

## CHROMATOGRAPHIC SEPARATION OF PHOSPHITE AND PHOSPHATE IONS

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## ABSTRACT

Separation of P (III) and P (V) is investigated. The influence of solvents and pH on the migration were studied. Their migrational behaviour is explained on the basis of solubilization and complexation. The degrees of separations were indicated by  $R_f$  values and the ions were quantitatively assayed photometrically.

## INTRODUCTION

LITERATURE on chromatographic separation of P (III) and P (V) is sparse, most of the work in the past having been confined to polymeric phosphates<sup>1-6</sup>. The separation of phosphite and phosphate though unusual, assume importance in agriculture. Phosphorus is an essential ingredient of fertilizers, and plants absorb both phosphite and phosphate. While the soluble phosphate is beneficial, phosphite can considerably inhibit oxygen uptake and hamper nitrogen fixation by the roots in certain cases<sup>7</sup>. This would therefore necessitate detection and estimation of small quantities of phosphite in the presence of major quantities of phosphate in fertilizers. Paper chromatography is an ideal tool for this.

The principle adopted for the separation is based on the stereochemical differences of the phosphorus ions in the two states of oxidation. Phosphite and phosphate have both tetrahedral configurations by  $sp^3$  hybridization<sup>8,9</sup>. However, while the tetrahedron of the phosphite is distorted, that of phosphate has a symmetrical distribution of valences. The distortion of a stereo-configuration usually imparts a greater covalent nature to phosphite and so it may be expected to complex with less polar solvents better than the phosphate, which is also borne out by our experiments; phosphite and phosphate migrate with  $R_f$  1.0 in water. But phosphate slows down in less polar solvents like alcohols. However the gap in migration was not large enough to result in good separation but the  $\Delta R_f$  could be enlarged by using mixed solvent systems. The present work makes use of the Rutter's technique<sup>10</sup> of circular paper chromatography and offers suitable explanations for the differential migrations of the two valences.

## EXPERIMENTAL

Analytical grade  $NaH_2PO_3$ ,  $Na_2HPO_3$ ,  $NaH_2PO_4$  and  $Na_2HPO_4$  were dissolved in water to prepare 1.0 M solutions. The pH of these solutions was found to be 4.5, 8, 5 and 9 respectively. 1  $\mu$ l. of each solution would contain 30.970  $\mu$ g. of phosphorus. Phosphite and phosphate solutions

were mixed in equal quantities as and when required. The solutions were spotted using an 'Agla' micro-meter syringe (least count  $\pm 0.2 \mu$ l). Chromatographic band of phosphite can be developed with 1% ferric chloride solution followed by 1% sulpho salicylic acid solution<sup>1</sup>. The technique is sensitive to 0.5  $\gamma$  of phosphorus. Phosphate ion gives a blue coloured band when sprayed successively with 5% ammonium molybdate solution containing 35% nitric acid and 0.25% stannous chloride dissolved in 20% HCl<sup>11</sup>. The reagent is sensitive to 1.25  $\gamma$  of phosphorus. The choice of eluents was guided by their polarity, viscosity and solubilization<sup>12</sup>.

## RESULTS AND DISCUSSION

In the case of *monobasic* salts of phosphite and phosphate the separation was poor as shown by the smallness of the  $\Delta R_f$ . In highly polar solvents like water, and aqueous ammonia or methyl amine (25% W/V), both ions moved with equal facility to the solvent boundary, the phosphite migrated as a well defined band, while the phosphate showed some diffusion. With less polar solvents like butanol and others, the migration was negligible for both the ions. At any rate no satisfactory separation was possible with these eluents (Table I).

The results obtained with *dibasic* salts of phosphite and phosphate were also more or less the same as with the monobasic salts. However, we were able to satisfactorily separate the two ions using methanol-ammonia, methanol-methylamine, dioxane-ammonia, and dioxane-methylamine.

Certain interesting points are revealed by the above study. Though both the ions migrate with the same  $R_f$  in water, the phosphate showed diffusion while the phosphite migrated as a well-defined band. The same was the case with strongly ammoniated water as eluent. This difference in quality of migration may be attributed to the relative strengths of the two acids. The  $pK_2$  of phosphoric acid is 7.21 and that of phosphorous acid is 6.15; in the second stage of dissociation  $H_3PO_3$  is about six times stronger than water and

about ten times stronger than  $\text{H}_3\text{PO}_4$ . We are of the opinion that the greater acidity goes to improve the quality of migration in the case of phosphite. The dibasic phosphate is likely to hydrolyse reversibly as  $\text{Na}_2\text{HPO}_4 \rightleftharpoons \text{NaH}_2\text{PO}_4$ , while  $\text{Na}_2\text{HPO}_3$  does not. Since phosphate changes composition during migration its course is marked by diffusion. That  $\text{Na}_2\text{HPO}_4$  undergoes reversible hydrolysis during migration was proved by spotting the chromatogram directly with  $\text{NaH}_2\text{PO}_4$ , when the same type of diffusion was observed, vide item 1 of Table I.

