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CHROMATOGRAPHIC BEHAVIOUR AND STRUCTURAL UNITS OF CONDENSED PHOSPHATES

II. INFLUENCES OF DEVELOPING SOLVENTS

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SUMMARY

Influences of the components (acidic solvents: water, ammonia water, trichloroacetic acid, and acetic acid; basic solvents: water and ammonia water) and pH values of developing solvents on R_F values, the ratio of the thickness of the mobile phase and the stationary phase, and free energy necessary to transport the structural units of condensed phosphates from the stationary phase to the mobile phase were examined. They are greatly influenced by the water content and pH value. The influences of the other components are slight.

INTRODUCTION

We reported in a previous paper¹ that the relationships between R_M values and the structural units (the middle PO_4 group, the end PO_4 group, and the single [orthophosphate] PO_4 group) of condensed phosphates were obtained and that the ratio of the thickness of the mobile phase to the stationary phase and the free energy necessary to transport 1 mole of each structural unit from the stationary to the mobile phase can be calculated by the use of these relationships.

Herein, we rewrite these relationships

for the metaphosphate ion consisting of m phosphorus atoms

$$R_M (m\text{-meta}) = -\log (A_M/A_S) + m\Delta\mu_{\text{middle}}/2.3RT \quad (1)$$

for the pyrophosphate ion

$$R_M (\text{pyro}) = -\log (A_M/A_S) + 2\Delta\mu_{\text{end}}/2.3RT \quad (2)$$

for the orthophosphate ion

$$R_M (\text{ortho}) = -\log (A_M/A_S) + \Delta\mu_{\text{ortho}}/2.3RT \quad (3)$$

for the polyphosphate ion consisting of n phosphorus atoms

$$R_M (n\text{-poly}) = -\log (A_M/A_S) + 2\Delta\mu_{\text{end}}'/2.3RT + (n-2)\Delta\mu_{\text{middle}}'/2.3RT \quad (4)$$

where (A_M/A_S) is the ratio of the thickness of the mobile phase to the stationary phase, and $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, $\Delta\mu_{\text{ortho}}$, $\Delta\mu_{\text{middle}}'$ and $\Delta\mu_{\text{end}}'$ are the free energy neces-

sary to transport 1 mole of a middle group of metaphosphate, an end group of pyrophosphate, and the ortho group, middle and end groups of polyphosphate, respectively.

In general, developing solvents used in paper chromatography (PC) and thin-layer chromatography (TLC) of condensed phosphates are mixed solutions of some alcohols and water. They are roughly divided into acidic and basic solvents, and trichloroacetic acid, ammonia water, etc., are added to the mixed solutions. Acidic solvents are suitable for the separation of ortho-, pyro-, and polyphosphates and basic solvents are suitable for the separation of metaphosphates.

EBEL² described the influence of the water content in the acidic and basic solvents on R_F values and the influence of the addition of a small quantity of ammonia water to the acidic solvents on R_F values. THILO AND FELDMANN³ described those influences and the influence of pH values of the solvents on R_F values. In this paper, we studied the influences of the water content and the other components and pH values of the solvents on R_F values, A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$. We did not examine the influences of $\Delta\mu_{\text{middle}}'$ and $\Delta\mu_{\text{end}}'$ because there seem to be mutual interactions between the middle and end groups of the polyphosphates.

The following influences are examined in this paper. (a) acidic solvents: water content, quantity of ammonia water, quantity of trichloroacetic acid, quantity of

