CHROM. 16,989

SIMULTANEOUS DETERMINATION OF ANIONS AND METAL CATIONS BY SINGLE-COLUMN ION CHROMATOGRAPHY WITH ETHYLENEDIAMINETETRAACETATE AS ELUENT AND CONDUCTIVITY AND ULTRA-VIOLET DETECTION

S. MATSUSHITA

4560 Central Research Laboratories, Toyo Soda Manufacturing Co., Ltd., 4560 Tonda, Shinnanyo-shi, Yamaguchi-ken (Japan)

(First received May 28th, 1984; revised manuscript received June 18th, 1984)

SUMMARY

A single-column ion chromatographic method for detecting and characterizing several metal ions and inorganic anions is described. Separations are achieved on columns packed with silica-based or polymer-based anion exchanger under identical conditions. As eluent, a chelating agent such as ethylenediaminetetraacetate is remarkably effective for the separation of inorganic anions and metal ions which are converted into metal chelate anions upon contact with the chelating agent. The eluted ions are detected with a UV detector in series with a normal conductivity detector; in the case of the UV detector, the eluent composition allows both absorbing and non-absorbing anions to be detected. Some practical applications are described.

INTRODUCTION

Chromatographic methods have widespread use in the separation and analysis of inorganic ions in a variety of aqueous matrices. Small *et al.*[1,2] invented a suppressor system for the separation of anions using a conductivity detector. Fritz *et al.* developed a non-suppressed method for the separation of anions[3] and cations[4,5].

Significant improvements in conductivity detectors have been made in recent years, enabling sensitive detection of ions without a suppressed system[6-9]. Chelating agents have been used in many published methods to achieve more selective chromatographic separtaions of metal ions, but their presence in the eluent often makes detection of the metal ions difficult. However, Yamamoto *et al.*[10] effected the chromatographic separation of inorganic anions and alkaline earth metal ions with ethylenediaminetetraacetate (EDTA) as eluent using a single anion-exchange column and conductometric detection. An alkaline earth metal ion, M^{2+}, forms a chelate anion, $MEDTA^{2-}$, immediately on contact with the EDTA eluent which contains H_2EDTA^{2-} and $HEDTA^{3-}$

$$M^{2+} + H_2EDTA^{2-} \rightarrow MEDTA^{2-} + 2H^+$$
$$M^{2+} + HEDTA^{3-} \rightarrow MEDTA^{2-} + H^+$$
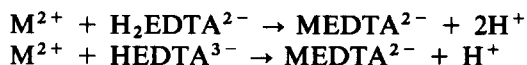
and is then separated on the anion-exchange column.

In the present work, eluents containing chelating agents such as EDTA, *trans*-1,2-cyclohexanediamine-N,N,N',N'-tetraacetate (CyDTA) and glycol ether diamine-N,N,N',N'-tetraacetate (GEDTA) have been investigated for the separation of several metal cations and inorganic anions. As in our previous work[11], ion selectivities on a silica-based anion exchanger TSKgel IC-Anion-SW are found to be somewhat different from those obtained on a conventional polymer-based material TSKgel IC-Anion-PW. The use of a UV detector, in series with a conductivity detector, greatly increases the amount of information obtainable on a given sample and can resolve many of the problems.

EXPERIMENTAL

A non-suppressor type Toyo Soda Model HLC-601 ion chromatograph with a corrosian-resistant eluent system was used[6]. The separator columns TSKgel IC-Anion-SW (50 × 4.6 mm I.D., Toyo Soda) were packed with a silica-based anion exchanger, and TSKgel IC-Anion-PW (50 × 4.6 mm I.D., Toyo Soda) with a polymer-based anion exchanger. Table I summarizes the physical and chemical properties of these columns.

TABLE I

PROPERTIES OF ANION SEPARATOR COLUMNS

Packing material	Polymer (PW)	Silica (SW)
Column length (cm)	5	5
Particle size (μm)	10	5
Functionality	Dimethylaminoethyl	Dimethylaminoethyl
Exchange capacity (mequiv./ml)	0.03 (dry)	0.40 (dry)
Plates per metre	> 18,000	> 28,000
Eluent pH range	1–13	2–7
Temperature (°C) max.	60	45

All injections were 100 μl and the flow-rate was kept at 1.0 ml/min under a pressure of 30–32 kg/cm^2. The columns and the conductivity detector were maintained at 30°C.