The situation was improved by partially replacing water with organic solvents, such as methanol or dioxane. With 50 : 50 dioxane-water, phosphate diffused with an  $R_f$  0.86 while the phosphite migrated with an  $R_f$  0.71. The diffusion band of phosphate overlapped that of the phosphite, making the separation ineffective. With 50 : 50 dioxane-ammonia as eluent the diffusion of phosphate was considerably retarded, whereas the migration of phosphite showed an  $R_f$  of 0.52. The differential migration in dioxane-ammonia eluent can be explained as follows :  $\text{Na}_2\text{HPO}_4$  may form

TABLE I  
Chromatographic behaviour of phosphite and phosphate mixture

No.	Eluent	Dielectric constant	$R_f$ values of		$R_f$ values of		$\Delta R_f$
			$\text{NaH}_2\text{PO}_3$	$\text{Na}_2\text{H}_2\text{PO}_4$	$\text{Na}_2\text{HPO}_3$	$\text{Na}_2\text{HPO}_4$	
1.	Water	78.54	1.0	1.0 <sup>d</sup>	1.0	1.0 <sup>d</sup>	
2.	Methanol	32.63	0.75	0.80 <sup>d</sup>	0.52 <sup>d</sup>	0.50 <sup>d</sup>	
3.	Ethanol	24.3	0.35 <sup>d</sup>	0.54 <sup>d</sup>	0.24 <sup>d</sup>	0.00 <sup>d</sup>	
4.	<i>n</i> -Propanol	20.1	0.00	0.45 <sup>d</sup>	0.00	0.00	
5.	<i>n</i> -Butanol	17.1	0.00	0.35 <sup>d</sup>	0.00	0.00	
6.	<i>n</i> -Amyl alcohol	13.9	0.00	0.25 <sup>d</sup>	0.00	0.00	
7.	Pyridine	12.3	0.00	0.62 <sup>d</sup>	0.00	0.00	
8.	Dioxane	2.21	0.00	0.00	0.00	0.00	
9.	Ammonia**		1.00	1.00 <sup>d</sup>	1.0	1.0 <sup>d</sup>	
10.	Methylamine <sup>w</sup>		1.0	1.0 <sup>d</sup>	1.0	1.0 <sup>d</sup>	
11.	MeOH : HCl (99:1)		0.92	0.95			
12.	<i>n</i> -PrOH : HCl (99:1)		0.80	0.75 <sup>d</sup>			
13.	<i>n</i> -AmOH : HCl (99:1)		0.79 <sup>d</sup>	0.67 <sup>d</sup>			
14.	MeOH : Water (90:10)		0.78	0.80 <sup>d</sup>			
15.	EtOH : Water (90:10)		0.49	0.61 <sup>d</sup>			
16.	EtOH : <i>n</i> -BuOH : HCl (49:50:1)		0.80	0.87 <sup>d</sup>			
17.	<i>n</i> -PrOH : <i>n</i> -BuOH : HCl (49:50:1)		0.80	0.77 <sup>d</sup>			
18.	Dioxane : Water (50:50)				0.71	0.86 <sup>d</sup>	
19.	Dioxane : Ammonia (40:60)*				0.80	0.57 <sup>d</sup>	0.23
20.	Dioxane : Ammonia (50:50)*				0.52	0.29 <sup>d</sup>	0.23
21.	MeOH : Ammonia (90:10)*				0.48	0.26 <sup>d</sup>	0.22
22.	MeOH : Ammonia (70:30)*				0.61	0.42 <sup>d</sup>	0.19
23.	Dioxane : Methylamine (50:50)*				0.50	0.35 <sup>d</sup>	0.15
24.	MeOH : Methylamine (90:10)*				0.46	0.27 <sup>d</sup>	0.19
25.	MeOH : Methylamine (80:20)*				0.49	0.34 <sup>d</sup>	0.15

\*\* 25% in water (W/V).

<sup>d</sup>—Diffuse.

\* Good separation.

TABLE II  
Quantitative estimation of phosphite and phosphate after separation  
Quantity spotted: 92.91  $\mu\text{g}$  each of phosphite and phosphate

No.	Eluent	Phosphite as P		Phosphate as P		P (V)
		Estimated*	Error	Estimated*	Error	P (III) + P (V) $\times 100 (\%)^{**}$
		( $\mu\text{g}$ )	( $\mu\text{g}$ )	( $\mu\text{g}$ )	( $\mu\text{g}$ )	
1.	Dioxane: Ammonia † (50:50)	90.1	-2.81	93.5	+0.59	50.93
2.	Dioxane: Methylamine † (50:50)	90.6	-2.31	91.7	-1.21	50.30
3.	Methanol: Ammonia					
	(a) 90:10	94.2	+1.29	90.9	-2.01	49.11
	(b) 70:30	90.3	-2.61	95.1	+2.19	51.30
4.	Methanol: Methylamine					
	(a) 90:10	93.8	+0.89	90.5	-2.41	49.10
	(b) 80:20	89.9	-3.01	95.7	+2.79	51.56

\* Average of four estimations. † 25% in water (W/V).

\*\* Redox ratio: 50% = No reduction or oxidation. More or less than 50% indicates oxidation or reduction respectively.

with ammonia a microcosmic salt-like complex<sup>13,14</sup>, with the result the diffusion of phosphate is retarded. Since the phosphite has no tendency to form a similar complex, it migrates with a different R<sub>f</sub> value. Other eluents like methanol-ammonia, methanol-methylamine, dioxane-methylamine work very similar to that of dioxane-ammonia system. With higher percentages of ammonia or methylamine, the influence of water comes into play and phosphate diffusion catches up with the phosphite band. However, good separations were possible with methanol and dioxane in admixture with ammonia (25% aq) or methylamine (25% aq).

#### Estimation of phosphite and phosphate after separation

The positions of phosphite and phosphate ions on the chromatogram were located with the help of pilot experiments wherever good separations were achieved. The bands were then cut out, shredded and the ions were extracted with 1% HCl. The phosphate ions in the extract was estimated photometrically by the modified heteropoly molybdenum blue method described by Lueck and Boltz<sup>15</sup>. The phosphite in the extract was oxidised to phosphate by digesting with fuming nitric acid and the resulting phosphate was estimated photometrically as before. The results are presented in Table II.

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