TABLE I

DEVELOPING SOLVENTS

Solvent	Ammonia water (28%, ml)	Trichloroacetic acid (g)	Acetic acid (glacial, ml)	Water content ^a (%)	Alcohol ^b	pH ^c
A1-30	0.25	5.0	1.0	30	iP	1.62
A1-40	0.25	5.0	1.0	40	iP	1.47
A1-50	0.25	5.0	1.0	50	iP	1.33
A2-0.50	0.50	5.0	1.0	40	iP	1.67
A2-0.75	0.75	5.0	1.0	40	iP	1.82
A2-1.00	1.00	5.0	1.0	40	iP	2.03
A2-1.25	1.25	5.0	1.0	40	iP	2.37
A2-1.50	1.50	5.0	1.0	40	iP	3.33
A3-1	0.25	1.0	1.0	40	iP	2.80
A3-3	0.25	3.0	1.0	40	iP	1.70
A4-2	0.25	5.0	2.0	40	iP	1.50
A4-3	0.25	5.0	3.0	40	iP	1.47
A5-0.20	0.20	2.0	1.0	40	iP	1.89
A5-1	1.0	2.0	1.0	40	iP	5.59
A5-2	2.0	2.0	1.0	40	iP	8.33
A5-4	4.0	2.0	1.0	40	iP	9.20
B1-30	1.0			30	iP-iB	—
B1-40	1.0			40	iP-iB	11.39
B1-50	1.0			50	iP-iB	—
B2-2	2.0			40	iP-iB	11.45
B2-4	4.0			40	iP-iB	11.86

^a Aqueous solutions (30, 40 or 50 ml) containing 3 components (acidic solvents) or 1 component (basic solvents) were diluted to 100 ml with alcohols.

^b iP = isopropyl alcohol; iP-iB = isopropyl-isobutyl alcohol (1:1).

^c pH = reading of the pH meter (glass electrode).

acetic acid; (b) basic solvents: water content, quantity of ammonia water; and (c) pH values of solvents.

EXPERIMENTAL

Developing solvents

The compositions of developing solvents are shown in Table I. pH values are also given.

Thin layers

In 60 ml of water were suspended 15 g of Avicel (FMC Corporation). Thin-layer plates were prepared as usual (250 m μ).

Samples of phosphates

A solution of mixed metaphosphates was prepared according to THILO AND SCHULKE⁴. Solutions of sodium salts of ortho-, pyro-, tripoly-, trimeta-, tetrameta-⁵, and hexameta-⁶ phosphates were used as standards.

Procedure

After the solutions of the phosphates had been spotted, the thin-layer plate was allowed to equilibrate with the vapour of the developing solvent for 2 h; the phosphates were then developed in a saturation chamber (ascending method). The temperature was maintained at 5° in an air bath (Coolnics, Komatsu-Yamato). When the solvent had travelled 10 cm from the application point of the phosphates, the plate was taken out and dried in air. The phosphates were then hydrolysed with an aqueous nitric acid solution (1:1) and visualised with ammonium molybdate and stannous chloride^{7,8}.

RESULTS AND DISCUSSION

R_F values obtained and A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ calculated are listed in Table II.

Acidic solvents

Water content (A1-30, A1-40, and A1-50). The plots of R_F value vs. water content, of R_M value vs. degree of condensation, and of A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ vs. water content are shown in Figs. 1, 2, and 3. In proportion as the water content increases, the R_F value increases by a great margin. A_M/A_S increases by a great margin, $\Delta\mu_{\text{ortho}}$ also increases, $\Delta\mu_{\text{middle}}$ slightly decreases and $\Delta\mu_{\text{end}}$ is almost constant. It is thought that the R_F value is greatly influenced by the water content and that this depends on the great increase of A_M/A_S .

Quantity of ammonia water (A1-40, A2-0.50, A2-0.75, A2-1.00, A2-1.25, and A2-1.50). The plots of R_F value vs. quantity of ammonia water, and of A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ vs. quantity of ammonia water are shown in Figs. 4 and 5. In proportion as the quantity of ammonia water increases, the R_F values of pyro- and metaphosphates slightly decrease, but the R_F value of orthophosphate is

TABLE II
 R_F VALUES OBTAINED AND A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, AND $\Delta\mu_{\text{ortho}}$ CALCULATED

