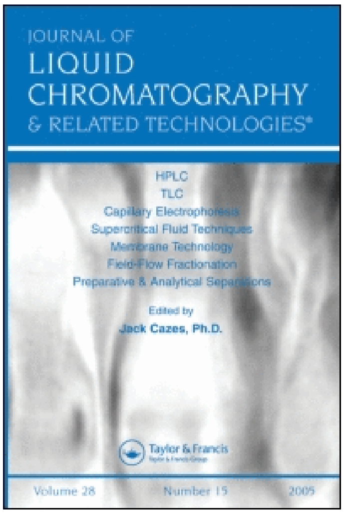

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INDIRECT DETECTION OF ANIONS WITH POTASSIUM HEXACYANOFERRATE(II) AND -(III) IN THE SUB-PG RANGE

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ABSTRACT

The use of potassium hexacyanoferrate(II) and -(III) as eluents for the determination of anions is shown. After eluent optimization the isocratic separation of Cl^-, HCO_2^-, $CH_3CO_2^-$, NO_3^- and SO_4^{2-} can be achieved within 6 minutes. As stationary phase a reversed phase 18 (RP-18) column was coated with dodecylamine.
With indirect UV detection limits of detection in the sub-pg range were obtained.

INTRODUCTION

For our research we need a method for the determination of the main anions (Cl^-, HCO_2^-, $CH_3CO_2^-$, NO_3^- and SO_4^{2-}) in atmospheric samples in the sub-pg range. Only small sample volumes (5 nl - 5 µl) are available so that no enrichment is possible. A short analysis time is desired because of high sample number.

Until now the two major methods for the determination of anions are suppressed conductivity detection [1-3] and single column ion chromatography with UV or conductivity detection [4-6].

With chemical suppression limits of detection in the sub ng/ml range can be achieved with typical injection volumes from 50 µl to 200 µl [7,8].

The lowest detection limits were obtained with direct detection methods in single column technique [9]. Pacakova et al. [10] determined 6 pg NO_3^- and 5 pg I^- with direct UV detection. A drawback of the direct detection mode is that only few anions can be determined. On the other hand indirect detection methods give universal detection of inorganic anions but show higher detection limits [11,12]. According to the investigations of Yeung [13] about indirect detection it is necessary to use low eluent concentrations to obtain low limits of detection. For lowering the eluent concentration eluents with a high eluting power are favoured [14].

$[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ are UV active multicharged anions [15] and can be used as strong eluents at very low concentrations (10^{-6} M).

Another requirement for low detection limits is the use of stationary phases with low ion exchange capacities because they allow the use of low eluent concentrations.

Permanent coated reversed phase columns as ion exchange columns are advantageous. Their ion exchange capacities can easily be controlled by changing the coating conditions and fitted to the separation problem [16-18].

Dodecylamine was chosen as coating reagent because it is adsorbed strong enough on the stationary phase for constant retention times. Furthermore dodecylamine is a primary amine resulting in weak exchange groups [19] being adsorbed on the stationary phase.

EXPERIMENTAL

Apparatus: The chromatographic system consisted of a metalfree IRICA HPLC pump Σ 871 (ERC, Alteglofsheim, Germany) and a metalfree Model 9125 injector Rheodyne (ERC).

The following detectors were used: A Spectro-Monitor 3100 UV photometric detector (LDC/Milton Roy, Hasselroth, Germany) and a S 3110 conductivity detector (Sykam, Gauting, Germany).

The injection volumes can be seen in the captions to the figures.

The four columns Novapak RP 18-3 µm (7 % C) (150 * 4.6 mm I.D.) (Millipore, Frankfurt, Germany), LiChrospher RP 18-5 µm (200 * 4.6 mm I.D.) (Merck, Darmstadt, Germany), Ultracarb RP 18-3 µm (20 % C) (150 • 4.6 mm I.D.) (Amchro, Sulzbach, Germany) and Optisil ODS (5µm) (18.5 % C) (125 * 4.6 mm I.D.) (ERC) were coated with 350 ml of 1 mM dodecylamine in methanol:water (10:90) (flow rate: 0.4 ml/min). The solution was adjusted to pH 4.0 (H_3PO_4). After the coating procedure the column was conditioned with the eluent ($[Fe(CN)_6]^{3-}$ or $[Fe(CN)_6]^{4-}$). As guard column a Millipore precolumn µBondapak™ RP 18 was used.

