

Note

Determination of some anions separated by thin-layer chromatography

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Procedures for determining cations have received great attention in analytical chemistry. Reported methods include atomic absorption or emission spectroscopy, polarography and others.

On the other hand, the situation is different where the determination of small amounts of anions is concerned. There exist various polarographic methods or methods using ion-selective electrodes, but these fail when the anions are to be determined in mixtures with various organic compounds. In such a case only separation methods, especially those based on chromatographic principles, may be of help.

Chromatographic separation of anions has received great attention as evidenced by a review by Brinkman *et al.*¹, which covers the papers published to 1972, and by further papers²⁻⁴. Similar problems were solved by electrophoresis^{5,6} and by isotachophoresis⁷ and even by gel chromatography on Sephadex⁸. All these papers discuss only the separation and identification of anions, not their determination. Only a few papers dealing with phosphates also cover their determination⁹⁻¹¹. The work reported here was aimed at solving the problem of determining nitrates in various organic materials (*e.g.*, molasses) where the hitherto known methods failed owing to the presence of interfering organic compounds and to low concentration of nitrates.

A recent paper reported the determination of nitrates by gas chromatography¹², in which aqueous nitrate ion is converted into nitrobenzene by reaction with benzene in the presence of a catalyst. The nitrobenzene is then quantitated by gas chromatography. This method would be adequate for our purposes, although it determines only nitrates and no other oxidizing anions.

We used thin-layer chromatography (TLC) to separate the nitrates and also performed the separation of some other oxidizing anions.

The determination was done by using diphenylamine, which reacts with these anions to give blue stains on the chromatograms. The stains may be evaluated densitometrically.

EXPERIMENTAL

The anions were separated on commercial plates of the Silufol 254 type using two solvent systems, *n*-propanol-conc. ammonia (2:1) and *n*-butanol-pyridine-water (5:3:3).

The anions were detected using several agents: (a) diphenylamine (0.2% soln. in conc. sulphuric acid); (b) 1% aqueous soln. of KI in 1.0 M HCl; (c) 1% aqueous soln. of FeCl₃; (d) aqueous 2 M AgNO₃ soln. with several drops of conc. ammonia added; (e) aqueous soln. of 2,6-dichlorophenolindophenol (a freshly prepared saturated soln.).

The model compounds were potassium salts, except the NO₃⁻, SeO₃²⁻, and SeO₄²⁻ anions (sodium salts), the Mo₇O₂₄⁶⁻ and VO₃⁻ anions (ammonium salts) and the ClO₄⁻ anion (Mg²⁺ salt) because of the low solubilities of the corresponding potassium salts.

In addition to TLC the separations were done by paper electrophoresis under following conditions: (a) 3M NH₄OH for 1.5 h at 220 V, paper Whatman 1 (6 V/cm); (b) 1 M CH₃CO₂H for 1.5 h at 220 V, paper Whatman 1 (6 V/cm).

The *R_F* values, the mobilities observed on electrophoresis, and the stain colors are listed in Table I.

TABLE I

THE *R_F* VALUES, MOBILITIES, AND STAIN-COLOR INTENSITIES OF ANIONS

S₁ = *n*-propanol-conc. ammonia (2:1). S₂ = *n*-butanol-pyridine-water (5:3:3). S₃ = 3 M NH₄OH. S₄ = 1 M CH₃CO₂H. D₁ = diphenylamine; D₂ = KI; D₃ = FeCl₃; D₄ = AgNO₃; D₅ = 2,6-dichlorophenolindophenol. X = tailing. Key to the detection: b = blue; sl = slight; y = yellow; g = green; bl = black; w = white; r = red; br = brown; rs = rose; v = violet; l = light.