Phosphates	A1-30	A1-40	A1-50	A2-0-50	A2-0-75	A2-1.00	A2-1.25	A2-1.50	A3-1	A3-3	A4-2
Ortho (O)	0.70	0.80	0.83	0.80	0.78	0.78	0.78	0.78	0.78	0.81	0.81
Pyro (Py)	0.38	0.56	0.73	0.54	0.47	0.43	0.38	0.36	0.55	0.61	0.61
Tripoly (TrP)	0.17	0.44	0.64	0.38	0.29	0.26	0.21	0.20	0.40	0.45	0.50
Trimeta (TrM)	0.12	0.36	0.65	0.30	0.22	0.19	0.17	0.18	0.34	0.37	0.38
Tetrameta (TrM)	0.06	0.20	0.54	0.16	0.12	0.09	0.09	0.09	0.19	0.24	0.21
Pentameta (PeM)	0.03	0.13	0.46	0.10	0.07	0.05	0.05	0.05	0.14	0.16	0.15
Hexameta (HexM)	0.01	0.07	0.38	0.06	0.03	0.02	0.02	0.02	0.07	0.08	0.08
Heptameta (HepM)		0.02	0.30	0.02	0.02				0.02 ^a	0.03 ^a	
Octameta (OctM)			0.22								
Nonameta (NonM)			0.15								
Decameta (DecM)			0.11								
A_M/A_S	1.26	3.03	5.81	2.45	2.52	2.54	2.04	2.28	3.10	3.90	3.75
$\Delta\mu_{\text{middle}}$	410	328	211	341	400	440	417	429	339	345	347
$\Delta\mu_{\text{end}}$	198	245	211	202	338	335	333	387	257	252	242
$\Delta\mu_{\text{ortho}}$ (cal/mole)	-345	-115	96	-271	-190	-185	-305	-244	-74	-50	-70

Phosphates	A4-3	A5-0-50	A5-1	A5-2	A5-4	BI-30	BI-40	BI-50	B2-2	B2-4
Ortho (O)	0.82	0.80	0.39	0.14	0.13	0.13	0.30	0.66	0.29	0.28
Pyro (Py)	0.60	0.54	0.17	0.04	0.04	0.05	0.20	0.47	0.15	0.15
Tripoly (TrP)	0.48	0.40	0.08	0.02	0.02	0.04	0.21	—	0.17	0.18
Trimeta (TrM)	0.38	0.37	0.21	0.25	0.26	0.23	0.59	0.86	0.58	0.57
Tetrameta (TrM)	0.23	0.21	0.11	0.13	0.13	0.11	0.48	0.80	0.44	0.45
Pentameta (PeM)	0.14	0.13	0.05	0.09	0.08	0.06	0.39	0.76	0.36	0.36
Hexameta (HexM)	0.08	0.07	0.02	0.04	0.04	0.03	0.32	0.71	0.28	0.29
Heptameta (HepM)			0.02	0.02 ^a	0.02 ^a		0.25	0.66	0.24	
Octameta (OctM)										
Nonameta (NonM)										
Decameta (DecM)										
A_M/A_S	4.14	4.24	3.61	2.18	2.67	2.65	4.04	13.20	3.70	4.10
$\Delta\mu_{\text{middle}}$	358	502	473	368	383	413	199	155	202	216
$\Delta\mu_{\text{end}}$	281	360	794	1120	1150	1084	769	747	842	870
$\Delta\mu_{\text{ortho}}$ (cal/mole)	-53	33	958	1490	1590	1592	1241	1060	1219	1302

^a Removed from the least squares calculation.

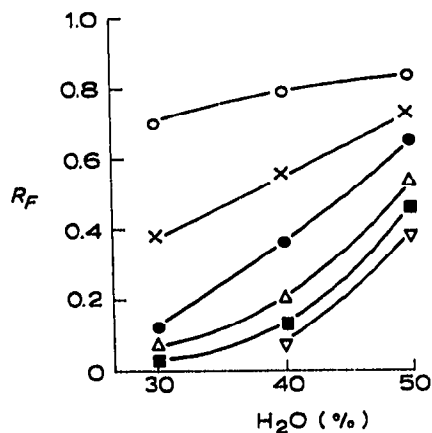


Fig. 1. Plots of R_F vs. water content. \circ , \circ ; \times , Py; \bullet , TrM; Δ , TtM; \blacksquare , PeM; ∇ , HexM.

