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New Approach for Inorganic Anion Analysis by Ion Exchange Chromatography Coupled with Evaporative Light Scattering Detection

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New Approach for Inorganic Anion Analysis by Ion Exchange Chromatography Coupled with Evaporative Light Scattering Detection

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ABSTRACT

The simultaneous separation of inorganic anions is possible by ion exchange chromatography (IEC) under an isocratic elution mode, using polymer exchanger Metrosep anion dual1 Metrohm (150 × 3 mm ID) followed by evaporative light scattering detection (ELSD). Several mobile

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phases [formic acid (FA)/ammonia, FA, hydrogen carbonate, trifluoroacetic acid (TFA), and heptafluorobutyric acid (HFBA)] were tested for optimization of the separation of inorganic anions. The choice of a mobile phase was proven by compatibility with ELSD volatility requirements.

Key Words: Ion exchange chromatography; Inorganic anions; Metrosep anion dual1; Evaporative light scattering detection; Isocratic analysis.

INTRODUCTION

Ion exchange chromatography (IEC) was first introduced by Small et al.^[1] about 27 years ago and has become a well-established technique. Its rapidity, sensibility, and the best selectivity characteristics that added the easy manipulated method, offer a large field of applications. The simultaneous analysis of common anions such as chloride, nitrate, phosphate, and 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 or without suppressed conductivity detection^[2,3] and UV indirect detection.^[4,5] Ion exchange chromatography replace, advantageously, the classic chemical methods already existing.

Several efforts have been focused on the development of new methodology by IEC; such as the research for the simultaneous determination of anions of widely different charges with isocratic elution mode. Sometimes it is necessary to use the gradient elution mode, and we are unable because the baseline conductance is influenced by the change of the eluent during the analysis. Dionex has introduced a system with chemical suppression consisting of decreasing the eluent conductivity,^[6] but the system is very expensive. The second area of research is the development of new system detection.

The IEC major interest allows us to contribute to the development of new methods for inorganic anions analysis by utilizing on-line coupled evaporative light scattering detection (ELSD) and IEC.

Evaporative light scattering detection is generally considered to be a very convenient and universal liquid chromatography (LC) detection for analytes without chromophoric groups and which are less volatile than the chromatographic eluent.

We have already used ELSD for the simultaneous determination of alkali and alkaline-earths under isocratic elution mode, with trifluoroacetic acid (TFA) as eluent on a Lichrosil IC CA2 column.^[7] These cations are also determined by gradient elution modes that present a great interest of compatibility with formic acid (FA) and ammonia or ethylene diamine on Hamilton PRP-X200.^[8] Evaporative light scattering detection is also used for organic



anions analysis, such as phosphonic acids, on Dionex IonPac columns with hydrogen carbonate ammonium as eluent.^[9] Glucosinolates have been also analyzed with formate ammonium on Vydac 302 IC.^[10] Inorganic anions are analyzed by Elfakir et al.^[11] on porous graphitic carbon (PGC) with TFA as an ion competitor. Furthermore, this detection mode has been proven useful for carbohydrates,^[12] cyclodextrins,^[13] and aminoacids.^[14]

The aim of this work is to evaluate a direct, isocratic, and simultaneous analysis of inorganic anions in standard aqueous media by IEC using evaporative light scattering detector. FA/ammonia, FA/ammonium hydrogen carbonate, TFA, and heptafluorobutyric acid (HFBA) are the eluents examined for the management of the inorganic anions retention with respect to the volatility requirements of ELSD.

EXPERIMENTAL

The LC apparatus consisted of a Gilson (Villiers-le-Bel, France) Model 302 isocratic pump, a Rheodyne (Berkeley, CA) Model 7125 injector with a 20 μ L sample loop and an ELSD (Sedere, Alfortville, France) Model Sedex 45. The usual settings were as follows: photomultiplier 9; evaporative temperature 40°C; air pressure 2.2 bar. Data were processed using a Shimadzu (Kyoto, Japan) Model CR5A integrator.

