CHROMATOGRAPHIC BEHAVIOUR AND STRUCTURAL UNITS OF CONDENSED PHOSPHATES

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SUMMARY

The relationships between the R_M values and the structural units of condensed phosphates were obtained. By using these relationships, the free energy necessary to transport one mole of each structural unit from the stationary phase to the mobile phase was calculated. The order of free energy was: middle PO_4 group $>$ end PO_4 group $>$ ortho PO_4 group in an acidic solvent, and middle PO_4 group $<$ end PO_4 group $<$ ortho PO_4 group in a basic solvent.

INTRODUCTION

Basic principles of phosphate structures[1]

By definition, the phosphates are those compounds of phosphorus in the anions of which each atom of phosphorus is surrounded by four oxygen atoms arranged at the corner of a tetrahedron. By sharing oxygen atoms between tetrahedra, chains, rings, and branched polymers of interconnected PO_4 tetrahedra can be produced. This means that there are only particular kinds of building blocks from which phosphates can be made, *viz.*: (1) the branching point; (2) the middle PO_4 group; (3) the end PO_4 group; and (4) the single (ortho- or monophosphate) PO_4 group. These are shown in Fig. 1.

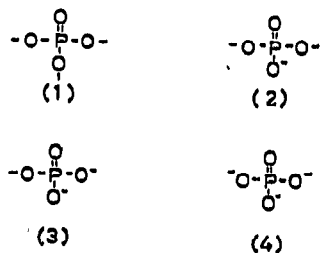By putting two end groups together, we obtain the pyrophosphate ion, and

Fig. 1. Structural units of condensed phosphates; (1) branching point, (2) middle PO_4 group, (3) end PO_4 group, (4) ortho PO_4 group.

adding middle groups between the end groups, we obtain a whole series of straight chain ions (polyphosphate ions).

Middle groups can also be connected to each other to give ring type ions (metaphosphate ions).

According to the "antibranching rule", the branching point will be exceedingly unstable in aqueous solution[2], so that in the case of chromatography of condensed phosphates, there is no need to consider the branching point.

Theory of chromatographic structural analysis

CONSDEN et al.[3] related the R_F value of a compound obtained from paper partition chromatography to the partition coefficient, a, of the compound between the phase used in the system by the equation:

$$a = (A_M/A_S) (1/R_F - 1) \tag{1}$$

where A_M/A_S is the ratio of the thickness of the mobile phase and the stationary phase.

From this relation, MARTIN[4] put forward the theory of chromatographic structural analysis in 1949.

BATE-SMITH AND WESTALL[5] modified MARTIN's relation:

$$\Delta\mu = RT \ln (A_M/A_S) (1/R_F - 1) \tag{2}$$

to the form:

$$\Delta\mu/2.3 RT = R_M + \log (A_M/A_S) \tag{3}$$

by the introduction of a new function:

$$R_M = \log (1/R_F - 1) \tag{4}$$

where $\Delta\mu$ is the free energy necessary to transport 1 mole of the compound from the stationary phase to the mobile phase.

Derivation of the formula relating R_M values to the structural units of condensed phosphates

(1) Metaphosphate ions (ring type ions). As mentioned above, metaphosphate ions consist of only middle groups.

For a metaphosphate ion consisting of m phosphorus atoms (m-metaphosphate ion, degree of condensation $= m$), we may assume that

$$\Delta\mu_{m\text{-meta}} = m\Delta\mu_{\text{middle}} \tag{5}$$

From the additivity principle of chromatographic behavior proposed by MARTIN[4], eqn. (3) therefore becomes

$$R_{M(m\text{-meta})} = -\log (A_M/A_S) + m\Delta\mu_{\text{middle}}/2.3 RT \tag{6}$$

(2) Pyrophosphate ion. Since the pyrophosphate ion consists of 2 end groups, we may assume that

$$\Delta\mu_{\text{pyro}} = 2 \Delta\mu_{\text{end}} \tag{7}$$

whence, eqn. (3) becomes

$$R_{M(pyro)} = -\log (A_M/A_S) + 2 \Delta\mu_{end}/2.3\ RT \tag{8}$$

(3) Orthophosphate ion. For the orthophosphate ion, one gets

$$R_{M(ortho)} = -\log (A_M/A_S) + \Delta\mu_{ortho}/2.3\ RT \tag{9}$$

(4) Polyphosphate ions (straight chain ions). As mentioned above, polyphosphate ions consist of 2 end groups with middle groups between them.

If a polyphosphate ion consists of n phosphorus atoms (n-polyphosphate ion, degree of condensation $= n$), we may assume that

$$\Delta\mu_{n\text{-poly}} = 2 \Delta\mu_{end'} + (n - 2) \Delta\mu_{middle'} \tag{10}$$

whence, eqn. (3) becomes

$$R_{M(n\text{-poly})} = -\log (A_M/A_S) + 2 \Delta\mu_{end'}/2.3\ RT \\ + (n - 2) \Delta\mu_{middle'}/2.3\ RT \tag{11}$$

$\Delta\mu_{middle}$, $\Delta\mu_{end}$, $\Delta\mu_{ortho}$, $\Delta\mu_{middle'}$, and $\Delta\mu_{end'}$ are the free energy necessary 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.

EXPERIMENTAL

Cellulose powder (Toyo-Roshi, > 300 mesh, 10 g) was suspended in a 1% cellulose acetate solution in N,N-dimethylformamide and acetone (1:1, 40 ml). The thin layer was prepared as usual[6].

