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ELECTROPHORESIS AND THIN-LAYER CHROMATOGRAPHY OF ORGANIC BASE POLYTHIONATES

QUALITATIVE ANALYSIS

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SUMMARY

Methods of qualitative analysis of organic base polythionates are described.

Paper electrophoresis in the 0.05 *M* triethylammonium bicarbonate buffer with a pH of 7.5 permits the analysis of polythionates of organic and inorganic bases. In this case, large amounts of colloidal sulphur and halogens do not interfere with the determination of higher polythionic acids. However, we failed to separate decylquinolinium tetrathionate by electrophoresis.

Thin-layer chromatography on a layer of Kieselgel G + 10% of Dowex 50 W-X8 in the K^+ form + 2% of potassium acetate makes it possible to analyse any polythionates, independent of the solubility and structure of the initial base, and the results of the separation of organic and inorganic polythionates agree well.

INTRODUCTION

Methods for the qualitative analysis of organic base polythionates and reaction mixtures by paper electrophoresis and thin-layer chromatography are described in this paper.

ELECTROPHORESIS

The study of the reactions involved in the formation of organic base tetra- and pentathionates during acidification of thiosulphate is greatly hindered by the absence of physico-chemical methods of analysis.

Current methods of analysing potassium or sodium polythionates[1,2] when applied to the separation of organic polythionates give unsatisfactory results.

The purpose of the present paper is to develop a method of analysis of organic base polythionates[3-5] and reaction mixtures.

Separation was carried out on FN-II paper with a "Labor" Model OE-202 vertical electrophoresis apparatus.

The following aqueous solutions were investigated as buffers: 0.05 *M* ammonium carbonate brought to pH 9.0 with ammonia; 0.05 *M* ammonium bicarbonate solution; 0.05 *M* sodium sulphate solution and 0.05 *M* triethylammonium bicarbonate solution, pH 7.5. The best separation was obtained by using the 0.05 *M* triethylammonium bicarbonate buffer.

Sodium sulphide and thiosulphate; potassium trithionate, potassium diethylnonylammonium and trioctylammonium tetrathionates; potassium and sodium pentathionates; potassium hexathionate; triethylnonylammonium chloride; decylquinolinium bromide; triethylbutylammonium iodide; trioctylammonium, diethylnonylammonium and triisoamylammonium polythionates were employed as markers and materials for investigation.

The material studied and markers were applied to the starting line in the form of 0.1 *M* solutions in methanol or water in a quantity varying from 1.0 μl to 15 μl in 2 cm length strips with 1 cm intervals; this constituted from $0.1 \cdot 10^{-6}$ mole to $1.5 \cdot 10^{-6}$ mole of material. The maximum sensitivity of detection of the polythionates was about $0.5 \cdot 10^{-8}$ mole or, depending on the molecular weight of the polythionate investigated, from 10 to 2 μg.

TABLE I

DISTANCES OF POLYTHIONIC ACID, HALOGEN, THIOSULPHATE AND SULPHIDE IONS FROM THE STARTING LINE AT DIFFERENT PERIODS OF TIME

Time	*Ion*									
	Cl^-	Br^-	I^-	$S_2O_3^{2-}$	S^{2-}	$S_3O_6^{2-}$	$S_4O_6^{2-}$	$S_5O_6^{2-}$	$S_6O_6^{2-}$	$S_7O_6^{2-}$
1 h	203	203	197	200	202	193	165	147	136	113
1 h 30 min	332	330	325	320	318	292	251	232	208	191
1 h 45 min	372[a]	370[a]	370[a]	360	368	318	290	270	256	246

[a] Spots are located at the bottom of the sheet.

At different periods of time the ionic mobility of the polythionic acids and halogen ions, together with thiosulphate and sulphide was studied at an electrical field intensity equal to 20 V/cm. Distances covered in mm by the given ions from the starting line are reported in Table I.

The increase in temperature and the degree to which the paper is soaked with buffer solution has a decided influence on the ionic mobility, a different distance being covered by the same material on the same sheet as a result of some irregularity of spraying and heterogeneity of the paper, but the relative ionic mobility is in good accordance (Table II). The mobility of the $S_4O_6^{2-}$ ion was taken as a unit for calculation.

