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Separation of arsenite and arsenate ions by paper chromatography

Study of a methanol-ammonia-water solvent system

The possibility of separating elements in different valency states is of importance in many fields of scientific research. A simple sensitive method for this purpose is paper chromatography. The possibility of separating arsenic in the two valency states is of importance in toxicological chemistry because of the different toxicity of arsenic-(III) and (V) compounds. There are not many publications available dealing with the chromatographic separation of the two valency states of arsenic.

The main difficulty in ascending paper chromatography of the two valency states of arsenic is the interference of some metals, especially Sb(III, V), Sn(II, IV), Fe, Cu and Zn. Also the R_F values for separating As(III) and As(V) were not sufficient. This fact was proved by a systematic study using mixtures of acetone, methyl ethyl ketone or methanol, and hydrochloric or nitric acid as solvents¹.

POLLARD, MCOMIE AND BANISTER² separated As(III) from As(V) with R_F 0.55 and 0.75, respectively, using ascending paper chromatography in the solvent mixture conc.HCl-water-ether-methanol (4:15:50:30, by vol.). Carefully standardized conditions were necessary in order to obtain completely reproducible R_F values. By the ascending method with butanol saturated with 1 N HCl, LEDERER³ separated successfully As(III) and As(V) from Ge(IV) with R_F 0.84, 0.52 and 0.26, respectively. The mixtures with butanol as solvent required several hours for development. OVERBY, BOCCHIERI AND FREDRICKSON⁴ described a paper chromatographic method for qualitative and quantitative estimation of arsenate-⁷⁴As, arsenite-⁷⁴As, arsenilic-⁷⁴As acid and related organic arsenicals. The two solvent systems: acetonitrile-HNO₃-water (78:2:20, by vol.; with an up-flow for 2 h or down-flow for 3 h) and isopropyl alcohol-water (7:3, by vol.; using an overnight or 8-h descending development) are described to give good separations of sodium arsenite from sodium arsenate.

The method depending on the use of an alkaline solvent mixture (ethanol-pyridine-ammonia (0.88)-water (60:20:4:16, by vol.)) was described by ELBEIH AND ABOU-ELNAGA⁵ for the analysis of anions based on paper chromatography. The R_F values for the separation of arsenate from arsenite were not sufficient (R_F AsO₄³⁻ = 0.03 and AsO₃³⁻ = 0.12). YAMAGUCHI⁶ recommended the solvent system methanol-1 N NH₄OH (3:1) for the effective separation of arsenite from arsenate (with R_F 0.54 and 0.00, respectively).

Since a speedy separation is desirable in toxicological analysis, attention has been focussed upon fast-moving solvents. Therefore we studied in detail the chromatographic behaviour and possibility of separating the two valency states of arsenic using the methanol-ammonia-water solvent system.

Experimental

Paper chromatography. Solutions of arsenites and arsenates were prepared from analytical grade reagents of arsenic trioxide or arsenic pentoxide or sodium hydrogen arsenate, respectively. Before being used the paper strips were thoroughly washed with 1 N HNO₃ and water (redistilled with a quartz apparatus) until the water eluant was not acidic. 20 µg (20 µl) of As were placed on a purified strip of Whatman No. 1

paper (23×15 cm) and developed by the ascending method in a solvent mixture of methanol–ammonia–water. Before being used, methanol was dried by means of metallic sodium and distilled in the usual way. The length of the run was 18 cm from the starting line. After development the chromatograms were removed and the solvent was allowed to evaporate in a current of air at room temperature.

Detection. An ammoniacal silver nitrate solution ($2 N$ AgNO_3 in 10 % ammonia) was used to visualize the spots. Silver arsenite gives a visible yellow spot and silver arsenate a pale brown one. The detection was more sensitive when U.V. light was used. The U.V. light source was an Analytic Quartzlamp, Hanau, $\lambda = 360$ nm. The spots were then blue. They are distinctly visible for about 15 min, then the paper darkens. For the other metals tested a 0.1 % solution of 4-(2-thiazolylazo)resorcinol (TAR) in ethanol (96 %) was used. The paper strips were exposed to ammonia vapour after being sprayed with this reagent.

Results

Ionic mobility of sodium and ammonium arsenites as well as arsenates was studied as a function of the ammonia concentration at a constant methanol–water ratio of 8:2 by volume (see Fig. 1). The concentration of ammonia in methanol was in the range $2 \times 10^{-4} N$ to $8 \times 10^{-1} N$ NH_4OH . The R_F values were calculated to the second organic front. The maximum mobility of sodium arsenite, as well as arsenate, was found at a concentration of $4 \times 10^{-4} N$ NH_4OH and was practically constant up to a concentration of $8 \times 10^{-4} N$. With a further increase in concentration of ammonium hydroxide, the R_F values of arsenite as well as arsenate decreased. On the other hand, the mobility of ammonium arsenate and arsenite under the same conditions was somewhat different. The constant R_F values of ammonium arsenite were observed from $2 \times 10^{-4} N$ to $6 \times 10^{-3} N$ NH_4OH and were slightly decreased with a further increase in concentration of ammonium hydroxide. The decrease of R_F values of ammonium arsenate was considerable, as can be seen in Fig. 1. From $2 \times 10^{-1} N$ NH_4OH the R_F values of ammonium arsenite and arsenate were practically