The solution of mixed metaphosphates was prepared according to THILO AND SCHÜLKE[7]. The solution of mixed polyphosphates was prepared according to JAMESON[8]. Solutions of sodium salts of ortho-, pyro,- tripoly-, tetrapoly-, trimeta-, and tetrametaphosphates were used as standards.

The composition of the developing solvents used were as follows:

Acidic solvent[7,9]: 60 ml of methyl alcohol; 10.3 ml of trichloroacetic acid solution (100 g of trichloroacetic acid diluted to 500 ml with water, and then 22.7 ml of ammonia–water (d 0.918) is added); 5 ml of acetic acid solution (20 ml of 96% acetic acid and 80 ml of water); and 8 ml of water.

Basic solvent[10]: 40 ml of isopropyl alcohol; 20 ml of isobutyl alcohol; 39 ml of water; 1 ml of conc. ammonia–water.

After the solutions of the phosphates had been spotted on the thin layer, the thin layer was allowed to equilibrate with the vapor of developing solvent for more than one hour, the phosphates were then developed in a saturation chamber. The temperature was maintained at 5° in an air bath (Coolnics, CTG-1B, Komatsu-Yamato). When the solvent had run for 10 cm from the point of application of the phosphates, the plate was taken out and dried in air. The phosphates were then hydrolyzed with an aqueous nitric acid solution (1:1) and visualized with ammonium molybdate and stannous chloride[10,11].
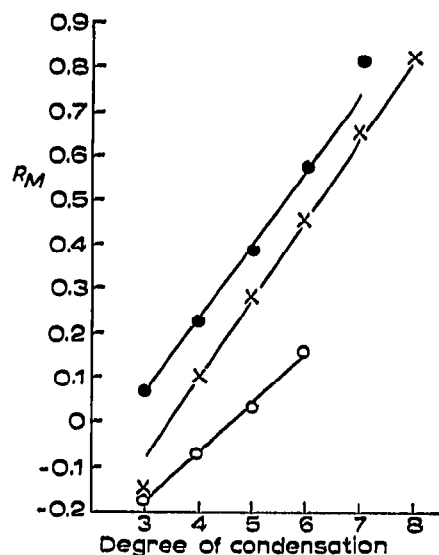
Fig. 2. R_M vs. degree of condensation plots; ●, $R_{M(m\text{-meta})}$ in an acidic solvent, ○, $R_{M(n\text{-poly})}$ in an acidic solvent, ×, $R_{M(m\text{-meta})}$ in a basic solvent.

RESULTS AND DISCUSSION

The R_F values obtained and the R_M values are shown in Table I. R_M vs. degree of condensation plots are shown in Fig. 2.

The following relationships were obtained, by using the method of least squares.

$$R_{M(m\text{-meta})} = -0.438 + 0.168\, m \tag{12}$$

$$R_{M(n\text{-poly})} = -0.290 + 0.111\,(n - 2) \tag{13}$$

TABLE I

R_F AND R_M VALUES

Phosphates	Acidic solvent		Basic solvent	
	R_F	R_M	R_F	R_M
Ortho	0.85	−0.7533	0.35	0.2688
Pyro	0.70	−0.3680	0.16	0.7202
Trimeta	0.46	0.0696	0.59*	−0.1581
Tetrameta	0.37	0.2311	0.44	0.1047
Pentameta	0.29	0.3889	0.34	0.2881
Hexameta	0.21	0.5754	0.26	0.4543
Heptameta	0.13*	0.8256	0.18	0.6585
Octameta	—	—	0.13	0.8256
Tripoly	0.60	−0.1761	0.23	0.5248
Tetrapoly	0.54	−0.0696	0.22	0.5497
Pentapoly	0.48	0.0348	—	—
Hexapoly	0.41	0.1581	—	—

* Notes: (1) R_M is taken from the table of R_F and R_F functions in ref. 12. (2) Values marked* seem to be abnormal and are removed from the least squares calculation.

TABLE II

SUMMARY OF RESULTS

Developing solvent	A_M/A_S	$\Delta\mu_{middle}$ (cal/ mole)	$\Delta\mu_{end}$ (cal/ mole)	$\Delta\mu_{ortho}$ (cal/ mole)	$\Delta\mu_{middle}'$ (cal/ mole)	$\Delta\mu_{end}'$ (cal/ mole)
Acidic	2.74	214	18	−465	141	94
Basic	4.18	230	853	1130	—	—

in an acidic solvent, and

$$R_{M(m\text{-meta})} = -0.621 + 0.181\,m \tag{14}$$

in a basic solvent.

We could not obtain a relation for $R_{M(n\text{-poly})}$ in a basic solvent, because we could not obtain R_F values for more than pentapolyphosphate in this experiment.

The results are summarized in Table II.

In an acidic solvent, the order of free energy is:

$$\Delta\mu_{middle} > \Delta\mu_{end} > \Delta\mu_{ortho}$$

and in a basic solution:

$$\Delta\mu_{middle} < \Delta\mu_{end} < \Delta\mu_{ortho}.$$

The difference between $\Delta\mu_{middle}$ and $\Delta\mu_{middle'}$, $\Delta\mu_{end}$ and $\Delta\mu_{end'}$ is thought to depend on the mutual interactions between the middle and end groups of the polyphosphates.

ACKNOWLEDGEMENTS

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