After drying, the electropherograms were examined under BUV-15 bactericide lamps with a UVS-1 light filter, and the spots of the polythionates were marked beginning from tetrathionic acid and higher, and then the electropherograms were developed in a 0.1 *M* ammonia solution of silver nitrate; in this case S^{2-}, $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$ and higher polythionates produced brown spots, and the $S_5O_6^{2-}$ was seen in the form of a black-and-brown spot. Among the halogens only the I^- ion is immediately discerned in the form of a yellow spot, but after a short exposure to an

TABLE II

RELATIVE IONIC MOBILITIES

Colloidal sulphur, present in some solutions, remained on the start.

Time	*Ion*									
	Cl^-	Br^-	I^-	$S_2O_3^{2-}$	S^{2-}	$S_3O_6^{2-}$	$S_4O_6^{2-}$	$S_5O_6^{2-}$	$S_6O_6^{2-}$	$S_7O_6^{2-}$
1 h	1.23	1.23	1.19	1.21	1.22	1.17	1.00	0.98	0.82	0.68
1 h 30 min	1.32	1.31	1.29	1.27	1.27	1.16	1.00	0.92	0.83	0.76
1 h 45 min	1.28	1.28	1.28	1.24	1.25	1.10	1.00	0.93	0.88	0.85

intense light the Cl^- and Br^- ions become dark violet and the I^- ion is seen as a violet spot. The electropherograms not developed with silver solution can be preserved for several months and the intensity of spots does not change, probably due to the decomposition of the polythionates to thiosulphate and sulphur, but those developed with silver solution become completely black in the course of a few days.

As is evident from Tables I and II, the ionic mobility of the Cl^-, Br^-, I^- ions practically coincides with that of a thiosulphuric acid ion, but halogen ions are located at a greater distance from the polythionic acid ions and were not used as markers in the subsequent operations. The sulphite ion which may be present in organic polythionates is not observed in either ultraviolet light or upon spraying with the ammoniacal silver nitrate solution.

The formation of polythionic acids during acidification of thiosulphate in the presence of organic bases occurs very rapidly, in practice, taking from a fraction of a second to 1–2 min; at the same time if 20 ml of dilute hydrochloric acid (1:4) are added to a solution of 7.4 g of sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$) in 25 ml of water the main components of the reaction mixture are thiosulphuric and trithionic acids.

TABLE III

EXPERIMENTAL RESULTS OF ELECTROPHORESIS OF A REACTION MIXTURE

Time	*Point No.*	*Ions*					
		$S_7O_6^{2-}$	$S_6O_6^{2-}$	$S_5O_6^{2-}$	$S_4O_6^{2-}$	$S_3O_6^{2-}$	$S_2O_3^{2-}$
0 min	1						m[a]
1 min	2				+[b]	m	m
4 min	3				+	m	m
11 min	4			+	+	m	m
20 min	5			+	+	m	m
26 min	6			+	+	m	m
32 min	7		+	+	+	m	m
39 min	8		+	+	+	m	m
43 min	9		+	+	+	m	m
67 min	10		+	+	+	m	m
80 min	11		+	+	+	m	m
48 h	12	+	+	+	+	m	m
72 h	13	+	+	+	+	m	m
96 h	14	+	+	+	+	m	m

[a] m is the main reaction product.

[b] + = present in the reaction mixture.

Polythionic acids are formed within several minutes, the heptathionic acid only after 48 h.

Table III shows the experimental results obtained by electrophoresis of the reaction mixture.

The zero point was taken immediately after mixing the solution, the supernatant liquid being used for the analysis. A small amount of colloidal sulphur did not hinder the determination as it remained on the start. The quantity of the substance applied to the start constituted about $1 \cdot 10^{-5}$ mole, the quantity of thiosulphuric and trithionic acids in solution being essentially greater than necessary for their good separation and, moreover, the solution contained a high concentration of chloride ions.

THIN-LAYER CHROMATOGRAPHY

SEILER[6] and SEILER AND ERLENMEYER[7] developed methods of analysis for halogen and sulphur compounds on silica gel in the form of their potassium and sodium salts and used solvent systems containing dioxane and ammonia. POLLARD *et al.*[2], in paper chromatography studies, noted that several spots are formed during polythionate analysis in the presence of several cations.