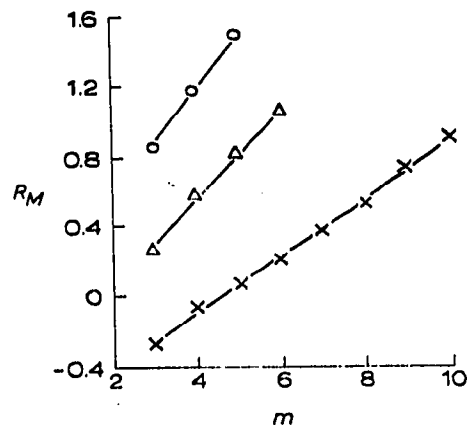


Fig. 2. Plots of R_M vs. degree of condensation (m). \circ , 30%; Δ , 40%; \times , 50%.

almost identical. A_M/A_S and $\Delta\mu_{ortho}$ are almost the same, but $\Delta\mu_{middle}$ and $\Delta\mu_{end}$ seem to increase slightly.

Quantity of trichloroacetic acid and acetic acid (*A3-1*, *A3-3*, and *A1-40*, and *A1-40*, *A4-2*, and *A4-3*). The plots of R_F value vs. quantity of trichloroacetic acid and acetic acid, of A_M/A_S , $\Delta\mu_{middle}$, $\Delta\mu_{end}$, and $\Delta\mu_{ortho}$ vs. quantity of trichloroacetic acid and acetic acid are shown in Figs. 6, 7, 8, and 9. Even if the quantity of trichloro-

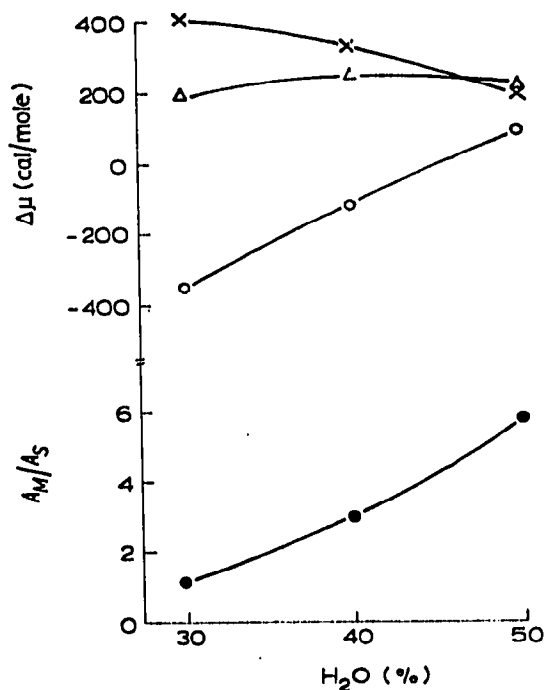


Fig. 3. Plots of A_M/A_S , $\Delta\mu_{middle}$, $\Delta\mu_{end}$, and $\Delta\mu_{ortho}$ vs. water content. \times , middle; Δ , end; \circ , ortho; \bullet , A_M/A_S .

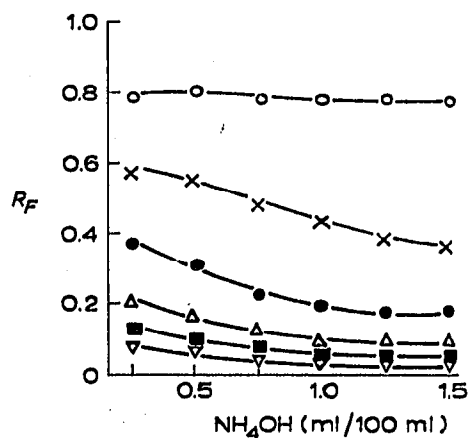


Fig. 4. Plots of R_F vs. quantity of ammonia water. ○, ○; ×, Py; ●, TrM; Δ, TtM; ■, PeM; ▽, HexM.