A Toyo Soda Model UV-8 variable wavelength UV detector was used. A Perkin-Elmer Model 2000 atomic absorption spectrometer was employed to analyze metal ions.

Unless otherwise stated, all chemicals were prepared from reagent grade materials (Wako, Osaka, Japan). Standard metal ion solutions were standardized by titration with EDTA. Standard anion solutions were prepared from the regent grade salts.

All eluents were prepared in distilled, deionized water and filtered through a 0.22-μm membrane filter before use. The pH of the eluent was adjusted with potassium hydroxide. The eluent reservoir was equipped with a soda lime tube.

All practical samples were prepared in distilled, deionized water and injected through a 0.22-μm membrane filter onto the chromatograph.

RESULTS AND DISCUSSION

Chelating agent

The eluent must be carefully chosen when a conductivity detector is to be directly coupled to anion-exchange column. In general, a good eluent is an organic anion which has a high selectivity coefficient for the anion exchanger. A low eluent concentration is desirable because of the correspondingly low background conductance. If a selective chelating agent can be employed, it should be possible to separate many ions.

Because diaminetetraacetates such as EDTA, CyDTA and GEDTA have a 4— charge they are expected to have a high affinity for the anion exchanger and to be effective eluting agents. These chelating agents are less mobile than inorganic anions and therefore have lower equivalent conductances. In addition, an UV detector can be applied because these complexing agents have weak absorbance in the 210–240 nm region.

Table II lists the adjusted retention times for eight ions with three chelating agents on TSKgel IC-Anion-SW. Solutions of the chelating agents can be conveniently prepared by dissolution in deionized water followed by adjusting the pH to 6.0 with potassium hydroxide solution. From the dissociation constants[12] it can be calculated that at this pH the EDTA eluent contains 59% H_2EDTA^{2-} and 41% $HEDTA^{3-}$, the CyDTA eluent 57% H_2CyDTA^{2-} and 43% $HCyDTA^{3-}$ and the GEDTA eluent 10% H_2GEDTA^{2-}. The results confirm that EDTA is a more successful eluent than GEDTA and CyDTA on TSKgel IC-Anion-SW at the same concentration and pH. CyDTA is somewhat expensive, but yielded a similar separation. FEDTA was not suitable for the divalent ions studied owing to its lower eluting power, even at the higher pH value of 7.7.

TABLE II

RETENTION TIMES (min) OF ANIONS WITH VARIOUS ELUENTS ON TSKgel IC-Anion-SW

Ion	EDTA 2 mM, pH 6.0	CyDTA 2 mM, pH 6.0	GEDTA 2 mM, pH 6.0
Ca^{2+}	5.8	7.4	——*
Mg^{2+}	6.8	4.2	——*
Ni^{2+}	8.4	8.8	——*
Zn^{2+}	6.4	9.4	——*
Cu^{2+}	9.4	9.8	——*
Cl^-	3.0	3.8	7.0
NO_3^-	3.6	4.2	10.2
SO_4^{2-}	9.0	9.8	——*

* Peak had too long a retention time.

Elution behaviour with EDTA

The elution behaviour with EDTA as eluent can be analyzed as follows. The anion-exchange equilibrium is represented by

$$_xS^{y-}R_y + {}_yE^{x-} \rightleftharpoons {}_yE^{x-}R_x + {}_xS^{y-} \tag{1}$$

where E^{x-} represents the eluent anion (EDTA), S^{y-} the sample anion and R_y, R_x the exchange sites on the resin used by the ions. The selectivity coefficient, K_S^E, for this reaction is:

$$K_S^E = \frac{[E^{x-} R_x]^y [S^{y-}]^x}{[E^{x-}]^y [S^{y-} R_y]^x} \tag{2}$$

At low loading of sample ion the resin capacity, [Cap], is approximately $[E^{x-} R_x]$. The capacity factor, k, is equal to the ratio $[S^{y-} R_y]/[S^{y-}]$. Thus eqn. 2 can be rewritten as:

$$K_S^E = \frac{[Cap]^y}{[E^{x-}]^y k^x} \tag{3}$$

The adjusted retention time, t, for an eluted peak is equal to $t_0 k$, where t_0 is the retention time of an unretained substance. Substituting t/t_0 for k in eqn. 3 and taking logarithms gives:

$$\log t = \frac{y}{x} \log [Cap] - \frac{y}{x} \log [E^{x-}] + \log t_0 - \frac{1}{x} \log K_S^E \tag{4}$$

The validity of eqn. 4 was tested by measuring the adjusted retention times of the eight ions at constant pH on the TSKgel IC-Anion-SW column at various concentrations of the EDTA eluent. As shown in Fig. 1, linear plots of log t versus log [EDTA] were obtained. The experimental values for the monovalent anions were -0.33 and for divalent inorganic anions were -0.66. These results indicte that the charge on the eluent anion is -3.0.
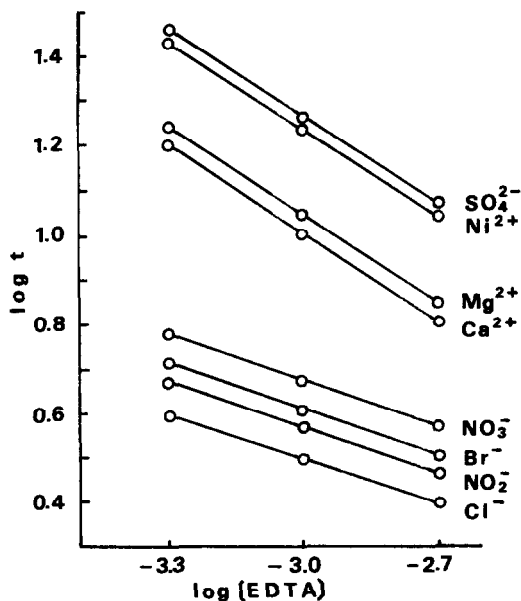

Fig. 1. Log of adjusted retention times *vs.* log of concentration of EDTA at pH 5.5 on TSKgel IC-Anion-SW. Flow-rate; 1 ml/min. Column temperature: 30°C.

The slopes of these plots for metal complex anions were very close to those for the divalent inorganic anions. Thus eqn. 1 for metal complex anions can be written as:

$$3MEDTA^{2-}R_2 + 2HEDTA^{3-} \rightleftharpoons 2HEDTA^{3-}R_3 + 3MEDTA^{2-} \qquad (5)$$

From the dissociation constants of EDTA, $pK_1 = 1.99$, $pK_2 = 2.67$, $pK_3 = 6.16$ and $pK_4 = 10.26$, it can be calculated that 2 mM EDTA solution at pH 5.5 contains 17.9% HEDTA^{3-}. Accordingly this eluent has an HEDTA^{3-} concentration of 3.6×10^{-4} M. The eluent equilibrium is dynamic and highly ionized HEDTA^{3-} is supplied from H$_2$EDTA^{2-}.

