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Note

Sensitive determination of nitrite and nitrate by ion-exchange chromatography*

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The simultaneous determination of nitrite and nitrate is of great importance, especially in water and foodstuffs, because the nitrite ion may be converted into nitrosamines which are of high toxicity. Furthermore, the interaction between nitrite and nitrate means that the concentration of nitrate must also be controlled. These anions occur in fertilizers, and in the meat industry where they are employed as curing agents.

The separation and determination of anions, especially inorganic anions, is now easily carried out by ion chromatography. However, despite automation of the method and the introduction of continuous regeneration, it is desirable that single-column methods be developed for this analysis: first to simplify the instrumentation, thereby decreasing its cost and maintenance, and allowing any laboratory owning a conventional liquid chromatograph to use it; secondly, to reduce the dead volume as a result of eliminating the suppressor column, thereby increasing efficiency and hence resolution.

Single-column ion chromatography using low capacity ion-exchange columns and dilute aqueous solutions of aromatic acids[1] or potassium hydroxide[2] as eluents has proved to be quite satisfactory. There are also methods which use ion-interaction or ion-pair chromatography coupled with direct or indirect ultraviolet (UV) absorbance detection and C_{18} reversed-phase, amino, cyano and organic polymer columns. A good review on this subject is given by Jackson *et al.*[3].

Our efforts were concentrated on achieving a simple method both from the instrumental and operational points of view. Considering that the two detectors most commonly used are the refractive index (RI) and UV detectors, we tried to use these for the determination of several anions, namely chloride, nitrite, nitrate and sulphate. In Table I the best detection limits found to date for these anions using the two detectors are indicated. For comparison we include the sensitivity found with the conductivity detector.

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TABLE I

DETECTION LIMITS (ng) FOR SEVERAL ANIONS WITHOUT PRECONCENTRATION

Detector	Cl^-	NO_2^-	NO_3^-	SO_4^{2-}
RI	20 (ref. 4)	80 (ref. 5)	30 (ref. 4)	30 (ref. 4)
Conductivity	2.5 (ref. 2)	1.5 (ref. 2)	1.5 (ref. 2)	60 (ref. 6)
UV				
Indirect	5 (ref. 1)	8 (ref. 1)	11 (ref. 1)	30 (ref. 7)
Direct	200 (ref. 8)	2 (ref. 9)	1 (ref. 9)	3000 (ref. 10)*

* Obtained with preconcentration.

We now present our method, which is based on the use of a new eluent, sodium perchlorate, and a high capacity ion-exchange column with a conventional liquid chromatograph and an UV detector. No preconcentration techniques are employed.

For comparison we include some values obtained with the methanesulphonate eluent.

EXPERIMENTAL

Instrumentation

A Spectra-Physics 8700 liquid chromatograph was used. The detectors included a Shimadzu UV-240 spectrophotometer, a Spectra-Physics SP8440 variable wavelength spectrophotometer and a Micromeritics 771 refractive index detector. The column was an Ionosphere tm A (250 × 4.6 mm) (Chrompack, The Netherlands); capacity about 1.0 mequiv g^{-1} packing.

Mobile phases

These comprised 0.02–0.06 *M* sodium perchlorate and 0.2 *M* sodium methanesulphonate. The reagents used were of analytical grade (Merck) and the water was purified on a Millipore Milli-Q system. All mobile phases were filtered through 0.45-μm filters, and degassed with helium.

Sample preparation

The water samples were filtered and injected directly onto the chromatograph. The samples of cooked ham were purchased from a supermarket and subjected to the following treatment[11]. A 30-g sample was finely comminuted, mixed thoroughly with 80 ml warm water at approximately 80°C and stirred for about 15 min. The flask was transferred to a steam-bath, and left for 2 h, with occasional shaking. The contents were allowed to cool and then made up to 1000 ml in a volumetric flask. A 25-ml aliquot was centrifuged and the supernatant decanted. This solution was filtered through a 0.45-μm filter and injected onto the chromatograph.

RESULTS AND DISCUSSION

The first results obtained showed that the UV detector could permit lower limits of detection than the RI detector, so we give only the results achieved with the former.

We began by recording the UV spectra (Fig. 1) for the two eluents. The low absorbance of the latter allow direct measurement of the UV absorbance of the ions

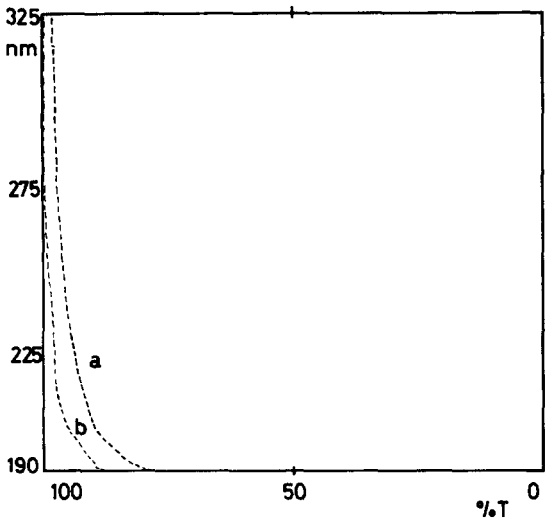


Fig. 1. Ultraviolet spectra for the eluents: a, 0.2 *M* methanesulphonic acid; b, 0.04 *M* sodium perchlorate.