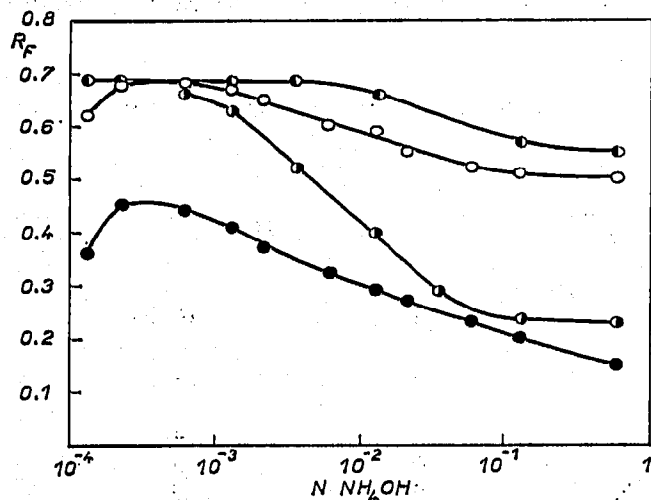


Fig. 1. Relationship between the R_F values of arsenite and arsenate ions and ammonium concentration in the methanol–ammonium hydroxide solvent system (8:2, v/v). (○) Na_3AsO_3 ; (●) $(\text{NH}_4)_3\text{AsO}_3$; (●) Na_3AsO_4 ; (●) $(\text{NH}_4)_3\text{AsO}_4$.

constant. We observed that the spots with the increasing concentration of ammonium hydroxide were more concentrated.

The effect of the methanol to water ratio on the mobility and separation of sodium arsenate and arsenite was studied at a constant concentration of $2 \times 10^{-1} N$ NH_4OH (Fig. 2). The R_F values increased with the hydration of methanol and this effect was greater in the case of arsenate than in that of arsenite. At the methanol-water ratio of 1:2:1 the curves intersect at an R_F value of 0.65.

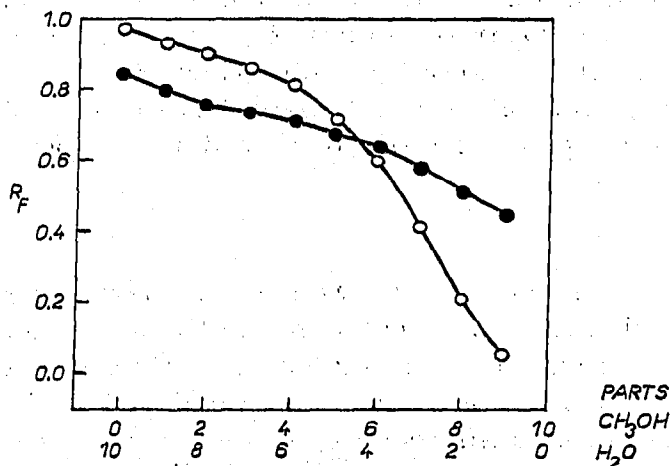


Fig. 2. Relationship between the R_F values of arsenite and arsenate ions in the methanol-ammonia-water solvent system and a different ratio of methanol-water at the constant concentration of $2 \times 10^{-1} N$ NH_4OH . (○) Na_3AsO_4 ; (●) Na_3AsO_3 .

The R_F values of Na_3AsO_3 , $(NH_4)_3AsO_3$, Na_2HAsO_4 , Na_3AsO_4 and $(NH_4)_3AsO_4$ after development in the solvent system methanol-1 N NH_4OH (8:2) are given in Table I. It was found that sodium nitrate tends to retard the movement of arsenate ions and this effect was greater at a lower ammonium hydroxide concentration of the solvent system. Addition of sodium nitrate (0.4 %) to the above-mentioned solvent system results in elongated spots and very low R_F values of arsenate ions (0.08), while the R_F values of arsenite ions did not change. The successful separation and identifi-

TABLE I

THE $R_F \times 100$ VALUES OF ARSENITE AND ARSENATE IN AMMONIACAL METHANOL, ETHANOL, AND ACETONE SYSTEMS

Compound	Solvent systems			
	MeOH-NH ₃ 1 N (8:2)	MeOH-NH ₃ 1 N- 0.4% NaNO ₃ (8:2)	(Me) ₂ CO-NH ₃ 1 N (8:2)	EtOH-NH ₃ 1 N (8:2)
Na_3AsO_3	51	50	12	31
$(NH_4)_3AsO_3$	57			
Na_2HAsO_4	22	8	0	3
Na_3AsO_4	21	8	0	3
$(NH_4)_3AsO_4$	25	8	0	3
Time of run for 18 cm	140 min, 20°	140 min, 20°	140 min, 17.2°	345 min, 17.2°

cation of Na_3AsO_3 and Na_3AsO_4 after developing in the solvent system methanol-1 *N* NH_4OH (8:2) are shown on a photograph of a chromatogram. 10 μg of As (10 μl) of each compound was applied (Fig. 3). On separation of a mixture of arsenite and arsenate ions a decreased R_F value of arsenite was found.