The distinct separation of anions is only possible in the presence of potassium or sodium ions, and in this case the sorbent or solvent system must not interact with them chemically.

Silica gel containing ion-exchange resin in the K^+ form and fixed with gypsum was used for the anion analysis.

Chromatographic separation was carried out on glass plates (100 × 200 and 200 × 200 mm) with a Kieselgel G sorbent layer + 10% of the ion-exchange resin Dowex 50 W-X8 (200–400 mesh) in the K^+ form + 2% of potassium acetate.

A 300 μ thick sorbent layer from an aqueous suspension was applied with an automatic applicator. 60 × 60 mm glass plates with the sorbent layer coated by hand were used for selecting the layer composition and the solvent system. On standard plates the chromatograms were developed in combined C- and BN-cells; on the non-standard ones—in the glass tanks with ground caps.

The use of a solvent system containing dioxan led to the formation of additional spots, as dioxane is rather quickly oxidised, and peroxides contributed to the formation of polythionates; for example, pure sodium thiosulphate gave several spots on the chromatogram.

The presence of ammonia in the solvent system led to the formation of twice as many spots, which suggests the displacement of anions in the form of ammonium salts and initial base salt. Cation-exchange resin in the K^+ form and potassium acetate, for the formation of a large excess of potassium ions during separation, were introduced into the sorbent layer in order to transform the organic base polythionates completely into potassium salts.

Acetate ions do not affect the separation and are not coloured during development.

In order to obtain an even front which is not discoloured with admixtures eluted from the layer by solvents, the plates were first washed up to their upper edge with the following solvent system: *tert.*-butanol–acetone–water (19:75:6).

Chromatograms were developed in a *tert.*-butanol–acetone–water (18:70:12) system. The solvent used for a 200 × 200 mm plate was 15 ml, for a 100 × 200 mm one it was 9 ml; separation time was 30–40 min. 0.1 *M* solutions of the substances being investigated, in water or methanol, were applied as 2–3 mm diameter spots in a quantity up to 1 μl–$1 \cdot 10^{-7}$ mole. Maximum detection sensitivity of anions constituted $1 \cdot 10^{-9}$ mole or from 1.0 to 0.2 μg.

If more than $0.5 \cdot 10^{-6}$ mole of the substance was applied the separation was unsatisfactory. The starting line (2 cm from the edge of the plate) and a start–front distance equal to 10 cm were chosen experimentally and gave the most distinct separation.

After drying the chromatograms were developed by spraying them with a 0.1 *M* ammonia solution of silver nitrate; they were also sprayed with an 0.1% solution of Bromcresol Green in 50% ethanol in order to determine SO_3^{2-} and $S_2O_8^{2-}$ ions. The luminophore L-36 was introduced into the sorbent layer for detecting $S_4O_6^{2-}$, $S_5O_6^{2-}$, $S_6O_6^{2-}$, $S_7O_6^{2-}$ ions without spraying. In this case polythionic acid ions are observed in the form of dark spots against a light background in ultraviolet light.

During the development by a silver salt solution S^{2-}, $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$, $S_5O_6^{2-}$, $S_6O_6^{2-}$, $S_7O_6^{2-}$ gave brown colourations; after exposure to the light halogen ions are seen as violet spots; SO_3^{2-} and $S_2O_8^{2-}$ ions produced yellow spots on a blue background after spraying with a solution of Bromcresol Green. The R_F values of the various anions are listed in Table IV.

TABLE IV

R_F VALUES OF DIFFERENT ANIONS

Start–front distance (cm)	*R_F value*												
	SO_3^{2-}	$S_2O_8^{2-}$	S^{2-}	$S_2O_3^{2-}$	Cl^-	Br^-	I^-	$S_3O_6^{2-}$	$S_4O_6^{2-}$	$S_5O_6^{2-}$	$S_6O_6^{2-}$	$S_7O_6^{2-}$	S_8
5.0	—	—	0	0	0.23	0.52	0.69	0.17	0.38	0.56	0.85	—	1.0
7.5	—	—	0	0	0.30	0.54	0.69	0.19	0.45	0.57	0.72	0.82	1.0
10.0	0.18	0.30	0	0	0.30	0.55	0.72	0.23	0.48	0.63	0.73	0.81	1.0
14.5	—	—	0	0	0.26	0.54	0.77	0.19	0.50	0.63	0.75	0.81	1.0

Considerable diffusion of the higher polythionic acid spots took place with long start–front distances and detection sensitivity decreased sharply.