TABLE II

WAVELENGTHS AT THE ABSORPTION MAXIMA OF THE UV SPECTRA FOR ANIONS

Anion	*Concn. (ppm)*	λ_{max} *(nm)*
Chloride	350	193
	35	< 190
Nitrite	9	209
Nitrate	7	200
Sulphate	93	< 190

TABLE III

EFFECT OF THE ELUENT CONCENTRATION ON THE RETENTION TIME AND RESOLUTION

Flow-rate: 2 ml/min.

Eluent concn. (mol/l)	t_R *(min)*				$R(Cl^-/NO_2^-)$	$R(NO_2^-/NO_3^-)$
	Cl^-	NO_2^-	NO_3^-	SO_4^{2-}		
Sodium perchlorate						
0.02	5.1	5.9	6.9		2.0	2.0
0.04	3.2	3.6	4.2	6.7	1.4	1.9
0.06	2.6	2.9	3.3		1.3	1.9
Methanesulphonic acid						
0.2	3.7	4.9	6.8		6.0	5.0

TABLE IV

EFFECT OF THE FLOW-RATE ON THE RETENTION TIME AND RESOLUTION

Flow-rate (ml/min) of 0.04 M $NaClO_4$	*t_R (min)*			*$R(Cl^-/NO_2^-)$*	*$R(NO_2^-/NO_3^-)$*
	Cl^-	*NO_2^-*	*NO_3^-*		
0.7	9.4	10.6	12.2	1.5	2.0
1.0	6.6	7.4	8.5	1.5	1.8
1.5	4.4	4.9	5.7	1.5	1.8
2.0	3.2	3.6	4.2	1.5	1.7

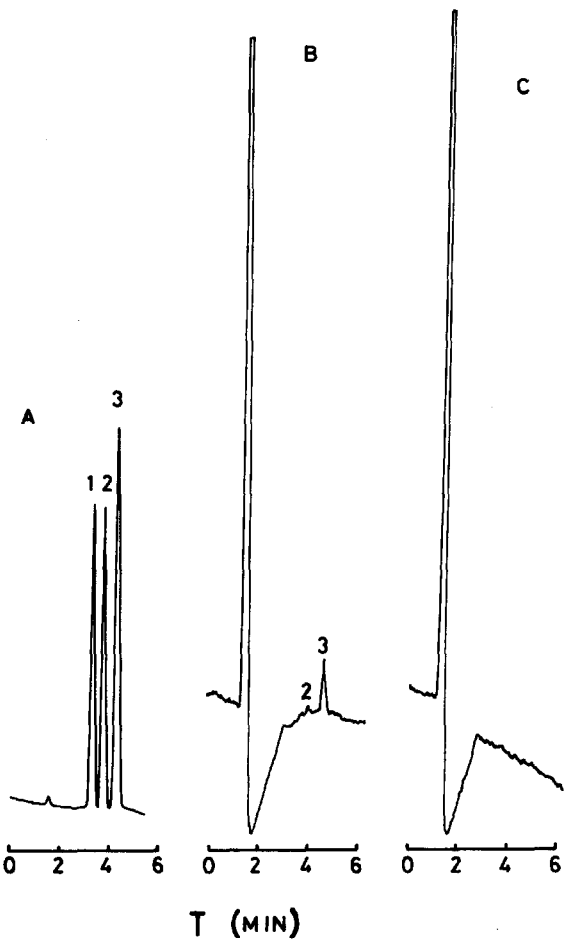


Fig. 2. A, Chromatogram of a standard solution containing 588 ppm chloride (1), 152 ppm nitrite (2) and 21 ppm nitrate (3). Mobile phase: 0.04 *M* $NaClO_4$, pH 5.5; flow-rate, 2 ml/min; sensitivity, 0.01 u.a.; atttenuation, 64; sample size, 100 μl; wavelength, 190 nm. B, Chromatogram of nitrite (2) and nitrate (3). Sensitivity: 0.0025 u.a. Wavelength: 209 nm. Other details as in Fig. 2A. C, Chromatogram of pure water under the same analytical conditions as in Fig. 2B.

studied. Fig. 1 shows that perchlorate ion does not absorb until close to 195 nm and only slightly at this wavelength. It also absorbs less than the methanesulphonate ion, especially at this short wavelength.

At the same time, we obtained the UV spectra of the different anions. In Table II the wavelengths of the absorption maxima for these anions are indicated. At 350 ppm, chloride has a definite maximum at 193 nm, but at 35 ppm the maximum does not fall within the measuring range. The same is true of the sulphate ion. However, with nitrite and nitrate ions a definite maximum is always obtained, though at slightly different wavelengths.