All solutions were prepared from analytical reagent grade chemicals in 18 Ω M water obtained from an ELGA purification system (Antony, France). Eluents constituents were purchased as follows: FA, TFA, HFBA from Merck (Darmstadt, Germany), ammonium hydrogen carbonate and ammonia from Fluka (St. Quentin Fallavier, France).

Solutes were purchased as follows: sodium chloride, sodium nitrate, ammonium sulfate from Prolabo (Paris, France), and phosphoric acid from Merck (Darmstadt, Germany). The concentration of each standard in the different injected samples was about 100–300 mg/L in aqueous solutions. The theoretical values of pH, ionic strength and ion developer, and co-ion concentrations of each eluent were calculated with PhoeBus, a software package for buffer studies (Sedere, Alfortville, France), and then the pH value was checked for each mobile phase with a Beckman pH meter (Model Φ 10, Gagny, France).

RESULTS AND DISCUSSION

The Metrosep anion dual1 (150 \times 3 mm ID) column was developed to achieve the separation of anionic species, based on methacrylate polymers, which have been chemically bound to quaternary ammonium as functional group.



Recently, we have realized the separation of inorganic anions on a Metrosep anion dual1 with conductimetric and UV-indirect as detection systems, with low concentrations of *ortho*-phthalic acid as ion developers.^[5] The latter is not compatible with volatility requirements of ELSD. In this regard, we have focused on the investigation of some ion developers that permit the use of ELSD. Formic and acetic acids are evaporable, but a concentration over 100 mM must be used to elute only monocharged anions [$k(\text{Cl}^-) = 6.5$]. We added a base reagent like ammonia to a solution of FA to decrease the retention time of all inorganic anions studied (phosphate, chloride, nitrate, and sulfate). Ammonia was chosen because of the volatility requirement of ELSD. To evaluate the effect of the mixture of ammonia and FA at different amounts of inorganic anions retention, we have used the potential of PhoeBus software to give the values concentrations of ionic strength, ion developer (HCOO^-), co-ion (NH_4^+), and pH of each composition of eluent. Four different eluent concentrations (FA/ammonia) mM/mM have been studied: 100/100, 100/75, 75/75, and 100/60. Table 1 reports the different values cited below.

The ionic strength of mobile phase decreases when the ammonia concentration is decreasing. The HCOO^- concentration is comparable to the ionic strength of each mobile phase composition and their pH values are very close to the range of use of Metrosep anion dual1. Figure 1 reports the retention factors, k , of inorganic anions with different compositions of eluent (FA/ammonia).

It appears that the retention factors of all inorganic anions studied decrease when the ionic strength increases, especially the ones of ammonia. Phosphate is presented in a mono-anionic species ($\text{p}k_{\text{a}1} = 2.12$; $\text{p}k_{\text{a}2} = 7.21$; $\text{p}k_{\text{a}3} = 12.67$). Nitrate is more retained than sulfate, this result was already shown by Mercier et al.,^[15] when they studied the separation of inorganic anions on a PGC support with FA as the eluent. We have obtained the same

Table 1. Ionic strength (mM), concentrations of ion developer [HCOO^- (mM)], and co-ion [NH_4^+ (mM)] and pH of different amounts of FA and ammonia constituted the eluent.