Chemicals: dodecylamine, 99 % (Fluka, Neu-Ulm, Germany)
potassium hexacyanoferrate(II) and -(III), p.a. (Merck)
methanol HPLC grade (Baker, Gross-Gerau, Germany)
All other reagents were of p.a. quality (Merck)

RESULTS AND DISCUSSION

We started experimenting with anion exchange columns with a fixed capacity (Hamilton PRP X-100 and DIONEX OMNIPAX 500). In spite of varying eluent concentrations combined with different methanol contents no satisfying resolution for the separation of the anions was achieved.

However, good separation was obtained with silica based RP columns coated with dodecylamine.

Dodecylamine adsorbed strong enough on the stationary phase resulting in constant retention times of the analysed anions over a period of three weeks. If regeneration is required, dodecylamine can easily be removed from the column with 100 % methanol.

The amount of dodecylamine adsorbed on the stationary phase is depending on the carbon load (% C) of the RP-18 columns. The carbon load influences to a high degree the retention behaviour of the anions.

In Table I the different retention times of some anions under the same experimental conditions on three RP-18 columns with different carbon loads are summarized. The carbon load of the column influenced the retention times especially of the late eluting anions. SO_4^{2-} eluted on the Novapak column (7 % C) after 15 minutes whereas the retention time for SO_4^{2-} on the Ultracarb column (20 % C) was 30 minutes.

The best result was achieved with Novapak RP-18. The low carbon load of 7 % C leads to a fast elution of SO_4^{2-} (15.2 min) without deterioration in peak separation of the earlier eluting anions.

$[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$ are multicharged anions and can be used at low concentrations (10^{-6} M). Indirect UV detection and indirect conductivity detection were favoured because the two ions are UV active anions [15] and have a high equivalent conductivity ($[Fe(CN)_6]^{4-}$: 110,4 * 10^{-4} [m^2 S mol^{-1}],

Table I: Retention time [min] of some anions on three RP-18 columns with different carbon loads (% C)

anion	Novapak (7 % C)	Optisil (18,5 % C)	Ultracarb (20 % C)
Cl^-	2,30	3,36	4,37
HCO_2^-	2,56	3,56	5,31
$CH_3CO_2^-$	3,26	4,37	6,55
NO_3^-	4,47	6,24	8,03
SO_4^{2-}	15,24	29,20	30,48

Experimental conditions: eluent: 23 µM $[Fe(CN)_6]^{3-}$, 1ml/min
detection: UV (205 nm)

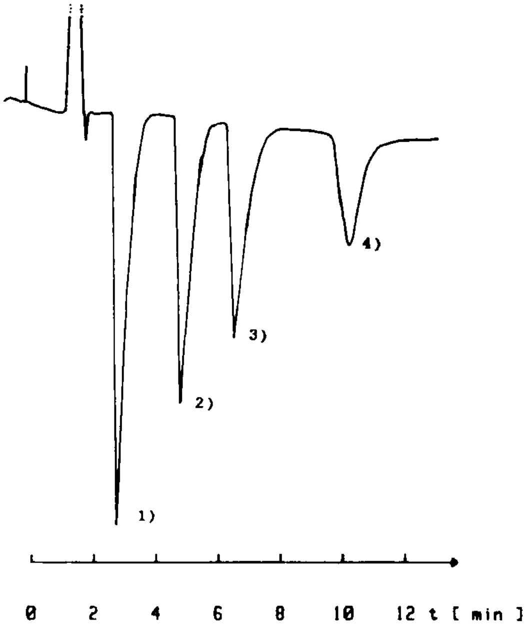

Fig.1: Chromatogram of an anion standard
(1 µg/ml each)
1) Cl^-, 2) ClO_3^-, 3) ClO_4^- 4) SO_4^{2-}
column: LiChrospher RP-18 (Merck) (200 • 4.6 mm I.D.)
coated with dodecylamine
eluent: 4.7 µM $[Fe(CN)_6]^{4-}$, 1.3 ml/min
detection: UV (205 nm), range: 0.01 AU
injection volume: 20 µl

