CHROM. 5198

Anion-exchange thin-layer chromatography of condensed phosphates

BERGER et al.[1] have reported the separation of polyphosphates on thin layers of Biorex 5 ion exchanger. TANZER et al.[2] have reported the separation of polyphosphates by anion-exchange thin-layer chromatography on polyethylene imine (PEI) impregnated Avicel coated thin layers.

CERRAI et al.[3] have found, in reversed-phase chromatography of metals on paper treated with di-(2-ethylhexyl)orthophosphoric acid (HDEPA), that the quantity $\log(1/R_F - 1)$ is related to the hydrogen ion concentration in the mobile phase. Furthermore, linear relationships between $-\log [H^+]$ and the R_M value have been obtained with ion-exchange resin papers (ALBERTI et al.[4]), zirconium phosphate paper (ALBERTI et al.[5]), ammonium tungstophosphate paper (PRÁŠILOVÁ AND ŠEBESTA[6]) and alginic acid thin layers (COZZI et al.[7]) etc. But there are a few such studies with respect to anions.

Condensed phosphates are one of the inorganic polymers (polyelectrolytes) and are the compounds of an homologous series with various numbers of phosphorus atoms per molecule. It is thought to be important and interesting to know the valency of condensed phosphates in solutions. So we reintroduce the relationship between R_M and valency in ion-exchange chromatography, and using this relation, we obtain the apparent valency of condensed phosphates.

Theoretical

The law of mass action and the derivation of the law of the distribution ratio[8]. When a chemical equation in ion exchange is as follows:

$$n_B A_X + B_L = B_X + n_B A_L \tag{1}$$

then the following law of mass action

$$K_T = a_{BX} \cdot (a_{AL})^{n_B} / (a_{AX})^{n_B} \cdot a_{BL} \tag{2}$$

holds even in the case of heterogeneous equilibrium.

In order to obtain an empirical formula, all activity coefficients are collected on the left-hand side of eqn. 2, and we then obtain

$$K_A^B = m_{BX} \cdot [A]^{n_B} / (m_{AX})^{n_B} \cdot [B] \tag{3}$$

Now, if the "distribution coefficient" is defined such that

$$D_A = m_{AX}/[A] \quad D_B = m_{BX}/[B] \tag{4}$$

then the eqn. 3 becomes

$$\log D_B = \log K_A^B + n_B \log D_A \tag{5}$$

Chromatographic structural analysis. BATE-SMITH AND WESTALL[9] modified "MARTIN's relation":

$$\Delta\mu = RT \ln (A_M/A_S) \cdot (1/R_F - 1) \tag{6}$$

into the form:

$$R_M = -\log(A_M/A_S) + \Delta\mu/2.3\ RT \tag{7}$$

by the introduction of a new function:

$$R_M = \log(1/R_F - 1) \tag{8}$$

Application of these equations to "ion-exchange thin-layer chromatography". Eqns. 5 and 6 are put into the relation:

$$\Delta\mu = 2.3\ RT \log D_B \tag{9}$$

whence, the following relation is obtained

$$R_M = -\log(A_M/A_S) + \log K_A^B + n_B \log D_A$$

$$= -\log(A_M/A_S) + \log K_A^B + n_B \log m_{AX} - n_B \log[A] \tag{10}$$

A_M/A_S and m_{AX} are characteristic constants for a kind of ion-exchange thin layer and K_A^B is characteristic constant for ion-exchange system, so eqn. 10 can be written as follows:

$$R_M = \text{const} - n_B \log[A] \tag{11}$$

Eqn. 11 is similar to the relation which was obtained by LEDERER AND KERTES[10].

On the other hand, eqn. 10 can be written as follows:

$$R_M = -\log(A_M/A_S) + n_B\{(\log K_A^B)/n_B + \log m_{AX} - \log[A]\} \tag{12}$$

so for an homologous series, eqn. 12 becomes

$$R_M = -\log(A_M/A_S) + n_B \cdot \kappa \tag{13}$$

under the condition that $[A]$ is constant and under the assumption that $(\log K_A^B)/n_B$ is constant (K_A^B is an exponential function of n_B). Eqn. 13 is similar to the relation which was reported in a previous paper[11].

Symbols
n_B = valency of ion B
A_X, B_X = ion A or B in the fixed ion exchanger phase
A_L, B_L = ion A or B in the solution phase
$[A]$, $[B]$ = so-called concentration of ion A or B in the solution phase
m_{AX}, m_{BX} = so-called concentration of ion A or B in the fixed ion exchanger phase
K_A^B = apparent equilibrium constant
A_M/A_S = the ratio of the thickness of the mobile phase and the stationary phase
$\Delta\mu$ = free energy necessary to transport 1 mole of the compound from the stationary phase to the mobile phase.

Experimental

TLC plates PEI-cellulose F precoated (Merck AG. Darmstadt, G. F. R., 0.10 mm) were employed. The plates were stored below 5°.