	Eluent FA/ NH_3 (mM/mM)			
	100/100	100/75	75/75	100/60
Ionic strength	99.86	75.09	74.89	60.18
HCOO^-	99.86	75.09	74.89	60.18
NH_4^+	99.86	75.00	74.89	60.00
pH	6.50	4.13	6.50	3.84



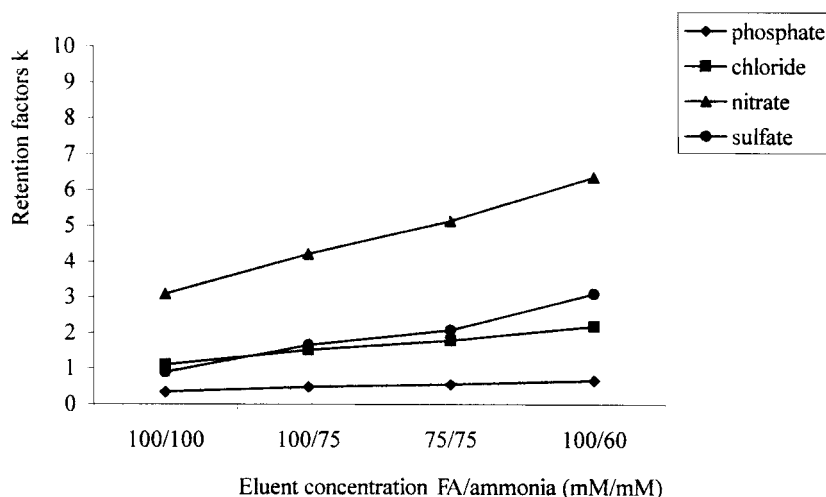


Figure 1. Retention factors, k , of inorganic anions on a Metrosep anion dual I Metrohm (150×3 mm ID) with a mobile phase consisting of aqueous solutions of FA and ammonia in variable amounts.

retention on silica support (PRP-X100) with FA/ammonia as eluent.^[16] This inversion is probably due to the polarisability of nitrate. Figure 2(a), (b), (c), and (d) depict, respectively, an example of separation obtained with FA/ammonia (75/75; 100/75; 100/100; and 100/60 mM/mM) for a standard mixture of (H_2PO_4^- , Cl^- , NO_3^- , and SO_4^{2-}).

As a second approach, we include a second ion competitor associated to ammonium. It is known, that hydrogen carbonate is considered an ion developer with respect to volatility requirement of ELSD.^[9] Separations of all inorganic anions were carried out by using a mobile phase consisted of FA and ammonium hydrogen carbonate at different ionic strengths (35, 45, 60, 75, and 100 mM) at different pH. In this case, we imposed the values of ionic strength and pH of eluent; the data of PhoeBus software gives the different ions (HCOO^- , HCO_3^- , and NH_4^+) concentrations at pH 4 and 7.7 (Table 2).

At pH 4, the ionic HCOO^- form is dominant, where as HCO_3^- is dominant at pH 7.7. Figures 3 and 4 show the variation of retention factors of the inorganic anions with pH 4 and 7.7, respectively, and the ionic strength of aqueous mobile phase. At fixed pH, the retention factors of inorganic anions decrease when the ionic strength of the mobile phase increases. We also noted, in the range of ionic strength studied, that nitrate is more strongly retained than sulfate. An opposite result is shown with the same eluent on PRP-X100