Fig. 2 shows a direct comparison of the pH effect on the two separator columns for the retention times of the nine ions under identical conditions. The retention times decreased with increasing pH accompanying the increase in concentration of HEDTA^{3-}. Eluents adjusted to pH < 4.5 on both columns were not suitable because the separation was too time-consuming.
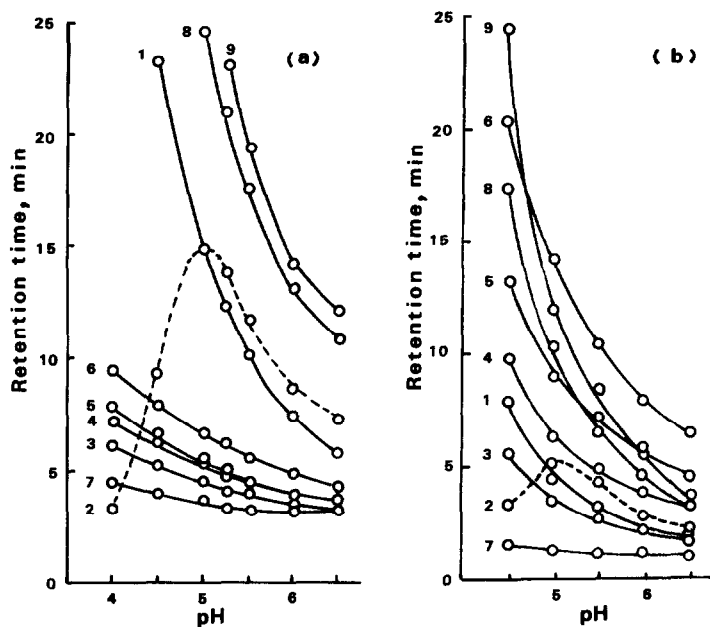

Fig. 2. Retention times of anions *vs.* pH of 1 mM EDTA eluent on TSKgel IC-Anion-SW (a) and TSKgel IC-Anion-PW (b). Ions 1, Ca^{2+}, 2, Mg^{2+}; 4, NO$_2^-$; 5, Br$^-$; 6, NO$_3^-$; 7, H$_2$PO$_4^-$; 8, Ni^{2+}; 9, SO$_4^{2-}$. Other details in Fig 1.

In the case of TSKgel IC-Anion-PW, a large chloride peak overlapped with a small calcium peak and interrupted the determination of Ca^{2+} in the pH range 5.0–6.0. Fortunately, the chloride peak on TSKgel IC-Anion-SW was eluteed early and hence the small amount of Ca^{2+} could be completely separated from the excess of chloride ion in this pH range. The retention time of Mg^{2+} was decreased at pH < 5.0. This means that considerable dissociation of MgEDTA^{2-} occurred below pH

5.0. $CaEDTA^{2-}$ and $NiEDTA^{2-}$ are stable even at pH 4.5. The pH effect in Fig. 2 is expected to be dependent on the conditional stability constants of metal EDTA chelates[12]. $MgEDTA^{2-}$, $CaEDTA^{2-}$ and $NiEDTA^{2-}$ are well characterized as 1:1 chelates with conditional stability constants at pH 4.5 of 1.25, 3.25 and 11.19, respectively[12]. Many transition metals with stability constants greater than 10.0 are also expected to be stable at pH 4.5.

The eluent pH should be controlled in the pH range 5.0–6.5 for the separation of Mg^{2+}. The separation of $H_2PO_4^-$ and Cl^- was difficult in this pH range. However, the separation of these ions could be accomplished with the use of 0.5 mM EDTA, pH 4.5, as eluent.
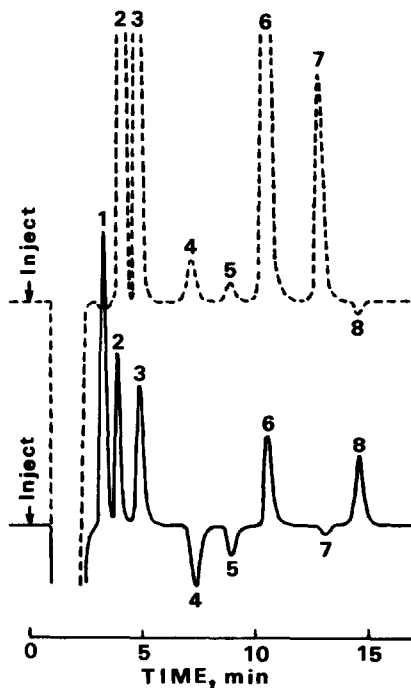

Fig. 3. Chromatograms of an eight-ion standard on TSKgel IC-Anion-SW with an UV detector at 210 nm (----) and a conductivity detector (———). Peaks: 1 = 5 ppm Cl^-; 2 = 5 ppm NO_2^-; 3 = 5 ppm NO_3^-; 4 = 5 ppm Ca^{2+}; 5 = 4 ppm Mg^{2+}; 6 = 4 ppm NCS^-; 7 = 5 ppm Ni^{2+}, 8 = 6 ppm SO_4^{2-}. Eluent: 1 mM EDTA at pH 6.0. Flow-rate: 1 ml/min. Column temperature: 30°C.