TABLE I

R_F VALUES

Phosphates	NaCl (f = 0.962)				
	0.5 N	0.8 N	1.0 N	1.2 N	1.5 N
Ortho	0.42	0.55	0.63	0.70	0.84[a]
Pyro	0.01	0.03	0.05	0.07	0.10
Tripoly	0.0[a]	0.01	0.02	0.04	0.07
Trimeta	0.12	0.35	0.59	0.69	—
Tetrameta	0.01	0.07	0.14	0.27	0.65[a]
Hexameta	0.0[a]	0.0[a]	0.02	0.04	0.16

Phosphates	NH₄Cl (f = 0.957)				
	0.5 N	0.8 N	1.0 N	1.2 N	1.5 N
Ortho	0.43	0.63	0.65	0.73	0.80
Pyro	0.02	0.03[a]	0.06	0.08	0.12
Tripoly	0.0[a]	0.02	0.03	0.05	0.07
Trimeta	0.15	0.41	0.56	0.65	0.80
Tetrameta	0.03[a]	0.08	0.17	0.24	0.51
Hexameta	0.0[a]	0.0[a]	0.02	0.04	0.11

[a] Removed from the least squares calculation.
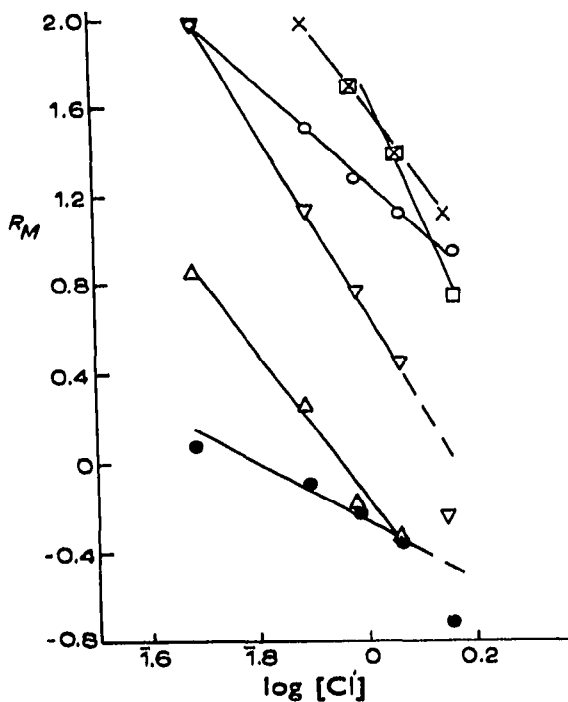
Fig. 1. R_M value vs. logarithm of concentration of NaCl plots. ● ortho; ○ pyro; × tripoly; △ trimeta; ▽ tetrameta; ☐ hexameta.
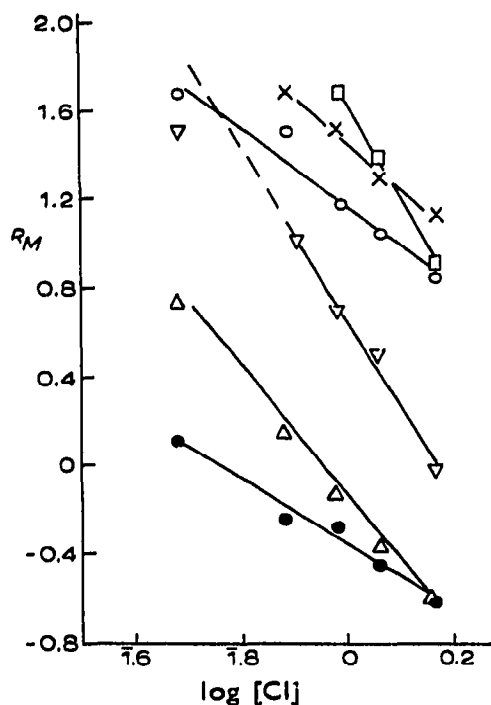
Fig. 2. R_M value vs. logarithm of concentration of NH_4Cl plots. ● ortho; ○ pyro; × tripoly; ○ trimeta; ▽ tetrameta; ☐ hexameta.

Aqueous solutions (1 mg/ml) of various sodium salts of phosphates were spotted such that about 0.5 μg of the salt was spotted on the thin layer.

The phosphates used were ortho-, pyro-, tripoly-, trimeta-, tetrameta-, and hexametaphosphates.

The developing solvents were aqueous solutions of NaCl (f = 0.962) and NH_4Cl (f = 0.957) of the following concentrations; 0.5 N, 0.8 N, 1.0 N, 1.2 N, 1.5 N.

After the solutions of phosphates had been spotted on the thin layer, the phosphates were 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[11].