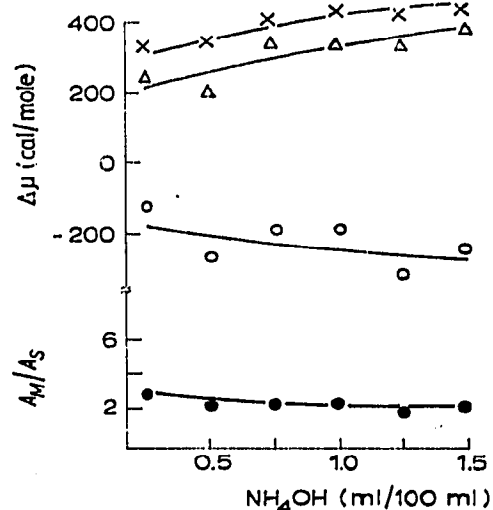


Fig. 5. Plots of A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ vs. quantity of ammonia water. ×, middle; Δ, end; ○, ortho; ●, A_M/A_S .

acetic acid and acetic acid is changed, R_F , A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ are almost constant.

Basic solvents (BI-30, BI-40, and BI-50)

Water content (BI-30, BI-40, and BI-50). The plots of R_F value vs. water content, of R_M value vs. degree of condensation, and of A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ vs. water content are shown in Figs. 10, 11, and 12. As in the case of the acidic

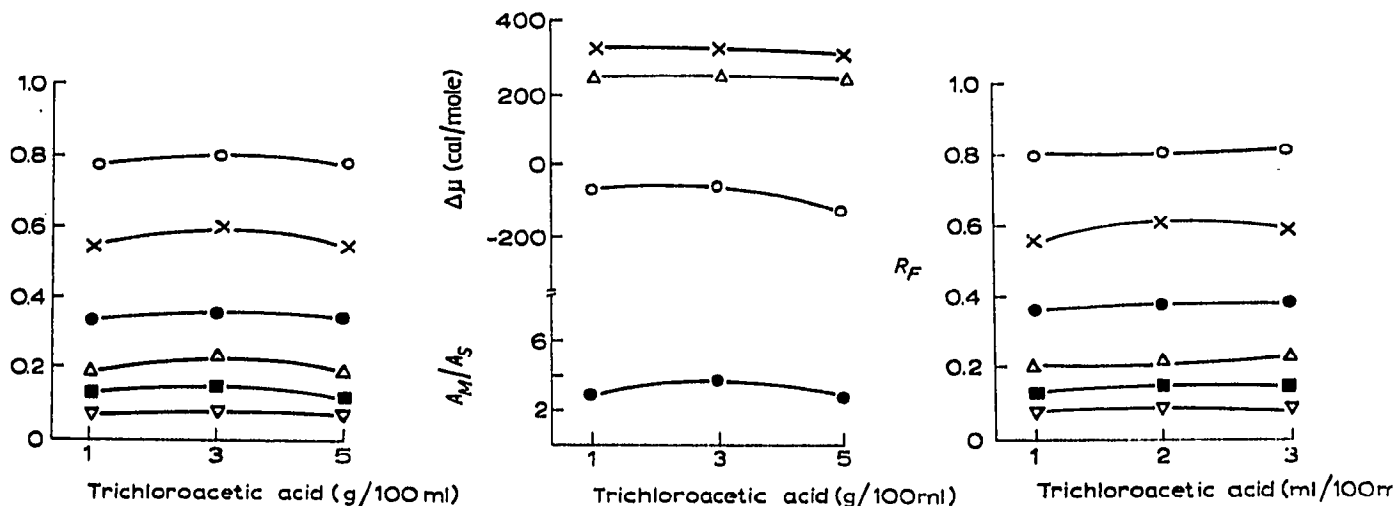


Fig. 6. Plots of R_F vs. quantity of trichloroacetic acid. ○, ○; ×, Py; ●, TrM; Δ, TtM; ■, PeM; ▽, HexM.

Fig. 7. Plots of A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ vs. quantity of trichloroacetic acid. ×, middle; Δ, end; ○, ortho; ●, A_M/A_S .

Fig. 8. Plots of R_F vs. quantity of acetic acid. ○, ○; ×, Py; ●, TrM; Δ, TtM; ■, PeM; ▽, HexM.

