CHROM. 15.158

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF INORGANIC ANIONS USING Fe^{3+} AS A DETECTION REAGENT

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(First received March 23rd, 1982; revised manuscript received July 5th, 1982)

SUMMARY

Fe^{3+} was examined as a detection reagent in the analysis of inorganic anions by high-performance liquid chromatography. The chromatographic conditions were: the stainless-steel tube was packed with TSK-GEL IEX-520 QAE: 0.05 M sodium acetate buffer (pH 5.48) containing 0.05 M sodium nitrate was used as eluent; and 0.8 M perchloric acid containing 0.05 M iron(III) perchlorate was chosen as a complex-forming reagent. Under these conditions, chloride, sulphate and thiocyanate ion were determined in the range 2–500 nmol, and phosphate, nitrite and thiosulfate ion in the range 8–500 nmol.

INTRODUCTION

A few methods for the simultaneous determination of inorganic anions by high-performance liquid chromatography (HPLC) have been reported[1–5]. Conductometry is generally used to detect the most ionic species by the method known as ion chromatography (IC)[6]. One of the disadvantages of this method is the lack of selectivity in the analysis of biological samples. UV detectors also lack selectivity, and cannot detect some major anions, such as chloride. phosphate and sulphate ions. because they have no absorption in UV region.

Recently, we reported the determination of free and bound sulphate[7] and thiocyanate[8] in human urine or serum by HPLC. The principle of this method was based on the formation of sulphate or thiocyanate complexes with Fe^{3+} (refs. 9 and 10), which was used as eluent and colour-developing reagent. It was also known that Fe^{3+} forms coloured complexes with chloride, phosphate and sulphate ions[10,11].

In this paper, we report the utility of Fe^{3+} as a detection reagent for the analysis of inorganic anions by HPLC, using a post-column derivatization method.

EXPERIMENTAL

Reagents

All chemicals used were of analytical grade. Water was redistilled after passage through anion-exchange resin.
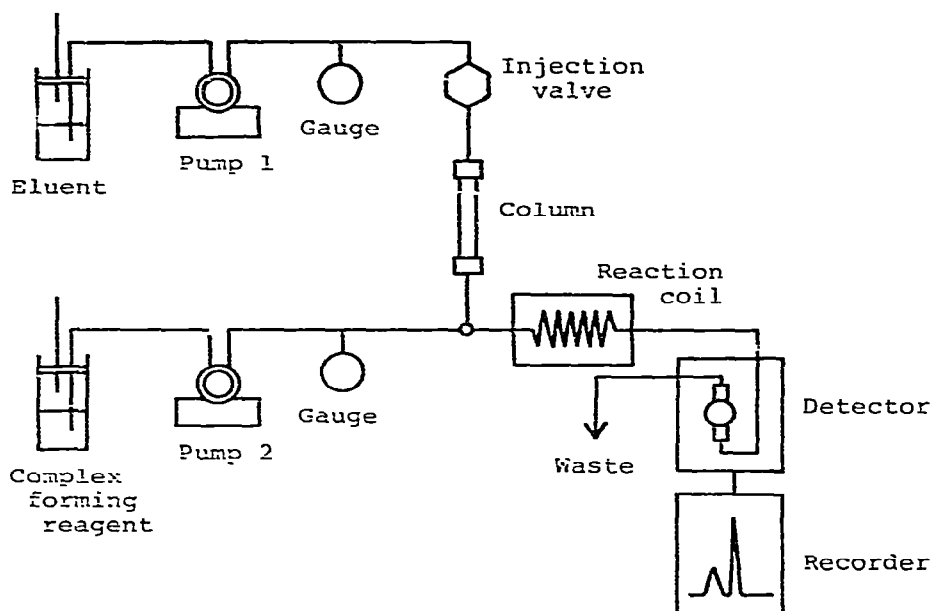
Fig. 1. Flow diagram of the chromatographic system.

Complex-forming reagent
    Perchloric acid (0.8 *M*) containing 0.05 *M* iron(III) perchlorate was used.

Eluent
    Sodium acetate buffer (0.05 *M*. pH 5.48) containing 0.05 *M* sodium nitrate was used.