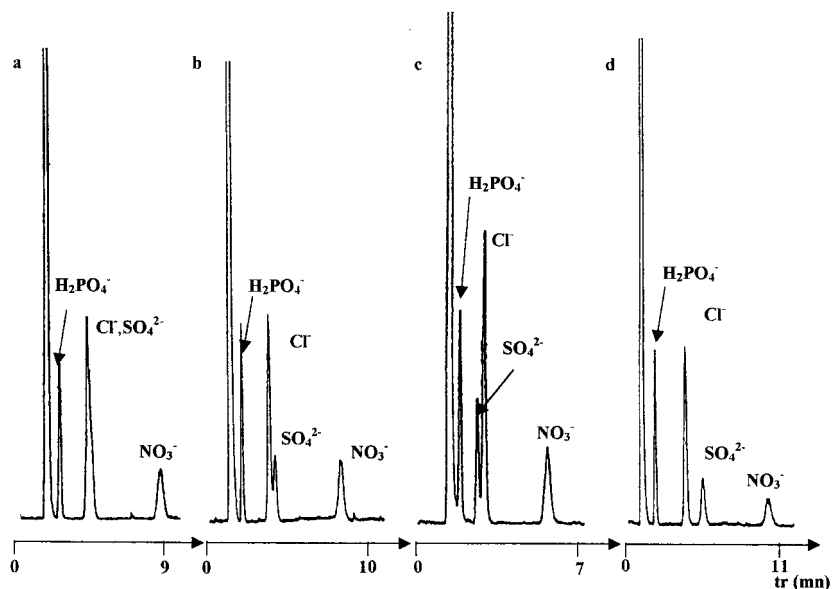


Figure 2. Separation of inorganic anions (phosphate, chloride, nitrate, and sulfate) in a standard mixture on a Metrosep anion dual1 (150 × 3 mm ID) column with FA/ammonia (mM/mM) in variable amounts; flow rate 0.7 mL/min; injected volume 20 μ L; detection: ELSD Sedex 45, 40°C, PM 9, P_{N_2} 2.2 bar. (a) FA/NH₃ 75/75; (b) FA/NH₃ 100/75; (c) FA/NH₃ 100/100; and (d) FA/NH₃ 100/60 (mM/mM).

Table 2. Concentrations of ionic developers [HCOO⁻, HCO₃⁻ (mM)] and co-ion [NH₄⁺ (mM)] of different amounts of FA and ammonium hydrogen carbonate at pH 4 and 7.7 of the eluents.

	Ionic strength (mM)				
	35	45	60	75	100
FA/NH ₄ HCO ₃ pH 4					
HCOO ⁻	34.81	44.76	59.67	74.59	99.44
HCO ₃ ⁻	0.18	0.24	0.33	0.41	0.56
NH ₄ ⁺	34.88	44.88	59.87	74.87	99.87
FA/NH ₄ HCO ₃ pH 7.7					
HCOO ⁻	0.32	0.39	0.49	0.62	0.77
HCO ₃ ⁻	34.26	44.04	58.70	73.38	97.80
NH ₄ ⁺	34.86	44.81	59.73	74.67	99.53



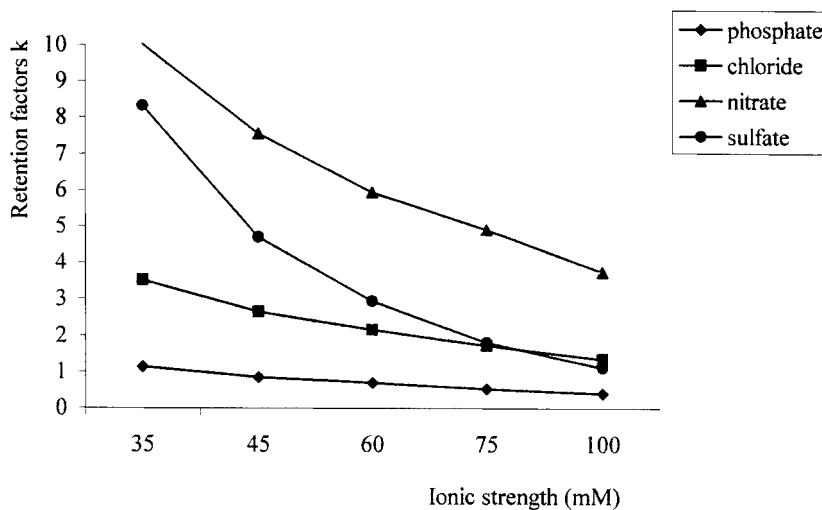


Figure 3. Retention factors, k , of inorganic anions on a Metrosep anion duall Metrohm (150×3 mm ID) with a mobile phase consisting of aqueous solutions of FA and ammonium hydrogen carbonate in variable amounts and pH 4 of eluents.