TABLE II

APPARENT VALENCY

Phosphates	NaCl	NH_4Cl
Ortho	1.31	1.46
Pyro	2.21	1.71
Tripoly	3.26	2.14
Trimeta	3.26	2.78
Tetrameta	4.08	3.83
Hexameta	5.56	4.45

Results and discussion

The R_F values of the sodium salts of ortho-, pyro-, tripoly-, trimeta-, tetrameta-, and hexametaphosphates when PEI plates were developed with various concentration of NaCl and NH_4Cl are given in Table I.

The R_M value vs. logarithm of the concentration plots are given in Fig. 1 (NaCl) and Fig. 2 (NH_4Cl). The apparent valency of the phosphate was obtained from the slope as shown in the Theoretical section. The apparent valency obtained is given in Table II.

In general, the apparent valency in NH_4Cl is smaller than that in NaCl. The two main reasons are thought to be: (i) the difference in pH of the solutions (NaCl(1 N)—5.60, NH_4Cl(1 N)—5.15) ; (ii) the difference in the ability of cations to form complexes.
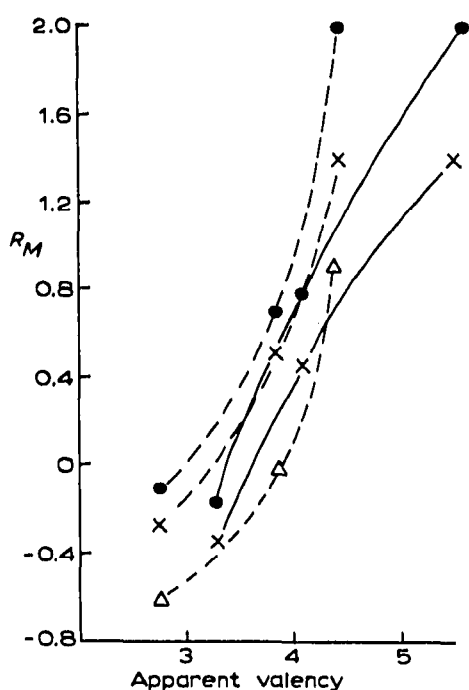

Fig. 3. R_M value vs. apparent valency plots. ● 1.0 N ; × 1.2 N ; △ 1.5 N ; ——— NaCl ; — — — NH_4Cl.

However, it was not possible to clarify which is the predominant reason in this experiment.

R_M value vs. apparent valency plots are shown in Fig. 3. Linear relations were not observed. The reason for this is thought to be that the assumption mentioned in the Theoretical section does not hold good.

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CHROM. 5206

Ion-exchange chromatography of dinucleoside-3'→5'-phosphates on chitosan-impregnated cellulose thin layers

In a preceding paper[1], we presented evidence that a number of nucleic acid constituents, such as 5'-mononucleotides, nucleosides, and nucleic bases, can be resolved by ion-exchange chromatography on chitosan formate-impregnated cellulose thin layers. The present communication will describe the separation of dinucleoside-3'→5'-phosphates, which are a phosphodiester type of nucleotide, on the layers.

Materials

Chitosan. The same chitosan as was used in the preceding experiments* was used again in the present investigation. The intrinsic viscosity $[\eta]$ of the chitosan was 8.25, which was determined in 0.5% formic acid solution at 25 ± 0.1°.**

Cellulose powder. Avicel SF, a finely powdered product of microcrystalline cellulose "Avicel" for use in TLC, was obtained from Funakoshi Pharmaceutical Co. and Asahi Kasei Co. (Tokyo, Japan).

*Dinucleoside-3'→5'-phosphates.**** All sixteen dinucleoside-3'→5'-phosphates were purchased from Nutritional Biochemicals Corporation (Cleveland, Ohio).

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* The commercial chitin purified by Hackman's method[2,3] was deacetylated with concentrated hot alkali by the procedure of Wolfrom *et al.*[4-6]. Quantitative analysis of the chitosan thus prepared gave 7.95% N, 1.71% N-acetyl and 1.15% ash; while 8.75% N and 0% N-acetyl were calculated for $C_6H_{11}NO_4$. These analytical data show that about 92% of the total nitrogen in the chitosan is present as the free amino group.

** The viscosity of chitosan markedly influences the chromatographic behavior in this procedure. An examination of the viscosity range of chitosan optimum for the chromatography is in progress, and the results obtained will be reported later.

*** The following abbreviations will be used: ApA, ApG, ApC, ApU = 3'-adenylyl esters of adenosine-5', guanosine-5', cytidine-5', uridine-5'; GpA, GpG, GpC, GpU = 3'-guanylyl esters of adenosine-5', guanosine-5', cytidine-5', uridine-5'; CpA, CpG, CpC, CpU = 3'-cytidylyl esters of adenosine-5', guanosine-5', cytidine-5', uridine-5'; UpA, UpG, UpC, UpU = 3'-uridylyl esters of adenosine-5', guanosine-5', cytidine-5', uridine-5'.