Instruments
    Pumps (Model PSU-2.5). a variable injection valve (Model VMD-350). pressure gauges (Model GN-100). a water-jacketed stainless-steel tube and a UV detector (Model D-340C) were obtained from Seishin Pharmaceutical (Tokyo, Japan). A recorder (Model ss-250F) was obtained from Sekonic (Tokyo, Japan). A water bath with a thermoregulator (Model BT-25) was obtained from Yamato Scientific (Tokyo, Japan). UV spectra were measured by a Hitachi 340 automatic recording spectrophotometer.

HPLC apparatus
    Fig. 1 is a flow diagram of the chromatographic system. A stainless-steel tube (150 mm × 4 mm I.D.) was slurry-packed with TSK-GEL IEX-520 QAE (silica type pellicular anion exchanger). Pump 1 was used to deliver an eluent at a flow-rate of 0.8 ml/min. The temperature of column and reaction coil was kept at 25°C. The complex-forming reagent was delivered by pump 2 at a flow-rate of 0.4 ml/min. The reagent from pump 2 was mixed with the effluent. The mixed solution was delivered to the reaction coil, and was then monitored with the UV detector at 340 nm. PTFE reaction coil was 2 m × 0.25 mm I.D.

TABLE I

COMPLEX FORMATION OF INORGANIC ANIONS WITH Fe^{3+}

Reactions were carried out in 0 8 M $HClO_4$ containing 0 05 M $Fe(ClO_4)_3$. UV spectra were measured within 5 min using a reagent blank as a reference. Detection limits were obtained by the system without a column illustrated in Fig. 1. Distilled water was used as a carrier solution from pump 1 keeping the flow-rate at 0.2 ml/min.

Anion	λ_{max} (nm)	Detection limit (nmol)	Anion	λ_{max} (nm)	Detection limit (nmol)
CrO_4^{2-}	305, 344	0.4	SO_3^{2-}	308	12.7
SCN^-	310	1.3	PO_3^{3-}	–	24.8
$Fe(CN)_6^{4-}$	305	1 6	$H_2PO_2^-$	–	28.8
$Fe(CN)_6^{3-}$	305	1.9	IO_3^-	–	73.7
SO_4^{2-}	306	2.8	CO_3^{2-}	–	141.3
Cl^-	335	4 8	Br^-	–	144.3
$P_2O_7^{4-}$	310	5.2	$B_4O_7^{2-}$	–	206.4
I^-	306, 350	5.6	BrO_3^-	–	931.7
$P_3O_{10}^{5-}$	310	6.4	CN^-	–	392.2
S^{2-}	–	7.1	SiO_3^{2-}	–	285.7
$S_2O_3^{2-}$	308	8.1	NO_3^-	–	–
NO_2^-	372, 360	10 8	F^-	–	–
PO_4^{3-}	310	11.8	ClO_3^-	–	–

RESULTS AND DISCUSSION

Complex formation between inorganic anions and Fe^{3+}
    Iron(III) perchlorate was used as a complex-forming reagent for each anion. and perchloric acid was chosen as a reaction medium, because of its lower complexing ability which would minimize ligand exchange during the analysis[12]. Iron(III) per-chlorate and perchloric acid concentrations were determined according to the con-ditions of sulphate ion analyses by Nakae et al.[11]. Tested inorganic anions formed iron(III) complexes exhibiting an absorption at ca. 300 nm. except for nitrate, fluoride and chlorate ions. Twenty-six inorganic anions were examined, and their detection limits were measured (Table I).
    Since considerable absorption by the reagent blank was observed at 300 nm[9]. the monitoring wavelength for these anions was set at 340 nm, where 60–70 % of the maximum absorbance was obtained. Fourteen inorganic anions from chromium(VI) oxide to sulphite ion shown in Table I exhibited strong absorption at 340 nm; the detection limits for these were 0.4–12 nmol by a peak height method, and for other inorganic anions were 73–930 nmol. These results suggest that Fe^{3+} is very useful as a reagent for the detection of certain anions such as phosphate. sulphate. chloride. nitrite, thiosulphate, iron(III) cyanide and iron(II) cyanide ions, which might be present in biological fluids, foods or environmental pollutants.