Anion	<i>R_F</i>		<i>U</i> ^a		Detection				
	S ₁	S ₂	S ₃	S ₄	D ₁	D ₂	D ₃	D ₄	D ₅
NO ₂ ⁻	0.43	0.23	20.8	0.0	b	v	y	—	b
NO ₃ ⁻	0.44	0.19	22.4	0.0	b	slv	—	—	rs
ClO ⁻	0.29	0.25	10.3	0.0	b	v	—	bl	rs
ClO ₃ ⁻	0.46	0.43	20.9	3.2	b	—	—	—	rs
ClO ₄ ⁻	0.50	0.72	3.7	0.0	—	—	—	w	rs
IO ₄ ⁻	0.26	0.28	11.3	7.7	b	v	b	w	—
BrO ₃ ⁻	0.61	X	22.1	2.9	b	v	—	w	slrs
CrO ₄ ²⁻	0.12	X	22.0	13.4	b	v	y	yrbl	—
Cr ₂ O ₇ ²⁻	0.17	0.40	23.0	13.1	b	v	—	ybr	—
[Fe(CN) ₆] ³⁻	0.14	0.20	25.4	16.2	b	v	bg	y	yg
[Fe(CN) ₆] ⁴⁻	0.00	0.14	21.4	16.2	—	—	b	w	—
S ₂ O ₈ ²⁻	0.46	X	23.3	5.1	b	v	—	br	rs
S ₂ O ₅ ²⁻	0.00	0.11	19.2	4.5	—	—	—	w	—
MnO ₄ ⁻	0.00	0.00	0.0	0.0	b	b	—	br	—
Mo ₇ O ₂₄ ⁶⁻	0.20	0.13	20.5	2.9	slb	slb	ly	w	—
WO ₄ ²⁻	0.07	0.06	15.2	1.3	b**	slv	—	ly	—
SeO ₃ ²⁻	0.13	0.15	14.6	0.0	slb	v	ly	w	rs
SeO ₄ ²⁻	0.08	0.12	—	—	slb	v	ly	w	rs
VO ₃ ⁻	0.00	0.00	13.4	8.0	b	y	y	y	rs

^a Mobility *U* (cm² s⁻¹ V⁻¹) × 10⁻⁵.

** After a prolonged period.

Determination

A sample containing 1–5 μg of anion was applied to a Silufol 254 plate. After the chromatogram was developed, preferably in *n*-propanol-ammonia, it was allowed to dry, and then sprinkled with a 0.2% soln. of diphenylamine in conc. sulphuric acid.

The blue colour obtained was evaluated by means of a ERI-10 densitometer (Carl Zeiss Jena, Jena, G.D.R.) with a yellow filter (max. 550 nm) and a slit of 18 mm.

Because the colour intensity varies with time, it was necessary to carry out the densitometric measurements within 2–4 min after the sprinkling with diphenylamine (*cf.* Fig. 1). The chromatogram was cut into strips, each strip being sprinkled with the detection agent immediately prior to the measurement.

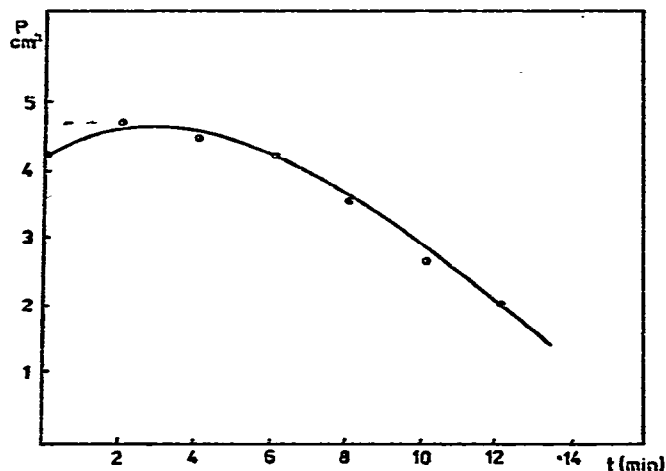


Fig. 1. The time dependence of intensity of the color stain formed by reaction of an oxidizing anion with diphenylamine (Silufol 254 plates).

The chromatogram was always fitted with a standard scale showing, preferably, a concentration of 1, 3, and 5 μg of the anion concerned. It is necessary to allow for a certain error in the results, in accordance with the time dependence of the colour intensity. In an interval of 2–4 min following the sprinkling the error corresponds to *ca.* 10%. As in most cases low concentrations of anions are involved the error does not appear considerable. The detection limit for readily reacting anions (such as NO_3^-) is 0.1 μg .

For the determination of NO_3^- and $[\text{Fe}(\text{CN})_6]^{3-}$ in molasses with the contents 1.0 and 0.1%, respectively, the standard deviation of the method was found to be $\sigma = \pm 6\%$ and $\sigma = \pm 8\%$, respectively.

RESULTS AND DISCUSSION

The method described can be used for determining the anions listed when present in concentrations down to hundredths of one per cent in various organic mixtures. The method is applicable to all anions that give a blue colour with diphenylamine. The stability of the colour could be improved by working with plates on a polyester substrate, as the aluminium substrate results in a reaction of aluminium with the sulphuric acid. The relationship of extinction to the concentration of anions shows a straight-line dependence in the range 0–5 μg .

The method has proved applicable to determining the concentration of anions

passing from fertilizers into soil and various plants and from these into further products. The method could also be used to follow the residual concentration in various oxidation processes run with nitric acid in chemical industry. Unfortunately, the separation of nitrates and nitrites is poorer than with other chromatographic methods.

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