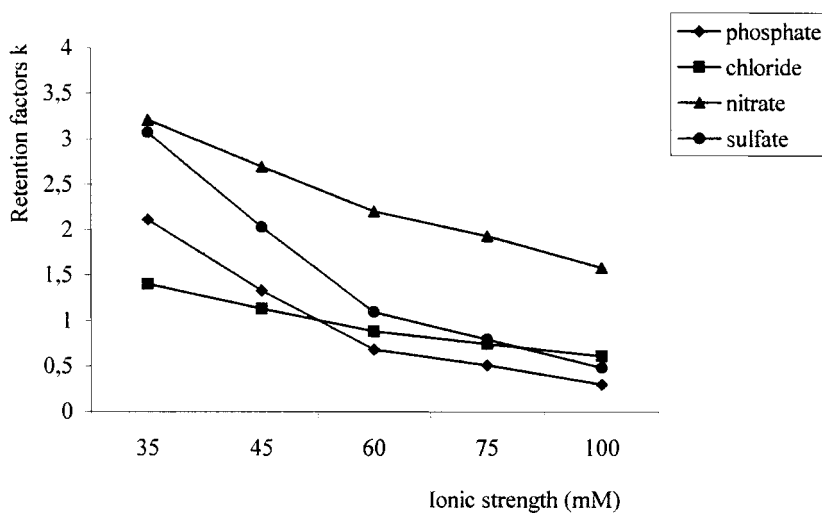


Figure 4. Retention factors, k , of inorganic anions on a Metrosep anion duall Metrohm (150×3 mm ID) with a mobile phase consisting of aqueous solutions of FA and ammonium hydrogen carbonate in variable amounts and pH 7.7 of eluents.

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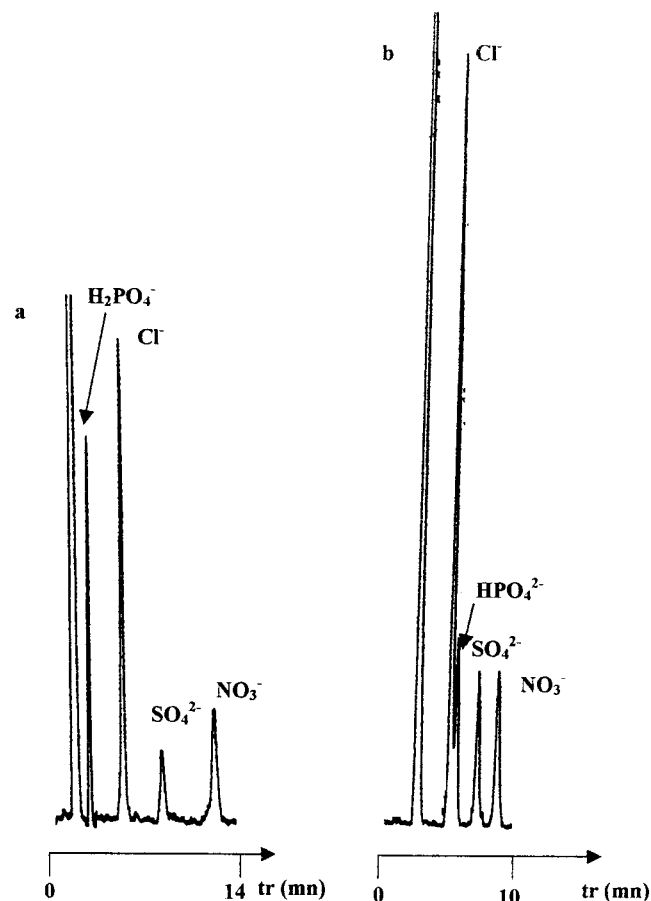


Figure 5. Separation of inorganic anions (phosphate, chloride, nitrate, and sulfate) in a standard mixture on a Metrosep anion dual1 (150×3 mm ID) column with FA and ammonium hydrogen carbonate. Flow rate 0.7 mL/min; injected volume 20 μL ; detection: ELSD Sedex 45, 40°C, PM 9, P_{N_2} 2.2 bar. (a) $\text{HCOOH}/\text{NH}_4\text{HCO}_3$, ionic strength 45 mM and pH 4; (b) $\text{HCOOH}/\text{NH}_4\text{HCO}_3$, ionic strength 45 mM and pH 7.7.

