NOTES

Studies on the Thin Layer Chromatography of Condensed Phosphates

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It is said that the chemistry of phosphates has been developed through the improvement of the techniques of the separation and the analysis of phosphates. The separation and the analysis of phosphates with various degrees of condensation have been tried by many methods.[1-3] Among them, the thin layer chromatography is one of the most effective, because a relatively short time of running and a small amount of samples are required and the separation is fairly good.

Thin layers are usually made by fixing silica gels or alumina powders with gypsum, but gypsum cannot be used for substances such as phosphates which react with it. Accordingly, the present authors have improved the method and the result will be presented.

Experimental

Silica gel (Wakogel B-O, 10 g) or cellulose powder (Toyo-Roshi >300 mesh, 10 g) was suspended in a 0.05% aqueous sodium polyacrylate (30 m*l*) or a 1% cellulose acetate solution in *N,N*-dimethylformamide (35 m*l*). The thin layers with the following combinations were prepared as usual; silica gel-sodium polyacrylate, silica gel-cellulose acetate, cellulose powder-sodium polyacrylate, and cellulose powder-cellulose acetate. As samples of phosphates with various degrees of condensation, sodium salts of orthophosphate, pyrophosphate, tripolyphosphate, trimetaphosphate, and tetrametaphosphate were used.

On the thin layer plates, 1 μl of 0.2% aqueous solution of each phosphate was spotted with a microsyringe, and developed in a closed chamber at room temperature. When the solvent was developed up to 10 cm from the spotting point, the plates were taken out and dried in air. Then the phosphates were hydrolyzed with an aqueous nitric acid solution (1 : 1) and colored with ammonium molybdate and stannous chloride.[4,5] The developing solvents are shown in Table 1.

TABLE 1. DEVELOPING SOLVENTS

Acidic solvent		Basic solvent	
No.	Main solvent	No.	Main solvent
A1	M	B1	M-*n*B
A2	E	B2	E-*n*P
A3	*i*P	B3	E-*i*B
A4	*i*P	B4	*n*P
A5	*i*P-*t*B	B5	*n*P-*i*B
A6	*i*P-D	B6	Py
A7	*i*P-EG		
A9	*t*B		
A11	D		
A12	A		
A0	A: 175 m*l*, AA: 55 m*l*, W: 45 m*l*		
B0[6]	M: 150 m*l*, Am: 45 m*l*, TC: 10 m*l*, W: 90 m*l*		

1) M: methanol, E: ethanol, *n*P: *n*-propanol, *i*P: isopropanol, *n*B: *n*-butanol, *i*B: isobutanol, *t*B: *t*-butanol, D: dioxane, Py: pyridine, EG: ethylene glycol monomethyl ether, A: acetone, AA: acetic acid, Am: ammonia water (28%), TC: aqueous trichloroacetic acid (10%), W: water

2) A1—A12 and B1—B6: for detail see S. Ohashi, Mukikagaku-Zensho IV-6 "Phosphorus," p. 42.

Results and Discussion

The R_f values and the time of running are shown in Tables 2, 3, 4, and 5.

When acidic solvents are used, the order of R_f values is

orthophosphate > polyphosphate > metaphosphate

1) S. Ohashi, Mukikagaku-Zensho IV-6 "Phosphorus," Maruzen, Tokyo (1965), p. 37.

2) J. R. Van Wazer, "Phosphorus and Its Compounds," Vol. I, Interscience Publishers, Inc., New York (1964), p. 441.

3) T. Kubo and E. Niki, *Bunseki Kagaku*, **11**, 1085 (1962).

4) E. Karl-Kroupa, *Anal. Chem.*, **28**, 1091 (1956).

5) D. N. Bernhart and W. B. Chess, *ibid.*, **31**, 1026 (1959).

6) H. Seiler, *Helv. Chim. Acta*, **44**, 1753 (1961).

TABLE 2. R_f VALUES
(Cellulose powder-Cellulose acetate)

No.	O	P	Tr	TrM	TeM	Time (min)
A0	.72	.10	.04	.10	.00	18
A1	.97	.90	.74	—	.30	17
A2	.99	.90	.80	—	.74	28
A3	.88	.60	.37	.31	.13	45
A4	.67	.21	.15	.08	.02	22
A5	.80	.55	.43	.37	.24	56
A6	.88	.52	.36	.22	.17	36
A7	1.00	.94	.91	—	.79	63
A9	.83	.46	.17	.10	.10	90
A12	.15	.03	.02	—	.37	29
B0	.00	.00	.00	.93	.90	15
B1	.50	.00	.00	.78	.68	19
B2	.49	.20	.26	.73	.63	40
B3	.45	.26	.33	.60	.56	45
B4	.29	.00	.00	.42	.30	35
B5	.44	.27	.33	.59	.52	55
B6	.15	.00	.02	.71	.53	30

O: orthophosphate, P: pyrophosphate,
Tr: tripolyphosphate, TrM: trimetaphosphate,
TeM: tetrametaphosphate

TABLE 3. R_f VALUES
(Cellulose powder-Sodium polyacrylate)

No.	O	P	Tr	TrM	TeM	Time (min)
A0	.63	.07	.02	.09	.00	14
A1	.87	.77	.62	—	.32	15
A2	.93	.83	.84	—	.32	26
A3	1.00	.48	.15	—	.00	27
A4	.62	.24	.12	.12	.05	24
A5	.83	.58	.50	.43	.25	57
A6	.73	.45	.31	.24	.14	34
A7	.94	.86	.82	—	.71	77
A9	.80	.45	.20	.15	.03	71
A11	.91	.88	.79	.17	.46	30
A12	.19	.07	.09	—	.34	17
B0	.57	.13	.60	.91	.90	17
B1	.42	.06	.08	.80	.74	21
B2	.49	.20	.26	.73	.63	40
B3	.45	.26	.33	.60	.56	45
B4	.32	.15	.13	—	.48	35
B5	.44	.27	.33	.59	.52	55
B6	.15	.00	.02	.71	.53	30

TABLE 4. R_f VALUES
(Silica gel-Cellulose acetate)

No.	O	P	Tr	TrM	TeM	Time (min)
A1	.28	.05	.20	—	.03	75
A4	.36	.06	.02	.00	.00	390
A9	.44	.05	.03	.06	.02	390
B0	.62	.13	.24	.77	.68	75
B2	.25	.05	.09	.56	.43	180
B3	.14	.02	.05	.26	.20	110

TABLE 5. R_f VALUES
(Silica gel-Sodium polyacrylate)

No.	O	P	Tr	TrM	TeM	Time (min)
A5	.51	.00	.10	.55	.54	360
A7	.39	.29	.30	—	.54	360
B0	.62	.00	.05	.83	.69	62
B2	.20	.05	.05	.61	.48	150
B3	.29	.00	.00	.46	.44	175
B6	.13	.02	.01	.58	.18	120

and when basic solvents are used, the order of R_f values is

metaphosphate > orthophosphate > polyphosphate.

These orders agree with those in the paper chromatography.[3] It was proved that the thin layer chromatography by the method proposed in this report was very useful for the separation and the analysis of condensed phosphates. The cellulose acetate, used as the binder, gave almost the same results for all the solvents, but when the system of silica gel, sodium polyacrylate, and acidic solvent was used, it is apt to take much time of running and caused tailing of spots.

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