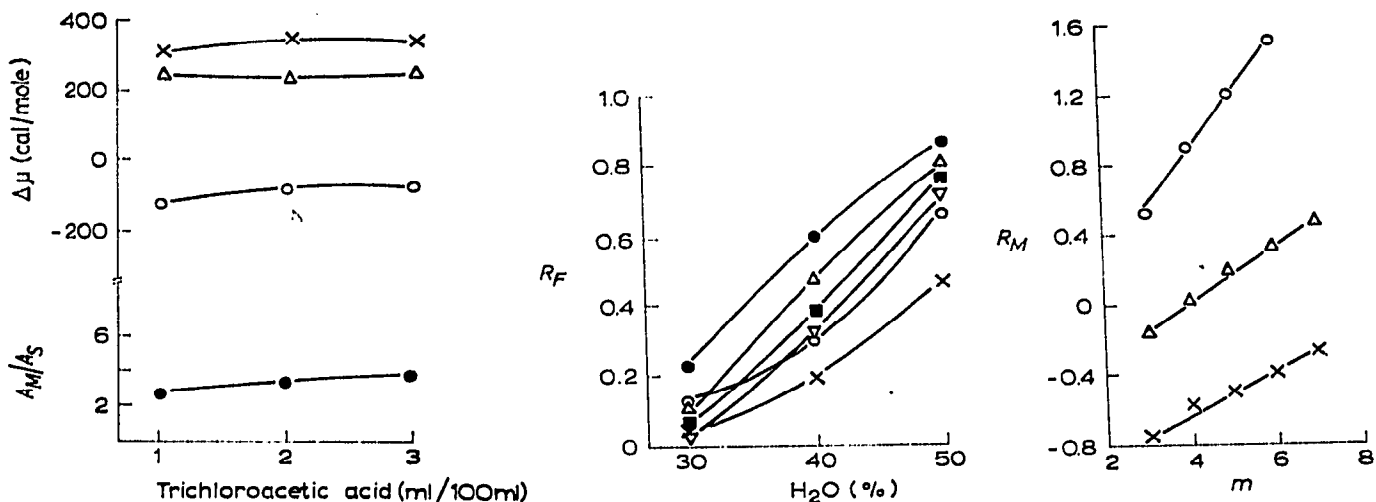


Fig. 9. Plots of A_M/A_S , $\Delta\mu_{middle}$, $\Delta\mu_{end}$, and $\Delta\mu_{ortho}$ vs. quantity of acetic acid. x, middle; Δ , end; \circ , ortho; \bullet , A_M/A_S .

Fig. 10. Plots of R_F vs. water content. \circ , \circ ; x, Py; \bullet , TrM; Δ , TtM; \blacksquare , PeM; ∇ , HexM.

Fig. 11. Plots of R_M vs. degree of condensation (m). \circ , 30%; Δ , 40%; x, 50%.

solvent, the R_F value increases by a great margin, in proportion to the increase of the water content. A_M/A_S increases by a great margin, but $\Delta\mu_{middle}$, $\Delta\mu_{end}$, and $\Delta\mu_{ortho}$ decrease slightly.

Quantity of ammonia water (B1-40, B2-2, and B2-4). The plots of R_F value vs. quantity of ammonia water, and of A_M/A_S , $\Delta\mu_{middle}$, $\Delta\mu_{end}$, and $\Delta\mu_{ortho}$ vs. quantity