Fig. 3. Chromatogram of a separation of arsenite and arsenate ions. Solvent system: methanol-1 *N* NH_4OH (8:2, v/v), ascending. Length of run: 18 cm; 140 min at 20°. Detection: 2 *N* AgNO_3 in 10% ammonium hydroxide. As(III) gives a yellow spot, As(V) a brown spot; under U.V. light, blue absorbency. 1 = Na_3AsO_3 ; 2 = Na_3AsO_4 ; 3 = mixture of Na_3AsO_4 and Na_3AsO_3 .

Sodium arsenate and arsenite were also developed in the ammoniacal ethanol and acetone solvent systems. These solvents were unsuitable for separating As(III) from As(V) because of very low R_F values of arsenite as well as of the arsenate remaining on the starting line (Table I); therefore many elements, especially iron, copper and antimony, interfere in the separation.

Conclusions

From our experiments it is evident that the best solvent system for the separation of arsenite and arsenate ions and for obtaining well developed spots of both is

TABLE II

THE $R_F \times 100$ VALUES OF SOME METALS OBTAINED IN THE METHANOL-AMMONIUM HYDROXIDE SYSTEM (8:2, v/v)

Sequence of separation: (Ba or Cu)—(Cr(VI) or As(V) or Zn)—(Mo(VI) or W(VI)—As(III)—Ag.

Metal (20 μg)	$R_F \times 100$	Metal (20 μg)	$R_F \times 100$	Metal (20 μg)	$R_F \times 100$
Ag	60	Al, Au, Bi	0	Ca	} strong tailing from the starting point
As(III)	52	Ce(III, IV)	0	Cd	
As(V)	20	Co(II)	0	Mg	
Ba	7	Cr(III)	0	Sr	
Cr(VI)	17	Fe(III)	0	Tl(I)	
Cu	10	Hg, Mn, Pb	0	V(V)	} 68 (the tailing spot)
Mo(VI)	30	Sb(III, V)	0	Ni	
W(VI)	30	Sn(II, IV)	0		
Zn	21	U(VI)	0		

methanol-1 *N* NH₄OH in the ratio 8:2 by volume. The *R_F* values of arsenite and arsenate ions were highly reproducible and were not greatly influenced by the basicity of the methanol but they were greatly affected by the methanol-water ratio.

This solvent system produced two fronts; the first one, the wet front, with *R_F* 0.65 and a second, organic, dry front. All *R_F* values were calculated to the second organic front. The first wet front did not change with the basicity of the solvent system (from 2×10^{-3} *N* - 2×10^{-1} *N* NH₄OH) and only slight changes were observed on increasing the aqueous part. With a methanol-water ratio of 5:5 the wet front had the *R_F* value 0.75. The wet front did not interfere with the *R_F* values of arsenic.

The advantage of the methanol-1 *N* NH₄OH (8:2) solvent system is the separation of arsenic from 13 elements which remain at the starting point. A further seven elements (barium, copper, chromium(VI), molybdenum, silver, tungsten and zinc) are separated from arsenic(III) according to their different *R_F* values. The *R_F* values found for some other metals are given in Table II. Calcium, cadmium, magnesium, nickel, strontium, thallium(I) and vanadium(V) interfere with their tailing spots. Chloride ions were detected in this solvent system with *R_F* 0.63 and nitrate ions with *R_F* 0.74. The time required for chromatographic analysis of arsenic does not exceed 2.5 h.

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- 1 V. MIKEŠUKOVÁ AND K. KÁCL, *XXIst Intern. Congr. Pure and Appl. Chem., Prague, Sept. 4-10, 1967, Toxicol. Chem., Abstr.* T-68.
- 2 F. H. POLLARD, J. F. W. MCOMIE AND A. J. BANISTER, *Chem. Ind. (London)*, (1955) 1598.
- 3 M. LEDERER, *Anal. Chim. Acta*, 11 (1954) 132.
- 4 L. R. OVERBY, S. F. BOCCIERI AND R. L. FREDRICKSON, *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 17.
- 5 J. J. M. ELBEIH AND M. A. ABOU-ELNAGA, *Anal. Chim. Acta*, 23 (1960) 30.
- 6 K. YAMAGUCHI, *J. Pharm. Soc. Japan*, 73 (1953) 1285.

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