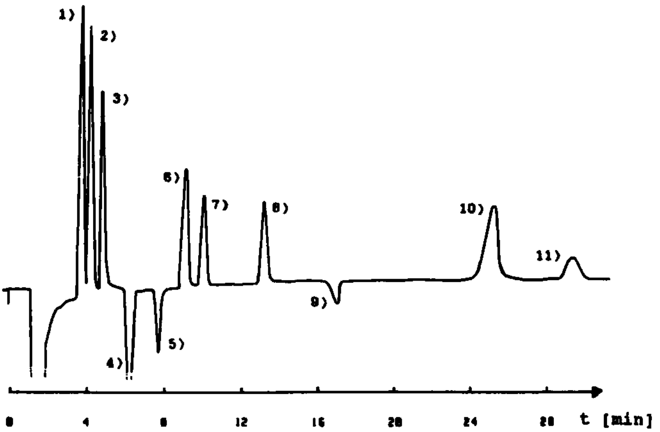

Fig.2: Chromatogram of an anion standard
1) Cl^- (4 µg/ml) 7) $ClCH_2CO_2^-$ (5 µg/ml)
2) HCO_2^- (5 µg/ml) 8) ClO_4^- (5 µg/ml)
3) $CH_3CO_2^-$ (5 µg/ml) 9) SCN^- (10µg/ml)
4) NO_3^- (4 µg/ml) 10) $Cl_2CHCO_2^-$ (5 µg/ml)
5) I^- (5 µg/ml) 11) SO_4^{2-} (5 µg/ml)
6) ClO_3^- (5 µg/ml)
column: LiChrospher RP-18 (Merck) (200 * 4.6 mm I.D.)
coated with dodecylamine
eluent: 23 µM $[Fe(CN)_6]^{3-}$, 1 ml/min
detection: a) UV (205 nm), range: 0.01 AU
b) conductivity, range: 0.3 µS
injection volume: 20 µl

$[Fe(CN)_6]^{3-}$: 100.9 * 10^{-4} [m^2 S mol^{-1}]) [20].

At 205 nm $[Fe(CN)_6]^{4-}$ has a molar absorptivity of 19870 [$l*mol^{-1}*cm^{-1}$]. At the same wavelength $[Fe(CN)_6]^{3-}$ has a molar absorptivity of 10780 [$l*mol^{-1}*cm^{-1}$].

There are two reasons to start the investigations with $[Fe(CN)_6]^{4-}$. First it shows a higher UV absorptivity and second it is the higher charged anion.

A chromatogram of an anion standard using $[Fe(CN)_6]^{4-}$ as eluent is shown in Fig 1. The strong eluting power of $[Fe(CN)_6]^{4-}$ is preferred for the late eluting anions (e.g. ClO_4^- and SO_4^{2-}) but