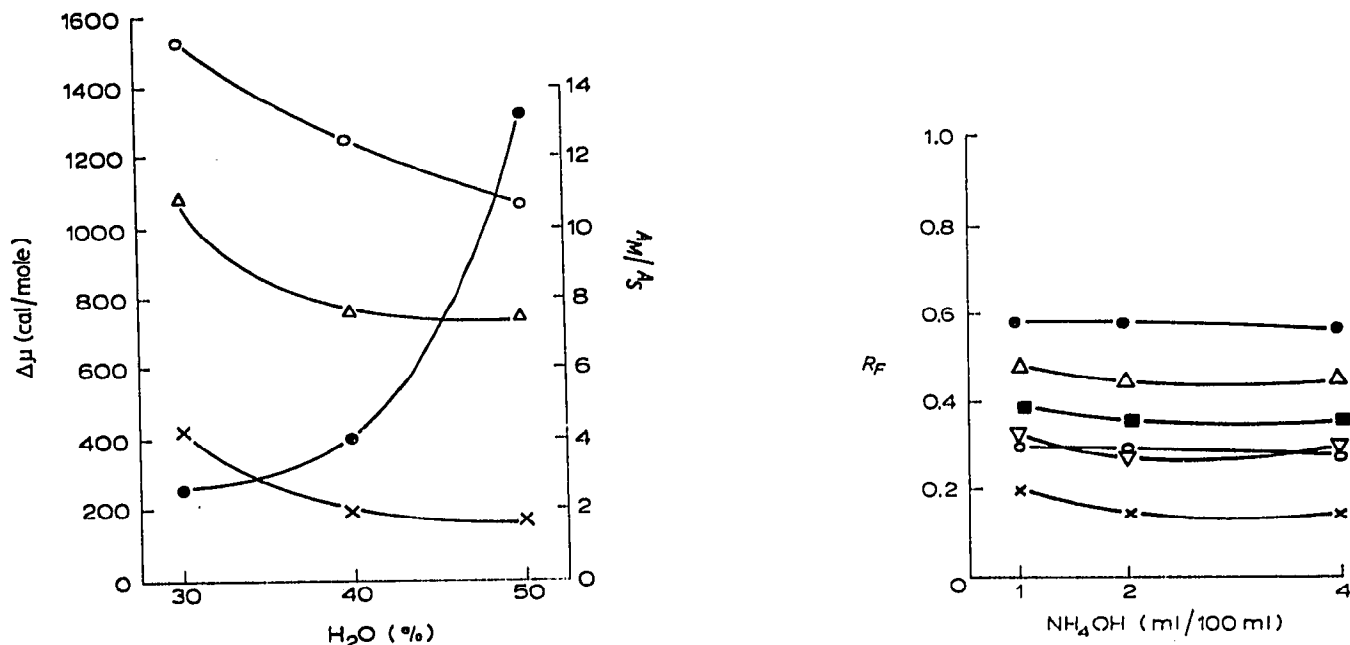


Fig. 12. Plots of A_M/A_S , $\Delta\mu_{middle}$, $\Delta\mu_{end}$, and $\Delta\mu_{ortho}$ vs. water content. x, middle; Δ , end; \circ , ortho; \bullet , A_M/A_S .

Fig. 13. Plots of R_F vs. quantity of ammonia water. \circ , \circ ; x, Py; \bullet , TrM; Δ , TtM; \blacksquare , PeM; ∇ , HexM.

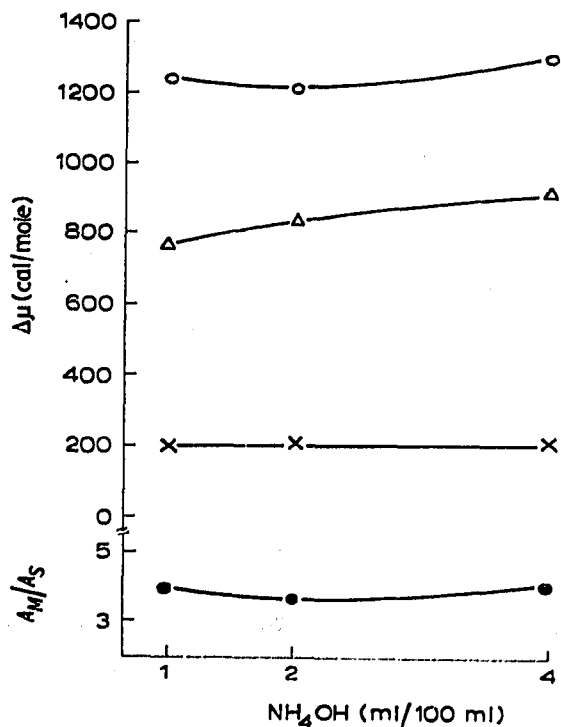


Fig. 14. Plots of A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ vs. quantity of ammonia water. \times , middle; Δ , end; \circ , ortho; \bullet , A_M/A_S .