Separation of inorganic anions by HPLC
    The separation of nine inorganic anions (thiocyanate, iron(III) cyanide, iron(II) cyanide, sulphate, chloride, iodide. thiosulphate, nitrite and phosphate) was examined using the chromatographic system illustrated in Fig. 1. Perchloric acid (0.8

M) containing 0.05 *M* iron(III) perchlorate was used as a complex-forming reagent. Sodium nitrate solution or sodium acetate buffer containing sodium nitrate, which exhibited little absorption at 340 nm, was chosen as eluent. The effect of sodium nitrate concentrations on the retention time and the separation of inorganic anions on a column of the pellicular anion exchanger, TSK-GEL IEX-520 QAE, were examined. Seven anions (thiocyanate, sulphate, chloride, iodide, thiosulphate, nitrite and phosphate) were not separated completely with 0.1 *M* sodium nitrate; however, these anions were separated completely within 30 min when the flow-rate was changed from 0.8 to 0.32 ml min (Fig. 2).

Iron(III) cyanide and iron(II) cyanide ion were not eluted with sodium nitrate in the concentration range 0.1–0.3 *M* within 60 min because they were strongly absorbed on the resin. In order to keep the pH constant, sodium acetate buffer containing sodium nitrate was examined. When 0.05 *M* acetate buffer (pH 5.48) containing 0.05 *M* sodium nitrate was employed as eluent, complete separation of these anions was obtained within 20 min (Fig. 3).

Complex-forming conditions

The effect of different concentrations of perchloric acid and iron(III) perchlorate on complex formation was examined. The eluent and its flow-rate were kept
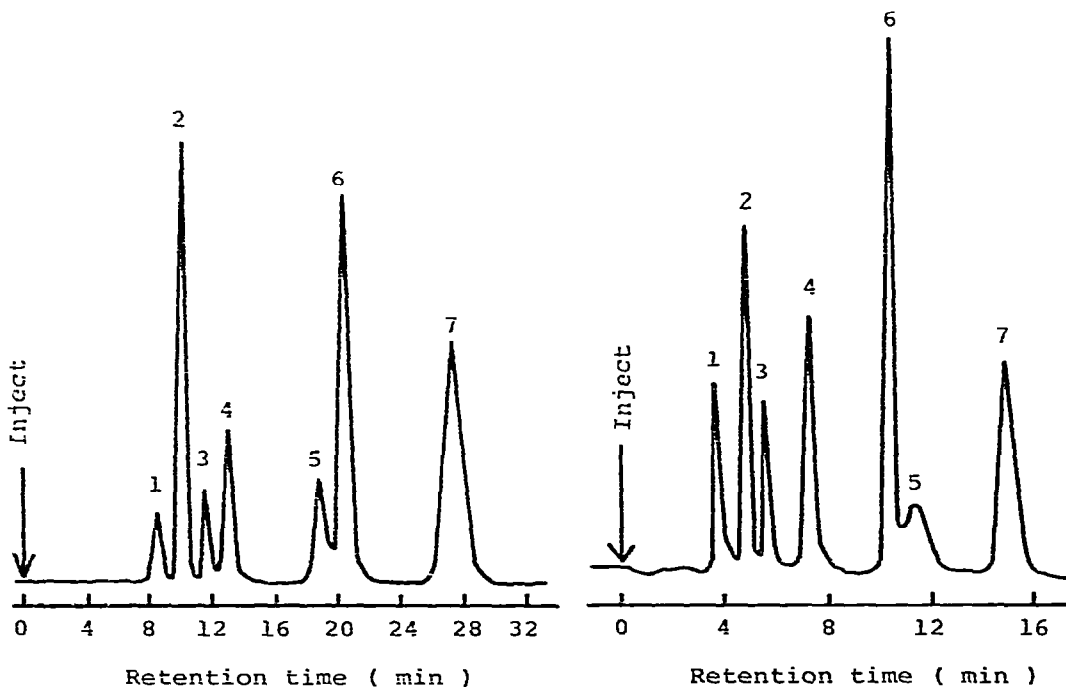

Fig. 2. Chromatogram of a standard mixture of inorganic anions. Eluent, 0.1 *M* NaNO$_3$; flow-rate, 0.32 ml min; other conditions are described in the text. Peaks: 1 = PO$_4^{3-}$; 2 = Cl$^-$; 3 = NO$_2^-$; 4 = SO$_4^{2-}$; 5 = S$_2$O$_3^{2-}$; 6 = I$^-$; 7 = SCN$^-$.

