, which has a lower retention than NO₃⁻ on Hamilton PRP-X200 when formate is used as eluent ion, will be eluted in void

volume when a nitrate salt is used in mobile phase. Also, the presence of NO₃⁻ in the mobile phase prevents its conductometric detection as analyte ion. Furthermore, the comparable values of nitrate, chloride or sulfate conductance are not conducive to a sensitive detection by direct conductivity of these anions when one of them is the eluent ion.

Fig. 4 depicts the simultaneous determination of monovalent cations (Na⁺ and K⁺) and associated anions (Cl⁻ and NO₃⁻) under an isocratic elution mode in less than 4 min. However, it was interesting to note that if necessary, it was possible to reduce drastically the ELSD response of semi volatile anions (Cl⁻ and NO₃⁻) just by increasing the drift tube temperature of the ELSD system (≥80°C) (data not shown).

4. Conclusion

The present paper illustrates a single run analysis for the determination of alkali and alkaline-earth cations using cation-exchange chromatography and ELSD under a gradient elution mode. The use of ELSD revealed that on a Hamilton PRP-X200 column, a stationary phase classically dedicated to cation analysis, a retention of inorganic anions was observed. Further experiments would be needed to characterise the anion retention mechanism and anion-exchange capacity, for though three different columns have shown the same retention properties (cation and anion), there are nevertheless differences

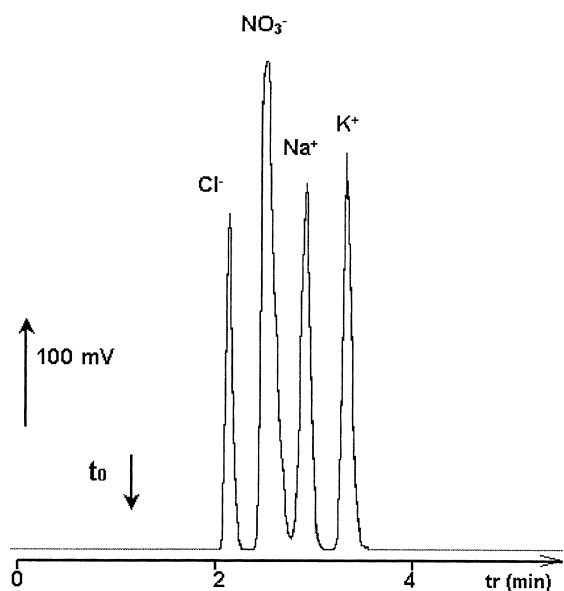


Fig. 4. Simultaneous analysis of Na^+ and K^+ and associated anions (Cl^- and NO_3^-). Column: Hamilton PRP-X200 (150 \times 4.1 mm I.D.). Eluent: ammonium formate at 10 mM ionic strength (pH 4); flow-rate: 1.0 ml min^{-1} ; injected volume, 100 μl ; solute concentration: 50 mg l^{-1} for Na^+ , K^+ and Cl^- and 100 mg l^{-1} for NO_3^- . Detection: ELSD Sedex 75, drift tube temperature 60°C, nebulizer gas pressure 3.6 bar, photomultiplier 9.

in anion retention and selectivity. Then quantitative analysis (sensitivity, precision, linearity) and sample matrix effects will be a necessary follow up.

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