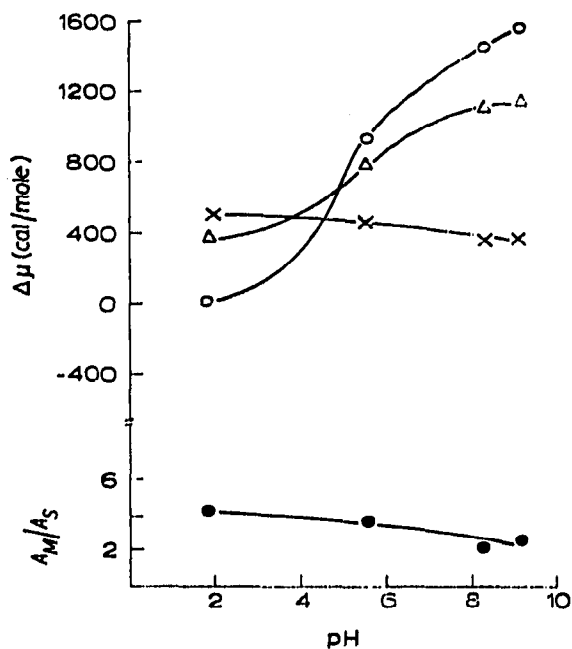
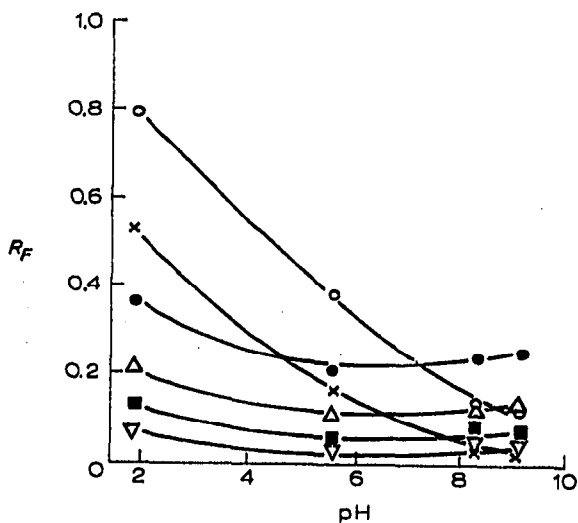


Fig. 15. Plots of R_F vs. pH of solvent. \circ , \circ ; \times , Py; \bullet , TrM; Δ , TtM; \blacksquare , PeM; ∇ , HexM.

Fig. 16. Plots of A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ vs. pH of solvent. \times , middle; Δ , end; \circ , ortho; \bullet , A_M/A_S .

of ammonia water are shown in Figs. 13 and 14. Even if the quantity of ammonia water is changed, R_F , A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, and $\Delta\mu_{\text{ortho}}$ are almost constant. This is not the case with the acidic solvent.

pH value of solvent (A5-0.20, A5-1, A5-2, and A5-4). The plots of R_F value vs. pH of the developing solvent, and A_M/A_S , $\Delta\mu_{\text{middle}}$, $\Delta\mu_{\text{end}}$, $\Delta\mu_{\text{ortho}}$ vs. pH of the developing solvent are shown in Figs. 15 and 16. When the pH of the developing solvent is increased, the R_F values of ortho- and pyrophosphates decrease by a large margin and the R_F of metaphosphates decreases slightly. A_M/A_S and $\Delta\mu_{\text{middle}}$ decrease slightly, but $\Delta\mu_{\text{end}}$ and $\Delta\mu_{\text{ortho}}$ increase by a large margin. As shown in Fig. 5, even if the quantity of ammonia water is increased, $\Delta\mu_{\text{end}}$ increases slightly and $\Delta\mu_{\text{ortho}}$ is almost constant as long as the pH change is not too large. Thus it is thought that the increase of $\Delta\mu_{\text{end}}$ and $\Delta\mu_{\text{ortho}}$ depends on the pH increase of the developing solvent, not on the increase of the quantity of ammonia water. The dependence of the R_F value of phosphates on the pH of the developing solvent is similar to that in the case of paper chromatography of metal ions (ortho- and pyrophosphates— Cu^{2+} , Ba^{2+} , and Pb^{2+} , metaphosphates— HgCl_2)⁹.

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