Detection

Typical chromatograms of the eight ions of interest on TSKgel IC-Anion-SW with the dual UV–conductivity detection system are shown in Fig. 3. For routine analyses of samples, conductometric detection is satisfactory in terms of speed and simplicity. However, UV detection offers lower detection limits and is thus well suited to the analysis of species in low concentrations.

Table III compares the retention times and detection limits for 21 ions with the dual UV–conductivity detection system. The elution behaviours of all metal cations listed were checked by atomic absorption spectrometry. Fractions were collected

TABLE III

RETENTION TIMES AND DETECTION LIMITS WITH UV AND CONDUCTIVITY DETECTION

Column: TSKgel IC-Anion-SW. Eluent: 1 mM EDTA, pH 6.0. Flow-rate: 1 ml/min.

Ion	Retention time (min)	Detection limit (ng)*	
		UV (210 nm)	Conductivity
$H_2PO_4^-$	3.2	60.0 (neg)	30.0 (neg)
Cl^-	3.4	13.2 (neg)	2.6
NO_2^-	3.8	0.6	5.2
Br^-	4.0	2.0	5.8
NO_3^-	5.0	0.8	5.4
I^-	8 1	2.8	6.6
SCN^-	10.6	4.4	8.8
ClO_4^-	11.1	100.0 (neg)	10.2
SO_4^{2-}	14.2	130.0 (neg)	10.0
SeO_4^{2-}	15.8	315.0 (neg)	25.0
$S_2O_3^{2-}$	22.7	20.0	13.0
Ba^{2+}	6.4	70.0	90.0 (neg)
Ca^{2+}	7.4	40.0	15.0 (neg)
Zn^{2+}	8.2	5.0	150.0 (neg)
Mg^{2+}	8.7	20.0	20.0 (neg)
Mn^{2+}	9.3	20.0	20.0 (neg)
Pb^{2+}	9.5	10.0	225.0 (neg)
Ni^{2+}	13.2	5.0	100.0 (neg)
Co^{2+}	13.5	10.0	140.0 (neg)
Cu^{2+}	14.8	10.0	250.0 (neg)
Fe^{2+}	22.5	60.0	65.0 (neg)

* Calculated as twice the random baseline noise. Based on three 100-μl injections of a sample solution containing 5 ppm of each ion. neg = Negative peak.

manually every 20 sec over the entire course of the chromatogram and were then subjected to atomic absorption spectrometry. It was confirmed that each metal cation was restricted to the corresponding peak in the chromatogram. The detection limits in Table III are defined as twice the standard deviation of the baseline noise.

Many metal EDTA complexes are colourless. However, they all exhibit strong absorption bands in the UV region of the spectrum. At pH < 7.0, EDTA solutions show weaker absorbance than those of metal EDTA complexes. For highly sensitive detection of many metals, a wavelength of 210 nm was employed when the pH range of the EDTA eluent was kept at 5.0–6.5. Unretained inorganic anions such as Cl^- and SO_4^{2-} appered as negative peaks upon UV detection owing to their lower selectively detected.

Applications

The use of the chromatographic technique described in the analysis of most common ions such as Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-} in various samples is summarized in Table IV. The samples were analyzed by anion chromatography on TSKgel IC-Anion-SW with the conductivity detector and the UV detector at 210 nm using 1 mM EDTA (pH 6.0) for the separation of Ca^{2+}, Mg^{2+}, Cl^-, NO_3^- and

TABLE IV

ANALYTICAL RESULTS (μg/ml) FOR VARIOUS SAMPLES

Values represent the average ± standard deviation for at least three analyses on the same day.