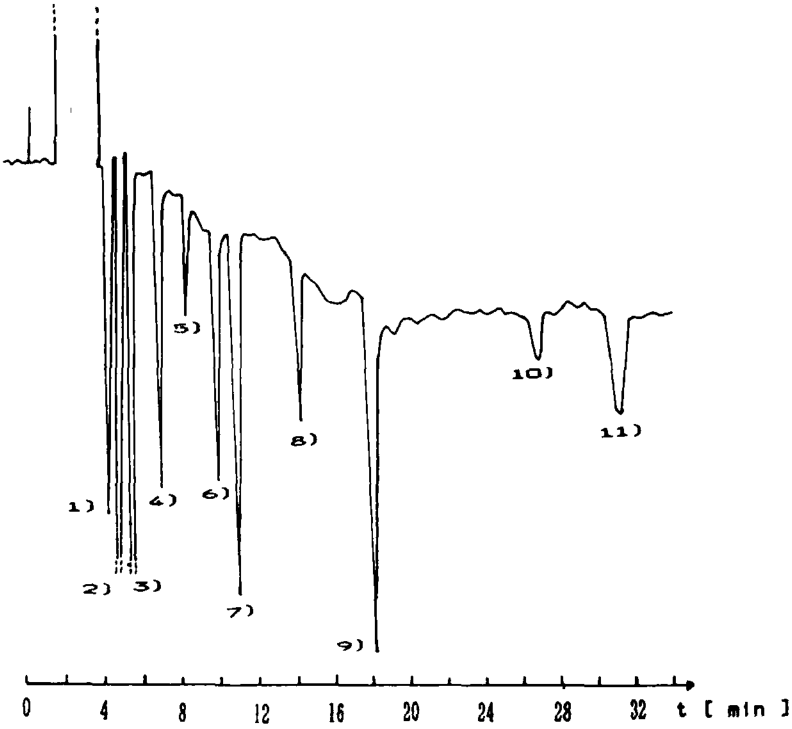

Fig. 2 (continued)

leads to a poor resolution of the earlier eluting anions Cl^- and HCO_2^-. The separation of these two anions could not be improved by varying the eluent concentration.

The $[Fe(CN)_6]^{3-}$ anion has a lower eluting power resulting in a better separation of Cl^- and HCO_2^-.

Fig 2a and 2b demonstrate the better resolution of the fast eluting anions. These two chromatograms show the same anion standard with indirect UV detection and indirect conductivity detection in tandem. With indirect UV detection NO_3^-, I^- and SCN^-

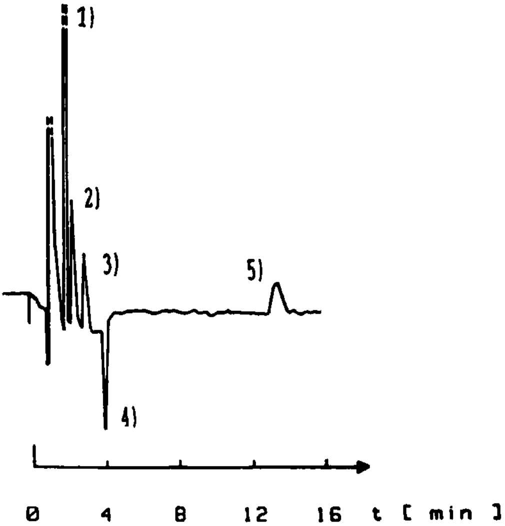

Fig.3: Chromatogram of a 1 μl rain drop
1) Cl^- (450 ng/ml) 4) NO_3^- (220 ng/ml)
2) HCO_2^- (240 ng/ml) 5) SO_4^{2-} (300 ng/ml)
3) $CH_3CO_2^-$ (710 ng/ml)
column: Novapak RP-18 (Waters) (150 * 4.6 mm I.D.)
coated with dodecylamine
eluent: 23 μM $[Fe(CN)_6]^{3-}$, 1 ml/min
detection: UV (205 nm), range: 0.002 AU
injection volume: 10 μl

Table II: Retention time [min] of some anions depending on the eluent composition
$[Fe(CN)_6]^{4-}/Fe(CN)_6]^{3-}]$ ratio [μM]

Anion	[0/23]	[4/19]	[7/16]	[9/14]	[12/12]
Cl^-	3,36	2,36	2,26	2,15	1,48
HCO_2^-	3,56	2,55	2,43	2,30	2,00
$CH_3CO_2^-$	4,37	3,25	3,13	2,57	2,18
NO_3^-	6,24	4,41	4,20	3,55	2,55
SO_4^{2-}	29,20	16,20	14,29	11,48	6,24

Experimental conditions: column: Optisil ODS (125 * 4.6 mm I.D.)
coated with dodecylamine
detection: UV (205 nm)
flow: 1 ml/min