Fig. 3. Chromatogram of a standard mixture of inorganic anions. Eluent, 0.05 *M* acetate buffer (pH 5.48) containing 0.05 *M* NaNO$_3$; flow-rate, 0.8 ml/min; other conditions are described in the text. Peaks: 1 = PO$_4^{3-}$; 2 = Cl$^-$; 3 = NO$_2^-$; 4 = SO$_4^{2-}$; 5 = S$_2$O$_3^{2-}$; 6 = I$^-$; 7 = SCN$^-$.
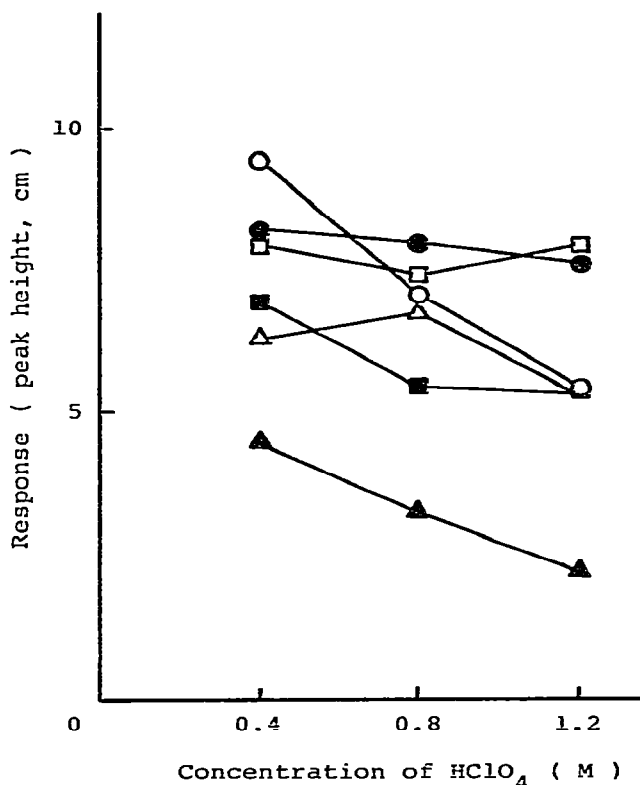
Fig. 4. Effect of concentration of $HClO_4$ on complex formation of inorganic anions in HPLC. using 0 05 M $Fe(ClO_4)_3$. Curves: $O = SO_4^{2-}$; $\bullet = Cl^-$; $\square = SCN^-$; $\blacksquare = NO_2^-$; $\triangle = PO_4^{3-}$; $\blacktriangle = S_2O_3^{2-}$

constant at 0.05 M acetate buffer (pH 5.48) containing 0.05 M sodium nitrate and 0.8 ml/min. respectively. The flow-rate of the complex-forming reagent was kept constant at 0.4 ml/min. The effects of various concentrations of perchloric acid and iron(III) perchlorate on the response for inorganic anions are shown in Figs. 4 and 5.

The response for the six anions increased with decreasing concentration of perchloric acid (Fig. 4), but the response for the reagent blank also increased. On the other hand, the response for these anions increased with increasing concentration of iron(III) perchlorate (Fig. 5).

Considering the background absorption, 0.8 M perchloric acid and 0.05 M iron(III) perchlorate were used in this system.

Under the chromatographic conditions and using the post-column method, it seemed that the iron(III) complexes of these anions (chloride. sulphate. thiocyanate. phosphate, nitrite and thiosulphate) were stable during the time passing the flow cell, because these absorbances were unchanged for 5 min. On the other hand, the absorbance of iodide ion increased, because of oxidation by Fe^{3+}.

Chloride. sulphate and thiocyanate ion were determined in the range 2–500 nmol, and phosphate, nitrite and thiosulphate ion in the range 8–500 nmol.