Sample	Ca^{2+} IC^* $(AA)^{**}$	Mg^{2+} IC^* $(AA)^{**}$	Cl^- IC^*	NO_3^- IC^{***}	SO_4^{2-} IC^*	PO_4^{3-} $IC^§$
Tap-water	5.1 ± 0.2 (4.6 ± 0.3)	0.6 ± 0.1 (0.5 ± 0.2)	5.2 ± 0.3	2.0 ± 0.1	5.7 ± 0.2	n.d
River-water	6.4 ± 0.3 (5.8 ± 0.4)	1.1 ± 0.1 (1.0 ± 0.2)	4.8 ± 0.3	4.3 ± 0.3	9.1 ± 0.3	n.d.
Snow	0.9 ± 0.1 (0.8 ± 0.2)	1.6 ± 0.1 (1.5 ± 0.2)	29.2 ± 1 2	0.8 ± 0.1	5.1 ± 0.2	n.d.
Rain-water	0.9 ± 0.1 (0.8 ± 0.2)	0.5 ± 0.1 (0.5 ± 0.2)	2.5 ± 0.2	0 7 ± 0.1	1.6 ± 0.1	n.d.
Sea-water	275 ± 9 (256 ± 12)	753 ± 23 (694 ± 13)	18,400 ± 550	0.4 ± 0.2	2380 ± 70	n.d
Soil extract	192 ± 8 (174 ± 15)	18.4 ± 0 7 (16.4 ± 0.8)	3.0 ± 0.1	0.3 ± 0.1	488 ± 13	n.d
Drink 1	20.4 ± 1.0 (18 5 ± 1.0)	6.1 ± 0.3 (5.7 ± 0.4)	656 ± 20	4 0 ± 0.2	12.0 ± 0.4	20 ± 0.5
Drink 2	18.0 ± 0.9 (15.5 ± 1.1)	0.4 ± 0 1 (0.3 ± 0.1)	578 ± 18	3.5 ± 0.2	10.3 ± 0.3	17 ± 0.6
Saliva	17.0 ± 0.8 (15.5 ± 1.1)	1.3 ± 0.2 (1.2 ± 0.2)	522 ± 15	31.8 ± 1.2	4 9 ± 0.2	470 ± 16

* Analysis by peak height with 1 mM EDTA (pH 6.0) using conductometric detection.
** Perkin-Elmer Model 2000 atomic absorption spectrometer.
*** Analysis by peak height with 1 mM EDTA (pH 6.0) using UV detection.
§ Analysis by peak height with 0 5 mM EDTA (pH 4.5) using conductometric detection; n.d. = not detected.

SO_4^{2-}. The analysis of PO_4^{3-} was performed under the same conditions except for the use of 0.5 mM EDTA (pH 4.5) eluent.

The calibration curves for Cl^-, NO_3^- and SO_4^{2-} were linear in the range 0.1–20 μg/ml and the linear range of other ions (Ca^{2+}, Mg^{2+} and PO_4^{3-}) was 0.3–30 μg/ml by peak height measurements using a conductivity detector. A lower linear range 0.02–5 μg/ml for NO_3^- was obtained using a UV detector at 210 nm.

The high sensitivity of the procedure for Ca^{2+} and Mg^{2+} makes it useful for determining "water hardness" because tap-, rain-, snow- and river-water vary appreciably in the levels, of these ions. The wide variation in levels of alkaline earth metal ions ranging from 0.5–20 μg/ml permits discrimination between them.

In a snow sample a high concentration of Cl^- and Mg^{2+} might be affected with sea-water. A sea-water sample was diluted 100-fold and injected.

A soil sample can easily be analyzed for extractable ions by adding 20 ml of water to 1 g of soil, shaking, filtering with a 0.22-μm membrane filter and directly injecting a 100-μl aliquot onto the chromatograph.