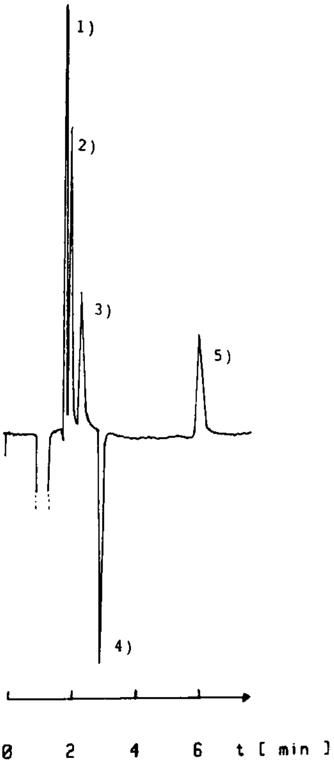

Fig.4: Chromatogram of an anion standard (1 µg/ml each)
1) Cl^-, 2) HCO_2^-, 3) $CH_3CO_2^-$, 4) NO_3^-, 5) SO_4^{2-}
column: Optisil ODS (ERC) (125 * 4.6 mm I.D.) coated with dodecylamine
eluent: 11.5 µM $[Fe(CN)_6]^{3-}$, 11.5 µM $[Fe(CN)_6]^{4-}$, 1 ml/min
detection: UV (205 nm), range: 0.02 AU
injection volume: 10 µl

appear as positive signals. These three anions absorb stronger than the eluent at 205 nm.

The capability of $[Fe(CN)_6]^{3-}$ as a useful eluent for our research is shown in Fig 3. It shows the determination of the main anions (Cl^-, HCO_2^-, $CH_3CO_2^-$, NO_3^- and SO_4^{2-}) in an individual rain drop (1 µl diluted with 10 µl water).

Table III: Limits of detection (signal/noise ratio = 3)

anion	UV		conductivity	
	pg	(ng/ml)	pg	(µg/ml)
Cl^-	30	(6)	620	(2.80)
HCO_2^-	50	(10)	410	(1.85)
CH_3CO^-	90	(20)	400	(1.80)
NO_3^-	60	(15)	550	(2.50)
SO_4^{2-}	110	(25)	720	(3.20)

Experimental conditions: eluent: 11.5 µM $[Fe(CN)_6]^{3-}$, 11.5 µM $[Fe(CN)_6]^{4-}$, Flow: 1 ml/min
column: Optisil ODS (125 * 4.6 mm I.D.) coated with dodecylamine
detection:UV (205 nm), range: 0.001 AU
conductivity, range: 0.3 µS
injection volume: 4.5 µl

The drawback of the $[Fe(CN)_6]^{3-}$ system is the long retention time of SO_4^{2-} especially on RP-18 columns with a high carbon load. Experiments showed that it is possible to reduce the retention time by addition of small amounts of $[Fe(CN)_6]^{4-}$ to the $[Fe(CN)_6]^{3-}$ eluent. An optimization of this mixed eluent system (high resolution of the earlier eluting anions but also short retention times for the later) was carried out.

In Table II the retention time of some anions depending on the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ ratio is shown. The best result was achieved if the eluent contained $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ at same quantities. In this case the separation of the early eluting anions is maintained whereas SO_4^{2-} elutes after 6.2 min. If the mixed eluent is containing more $[Fe(CN)_6]^{4-}$ than $[Fe(CN)_6]^{3-}$ the separation of Cl^- and HCO_2^- becomes worse.

Fig 4 shows a chromatogram of an anion standard with an $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ eluent.

The limits of detection for indirect UV and indirect conductivity detection are summarized in Table III. It shows the absolute limits, calculated at a signal/noise ratio of 3.

The comparison of the two detection methods shows lower detection limits for the indirect UV detection. The sub-pg range for the analysed anions was achieved.

CONCLUSION

RP-18 columns coated with dodecylamine are suitable as stationary phases for the separation of organic and inorganic anions. The retention times are depending on the different carbon loads (% C) of the RP materials. $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$ were used as strong eluents at low concentrations (10^{-6} M). Due to better separation of the early eluting anions (Cl^- and HCO_2^-), $[Fe(CN)_6]^{3-}$ is preferrable. Shorter analysis times without deterioration in peak separation can be achieved with a mixed eluent of $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$.

The retention time for SO_4^{2-} can be reduced from 30 min. to 6.2 min. A comparison between indirect UV and indirect conductivity detection shows lower limits of detections (sub-pg range) for the former.

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