The effects of the eluent concentration and the flow-rate on the separation of the anions studied are shown in Tables III and IV. The results indicate that satisfactory retention times and resolution were obtained. As expected, lower eluent concentrations and flow-rates give better resolution. For most problems, a concentration of sodium perchlorate of 0.04 *M* and a flow-rate of 2 ml/min gives sufficient resolution with a short retention time.

The methanesulphonate eluent gives better resolution than perchlorate eluent, but we must point out that in order to obtain a reasonable retention time with the former, its concentration must be of the order of 0.2 *M*. This is due to the fact that we utilized a high capacity anion-exchange column which quickly became damaged at high mobile phase concentrations.

Some chromatograms are presented in Fig. 2. Fig. 2A shows the good resolution obtained, although it is not optimized for sensitivity because the wavelength used is the best only for the chloride ion. Fig. 2B is a chromatogram of 0.02 ng nitrite ion, a level which we take as the detection limit. Fig. 2C shows a chromatogram of pure water under the same analytical conditions as in Fig. 2B.

If a variable wavelength UV detector is used the optimum wavelength for each ion can be adopted. With this technique we obtained the limits of detection for the anions studied (Table V). The relative standard deviation was 0.5% for 0.9 ppm nitrite and $n = 20$.

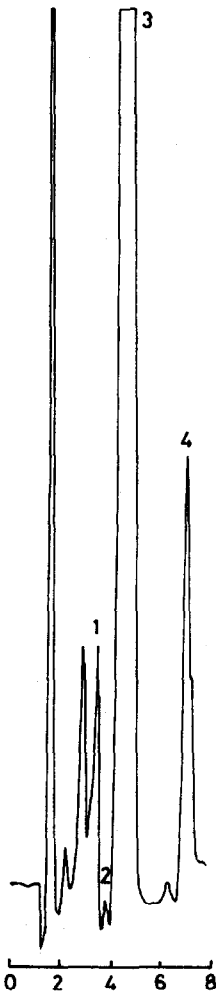
The analysis of nitrite in river-water gave no problems even in the presence of high levels of chloride or nitrate (Fig. 3). This is also true for the analysis of foodstuffs. Fig. 4 shows a typical chromatogram of a sample of an aqueous extract from cooked ham. The concentration obtained in the aqueous extract correspond to 1330 mg Cl^-, 53 mg NO_2^- and 17 mg NO_3^- per kg ham.

TABLE V

LIMITS OF DETECTION

Sample volume = 100 µl.

Anion	*λ (nm)*	*Detection limit*	
		ng	*ppb*
Chloride	190	0.3	3
Nitrite	209	0.02	0.2
Nitrate	200	0.1	1
Sulphate	190	1000	10,000



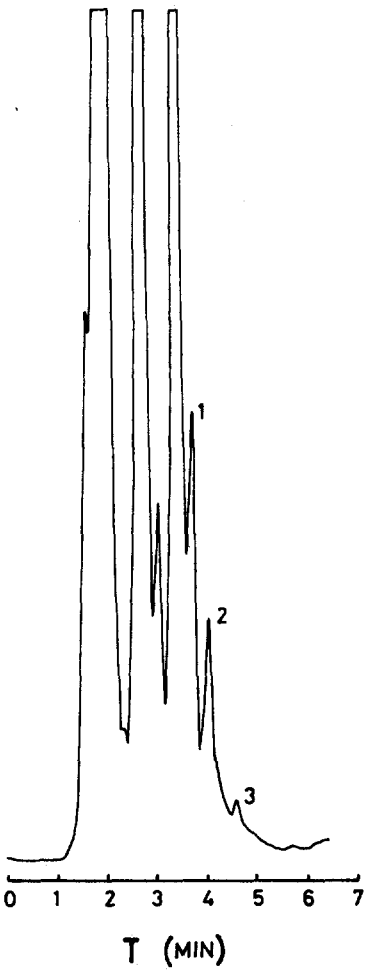


Fig. 3. Chromatogram of water from the Llobregat river (Barcelona). Peaks: 1 = chloride, ≈350 ppm; 2 = nitrite ≈20 ppb; 3 = nitrate, ≈50 ppm; 4 = sulphate, ≈ 0.5%. Attenuation: 1024. Other conditions as in Fig. 2B.

Fig. 4. Chromatogram of a sample of an aqueous extract from cooked ham. Peaks: 1 = chloride, ≈40 ppm; 2 = nitrite, ≈1.6 ppm; 3 = nitrate, ≈0.5 ppm. Sensitivity: 0.02 u.a. Other conditions as in Fig. 2B.

CONCLUSIONS

The use of sodium perchlorate as an eluent in conjunction with a high capacity anion-exchange column and a conventional liquid chromatograph with an UV detector permits, without preconcentration, a hundred-fold increase in the sensitivity for nitrite ion compared with existing methods. In addition, the sensitivity for chloride and nitrate ion is of the same order of magnitude as the best found to date. The method is suitable for the determination of nitrite and nitrate in water and meat samples.

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