stationary phase.^[16] We suppose it is due to the preference interaction of Metrosep anion dual1 with nitrate rather than with sulfate. On this support and an ionic strength of 75 mM and a fixed pH 4 or 7.7, the anions chloride and sulfate are co-eluted. After examining Figs. 3 and 4, it appears that the retention of inorganic anions at pH 7.7 is inferior to that at pH 4. It is explained by the dominant presence of HCO_3^- and the ionic strength of this ion.



Table 3. Ionic strength (mM), concentrations (mM) of ions developers (CF_3COO^-), and ($\text{C}_3\text{F}_7\text{COO}^-$) and pH of different mobile phases.

Eluent	TFA		HFBA	
	10 mM	15 mM	10 mM	15 mM
Ionic strength (mM)	9.80	14.55	9.99	14.99
CF_3COO^-	9.79	14.55	—	—
$\text{C}_3\text{F}_7\text{COO}^-$	—	—	9.99	14.99
pH	2.06	1.89	2.07	1.87

This study showed that the value of pH and ionic strength make it possible to modulate the selectivity between all inorganic anions studied, and to obtain various profiles of resolution and run time analysis. This makes it possible to select conditions enabling determination of most mixtures. The use of conductivity or direct/indirect UV detection with the ionic strengths studied of the eluents, would not be possible because of the high background noise; there is no problem with ELSD if the salt is volatile.

Figure 5(a) and (b) depict, respectively, separations obtained with $\text{FA}/\text{NH}_4\text{HCO}_3$ (ionic strength 45 mM, pH 4, and 7.7) for a standard mixture of chloride, phosphate, sulfate, and nitrate.

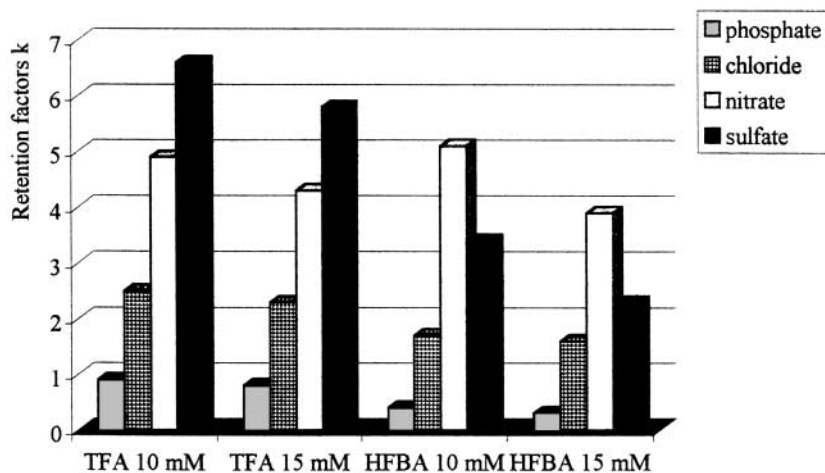


Figure 6. Retention factors, k , of inorganic anions on a Metrosep anion dual1 Metrohm (150×3 mm ID) with a mobile phase consisting of aqueous solutions of TFA and HFBA at different concentrations.