From Table IV it can be seen that S^{2-} and $S_2O_3^{2-}$ ions remain on the starting line, the Cl^- ion occupies an intermediate position between $S_3O_6^{2-}$ and $S_4O_6^{2-}$, the Br^- ion is between $S_4O_6^{2-}$ and $S_5O_6^{2-}$, the I^- ion practically coincides with the $S_6O_6^{2-}$ ion, colloidal sulphur present in polythionates forms a very diffused spot and is located mainly on the front line.

A high concentration of halogen, and especially I^- and Br^- ions, hinders the analysis of reaction mixtures owing to a complete overlapping of the polythionic acid spots.

Organic base polythionates and halides completely exchanged organic ions for K^+ ions on the ion-exchange resin. In this case even such slightly water-soluble polythionates as trioctylammonium and triisoamylammonium were completely converted into potassium salts.

THE COMPARISON OF METHODS OF ANALYSIS

A comparison of the methods of analysing organic base polythionates by paper electrophoresis and thin-layer chromatography is given in Table V.

It can be seen from Table V that the quantity of substance used for chromatography is 10 times less and the maximum detection sensitivity is 5 times higher as compared with that for electrophoresis. Time of analysis is 2–3 times shorter. But electrophoresis permits the separation of reaction mixtures with a high content of halides, thiosulphate and sulphide from polythionic acids, and even the measurement of their relative contents. In this case the large amount of colloidal sulphur which separates at the moment of reaction does not hinder the determination at all.

TABLE V

COMPARISON OF METHODS OF ANALYSIS

	Electrophoresis	*Thin-layer chromatography*
Time of analysis	1.5–2 h	30–40 min
Quantity of applied substance (moles)	$0.1 \cdot 10^{-6}$–$1.5 \cdot 10^{-6}$	$0.1 \cdot 10^{-7}$–$1 \cdot 10^{-7}$
Maximum detection sensitivity (moles)	$0.5 \cdot 10^{-8}$	$1 \cdot 10^{-9}$
Arrangement of anions:		
Start	colloidal sulphur	$S_2O_3^{2-}$, S^{2-}
	$S_7O_6^{2-}$	$S_3O_6^{2-}$
	$S_6O_6^{2-}$	Cl^-
	$S_5O_6^{2-}$	$S_4O_6^{2-}$
	$S_4O_6^{2-}$	Br^-
	$S_3O_6^{2-}$	$S_5O_6^{2-}$
	$S_2O_3^{2-}$, S^{2-}	$S_6O_6^{2-}$, I^-
	Cl^-, Br^-, I^-	$S_7O_6^{2-}$
Front	—	colloidal sulphur

However, it was not possible to achieve a distinct separation during analysis of N-alkylquinolinium polythionates by electrophoresis; for example, pure decylquinolinium tetrathionate gave a smeared spot which stretched from the start to the $S_4O_6^{2-}$ ion level.

Regardless of the nature of the organic base and the length and number of alkyl chains, organic base polythionates easily exchange an organic ion for a potassium ion, and ion-exchange adsorption–partition thin-layer chromatography makes it possible to determine their qualitative and quantitative composition.

The simultaneous application of these two methods of analysing organic and inorganic base polythionates provides the possibility of estimating the composition of the substances obtained more precisely and controlling the degree of purity, stability, and speed of formation of polythionic acids during thiosulphate acidification.

0.1 *M* sodium tetrathionate solution used as a marker contained about 90% of the original substance after a month while the rest was penta- and trithionic acids; the solution containing sodium thiosulphate, trithionate and tetrathionate gradually changed into a solution containing mainly sodium tri- and tetrathionate. Organic base polythionate solutions in methanol did not display visible changes in composition even after a year's storage.

CONCLUSIONS

1. A method of qualitative analysis of organic base polythionates by paper electrophoresis is described.

2. It was shown that polythionic acids produced during the acidification of thiosulphate are formed more slowly than in the presence of organic bases.

3. A technique for the analysis of organic and inorganic base polythionates and reaction mixtures by thin-layer chromatography has been worked out.

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