This ion chromatographic technique was applied to the determination of Ca^{2+} and Mg^{2+} in isotonic drinks. Fig. 4 shows the chromatogram of a 10-fold diluted drink. Citrate anion in the sample was eluted at 36 min, hence the separation of Ca^{2+} and Mg^{2+} could not be affected. Coexisting citrate anion had no appreciable effect even when present in twice the molar concentration of each metal ion.
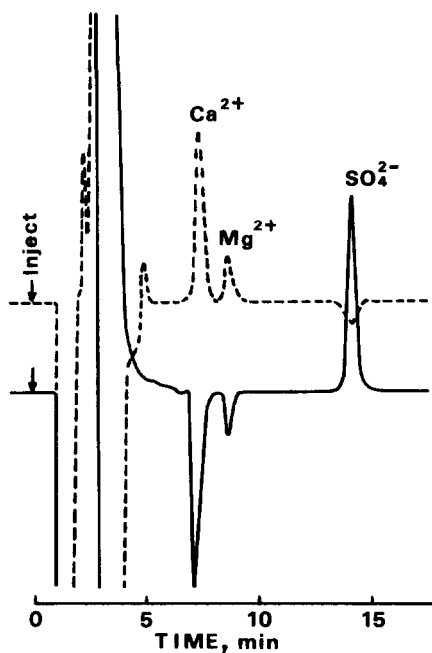
Fig. 4. Separation of calcium, magnesium and sulphate ions in an isotonic drink with an UV detector at 210 nm (----) and a conductivity detector (——). Other conditions as in Fig. 3
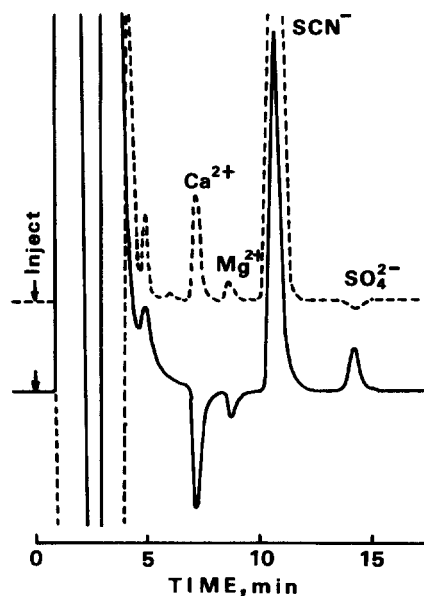
Fig. 5. Separation of calcium, magnesium, thiocyanate and sulphate ions in a smoker's saliva with a UV detector at 210 nm (----) and a conductivity detector (——). Other conditions as in Fig. 3.

A particularly interesting illustration of the value of the UV detector in providing selectivity and sensitivity is in its applications to the examination of saliva. The presence of thiocyanate anion in 10-fold diluted saliva is revealed as shown in Fig. 5. This smoker's saliva contained 94 μg/ml thiocyanate ion.

ACKNOWLEDGEMENTS

REFERENCES

1 H. Small, T. S. Stevens and W. C Baumann, *Anal. Chem.*, 47 (1975) 1801.
2 T. S. Stevens, J. C Davis and H. Small, *Anal. Chem.*, 53 (1981) 1488.
3 D. T. Gjerde, G. Schmuckler and J. S. Fritz, *J. Chromatogr.*, 187 (1980) 35.
4 J. S. Fritz, D. T. Gjerde and R. M. Becker, *Anal. Chem.*, 52 (1980) 1519.
5 G. J. Sevenich and J. S. Fritz, *Anal. Chem.*, 55 (1983) 12.
6 S. Matsushita, Y. Tada, N. Baba and K. Hosako, *J. Chromatogr.*, 259 (1983) 459.
7 J. M. Keller, *Anal. Chem* , 53 (1981) 344.

8 T Okada and T. Kuwamoto, *Anal. Chem.*, 55 (1983) 1001.
9 T Okada and T. Kuwamoto, *Anal. Chem.*, 284 (1984) 149.
10 M Yamamoto, H. Yamamoto, Y. Yamamoto, S. Matsushita, N. Baba and T. Ikushige, *Anal. Chem.*, 56 (1984) 832.
11 S. Matsushita, Y. Tada, K. Komiya and A. Ono, *Bunseki Kagaku (Jap. Anal.)*, 32 (1983) 562.
12 A. Ringbomb, *Complexation in Analytical Chemistry*, Wiley, New York, 1963.