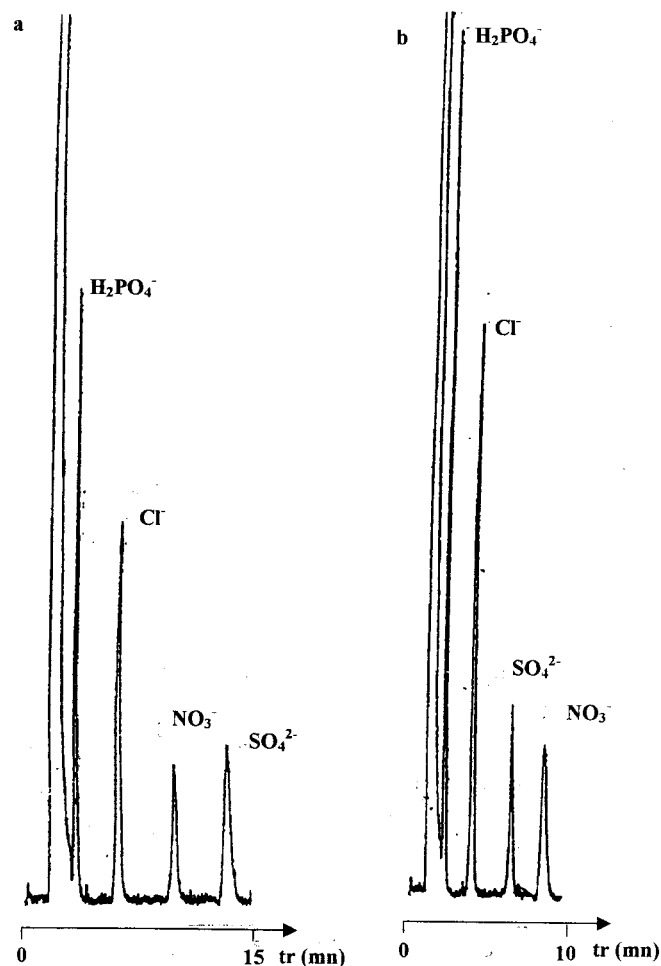


Figure 7. Separation of inorganic anions (phosphate, chloride, nitrate, and sulfate) in a standard mixture on a Metrosep anion dual1 (150×3 mm ID) column. Flow rate 0.7 mL/min; injected volume 20 μL ; detection: ELSD Sedex 45, 40°C, PM 9, P_{N_2} 2.2 bar. (a) TFA 10 mM; (b) HFBA 10 mM.

After evaluating the potential of sublimate salt, we have used another ion developer like perfluorinated carboxylate anion. TFA (10 and 15 mM) and HFBA (10 and 15 mM) are the eluents that we have tested on Metrosep anion dual1 columns. The use of two strong acids, TFA or HFBA, which are totally dissociated of the concentrations studied, causes variations in retention factors.



Table 3 depicts the data given by PhoeBus software, like ionic strength of aqueous mobile phase, concentrations of ion developers CF_3COO^- and $\text{C}_3\text{F}_7\text{COO}^-$, and correspondent pH of eluent at different concentrations of TFA and HFBA.

Figure 6 represents the variation of retention factors of inorganic anions as a function of nature of ion developer and its concentration. It appears that the retention decreases when the concentration increases, and $\text{C}_3\text{F}_7\text{COO}^-$ is more eluent than CF_3COO^- . We have noted an inversion elution order between nitrate and sulfate with the use of HFBA 10 mM. Figure 7(a) and (b) depict, respectively, separations obtained with TFA 10 mM and HFBA 10 mM for a standard mixture of chloride, phosphate, sulfate, and nitrate.

CONCLUSION

Evaporative light scattering detection has been a direct and simple mode of detection for inorganic anions on Metrosep anion dual1. Its use allows obtaining the opposite of usual conductive detection; satisfactory conditions for a rapid and isocratic analysis of inorganic anions (chloride, phosphate, nitrate, and sulfate). We have modulated the retention and the selectivity of the inorganic anions with an aqueous mobile phase containing FA and ammonium hydrogen carbonate, by varying the ionic strength of this phase and the pH also with FA and ammonia. The potentialities of two evaporable acids TFA and HFBA have been evaluated. The system is stable and simple and can be used in teaching and industrial laboratories in the absence of specialized and quite expensive equipment.

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