The precisions (coefficients of variation, $n = 7$) were 1.1, 0.8, 5.1. 3.8. 1.6 and
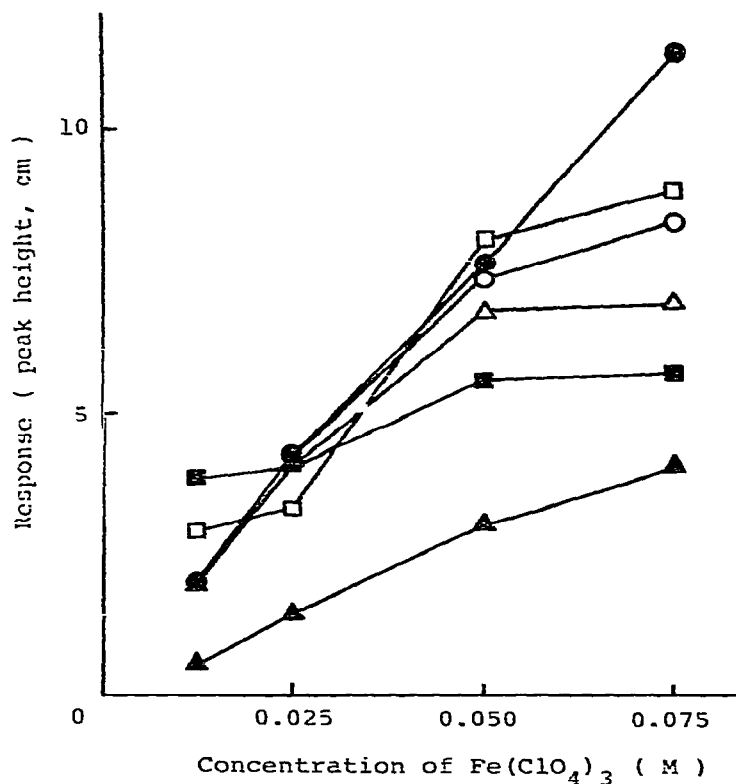
Fig. 5. Effect of concentration of Fe(ClO₄)₃ on complex formation of inorganic anions in HPLC, using 0.8 *M* HClO₄. Curves: $O = SO_4^{2-}$; $\bullet = Cl^-$; $\square = SCN^-$; $\blacksquare = NO_2^-$; $\triangle = PO_4^{3-}$; $\blacktriangle = S_2O_3^{2-}$.

1.2°₀ for 50 nmol chloride, 25 nmol sulphate, 25 nmol thiocyanate, 100 nmol phosphate, 100 nmol nitrite and 100 nmol thiosulphate, respectively.

APPLICATION

　　　Fig. 6 illustrates a chromatogram for the analysis of waste water. High levels of chloride and sulphate ions were detected in this sample.

　　　A chromatogram for the analysis of human urine is shown in Fig. 7. The second, third and fourth peaks were attributable to phosphate, chloride and sulphate ion, respectively, and the trace amount of thiocyanate ion (1.4 nmol) was determined with only 10 µl urine sample.

CONCLUSION

　　　Fe^{3+} was used as a detection reagent for the analysis of inorganic anions by HPLC. This method could determine major anions such as chloride, phosphate and sulphate, which have no absorption in UV region, and was selective in the analysis of biological samples. Thus it may be suggested that this method is useful for the simul-

Fig. 6. Chromatogram of waste water. Sample size. 10 μl. Peaks: 1 = Cl⁻ (200 nmol); 2 = SO_4^{2-} (15 nmol).
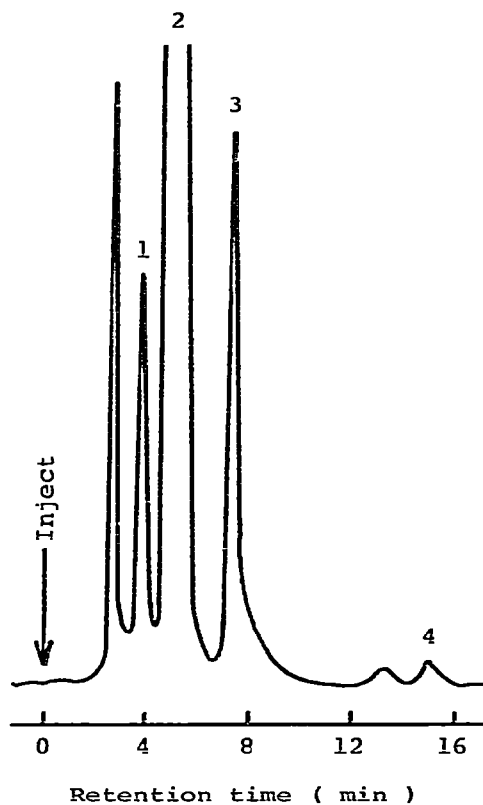
Fig. 7. Chromatogram of human urine. Sample size. 10 μl. Peaks: 1 = PO_4^{3-} (257 nmol); 2 = Cl⁻ (1800 nmol); 3 = SO_4^{2-} (100 nmol); 4 = SCN⁻ (1.4 nmol).

taneous determination of inorganic anions, such as phosphate, nitrite, chloride, sul⁊ phate, thiosulphate and thiocyanate, with sufficient sensitivity and simplicity.

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