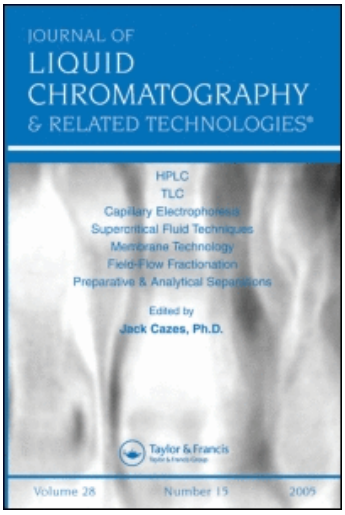

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Characterization of Inorganic Long-Chain Polyphosphate by a Sephadex G-100 Column Combined with an Autoanalyzer Detector

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CHARACTERIZATION OF INORGANIC LONG-CHAIN POLYPHOSPHATE BY A SEPHADEX G-100 COLUMN COMBINED WITH AN AUTOANALYZER DETECTOR

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ABSTRACT

A Sephadex G-100 column with an AutoAnalyzer detector was used in order to characterize a long-chain polyphosphate mixture. This technique enabled us to obtain the molecular weight distribution profile of the mixture within 2 h. A linear relationship between distribution coefficient values and the logarithms of the average chain lengths was obtained.

INTRODUCTION

Sodium phosphate glasses consist mainly of a mixture of long-chain polyphosphate molecules which are known to form complex ions with alkaline earth and other metal ions. They have found widespread industrial applications such as the softening of water (1). In order to characterize the molecular weight distribution of chain phosphates in sodium phosphate glasses, solubility fractionation (2) combined with chain length determination by pH titration (3) has been employed. However, this method is too tedious and cautions should always be taken to prevent hydrolysis of these polyphosphate molecules. A rapid and automatic procedure has been desired.

Ion-exchange chromatography combined with an AutoAnalyzer detector (4-6) developed in our laboratory has been applied to the

analysis of relatively short-chain polyphosphates. Gel chromatography on a cross-linked dextran gel, Sephadex (7,8) has been successfully applied to characterize inorganic polyphosphates. In this work, a Sephadex G-100 column combined with an AutoAnalyzer detector was applied to the routine analysis of the mixture of long-chain polyphosphates. This system has been proved to provide a molecular weight distribution profile of the mixture with a good reproducibility within 2 h.

EXPERIMENTAL

Preparation of sodium phosphate glasses (1)

Appropriate quantities of anhydrous mono- and disodium orthophosphate were mixed and heated in platinum dish at 1000°C. After the melts were heated for 2 h at 1000°C, they were quenched by pouring on a slab and quickly pressing another copper slab on top. By this procedure sodium phosphate glasses with various average chain lengths could be obtained (Table 1).

Determination of average chain length (3)

To a portion of the sample polyphosphate solution, hydrochloric acid was added to lower the pH of the solution to ca. 3. Then 0.1 M carbonate free sodium hydroxide solution was added and the pH of the solution was recorded. This titration was carried out automatically with a KEM Potentiometric Automatic Titrator Recorder model ATR-107 (Kyotodenshi Kogyo Ltd., Japan). From this titration, the amount of end phosphates, N_e, was calculated. The total amount of phosphate monomers, N_t, was determined colorimetrically using Mo(V)-Mo(VI) reagent (9,10). The average chain length, $\bar{n}$, was calculated according to eqn.(1).

$$\bar{n} = 2N_t/N_e \qquad (1)$$

Solubility fractionation with acetone (2)

14 g of phosphate glass (Sample 10 in Table 1) was dissolved in 140 ml of water, then 14 ml acetone was added. To this original

TABLE 1.

Data for the Sodium Phosphate Glasses

Sample	1	2	3	4	5	6	7	8	9	10
$(\bar{n})_{nom.}$ *	4.7	6.4	9.2	13.7	22.2	28.7	40.0	50.4	55.8	75.8

* Not corrected for cyclic phosphates.

solution an appropriate volume of acetone was added to cause it appear cloudy. This suspension was stirred for ca. 10 min and then centrifuged at ca. 2000 rpm for 15 min. An oily substance could be found below the solution which had become clear. After decantation the oily substance was dissolved into water. Seven fractions were taken and the amount of acetone used for each was gradually increased as fractionation proceeded. Data for the fractionation experiments are tabulated in Table 2.

Gel chromatography with AutoAnalyzer as a detector

Sephadex G-100 column (15x300 mm) was used. Eluent contained 0.1 M sodium chloride, 0.01 M sodium acetate, and 5×10^{-4} M acetic acid (pH 6). Sample solutions contained sodium chloride and buffer agent of the same concentrations as the eluent and sample phosphates of the concentration of ca. 10^{-3} M (as phosphate monomer). A 0.5 ml portion of the sample solution was applied to the column with a loop injector. The elution flow rate was kept to be 0.52 ml/min because the higher flow rate caused the shrinkage of the gel bed.

A portion of an effluent from the column was introduced to the AutoAnalyzer detector (Technicon). The details of the experimental conditions of the AutoAnalyzer detector have been described previously (5). For about 18 min the sample had to remain in the reaction coil to permit the hydrolysis of polyphosphate and a color reaction of the resultant orthophosphate with a molybdenum reagent.

TABLE 2.

Data for the Acetone Fractionation

Fraction	1	2	3	4	5	6	7*
Volume of acetone added (ml)	6	8	8	8	16	32	100
$\bar{n}$	-**	127	101	85	50	36	-

* Solid precipitate which may consist of cyclic phosphates was obtained.

** Since the quantity of the sample obtained was too small for titration, $\bar{n}$ could not be determined.

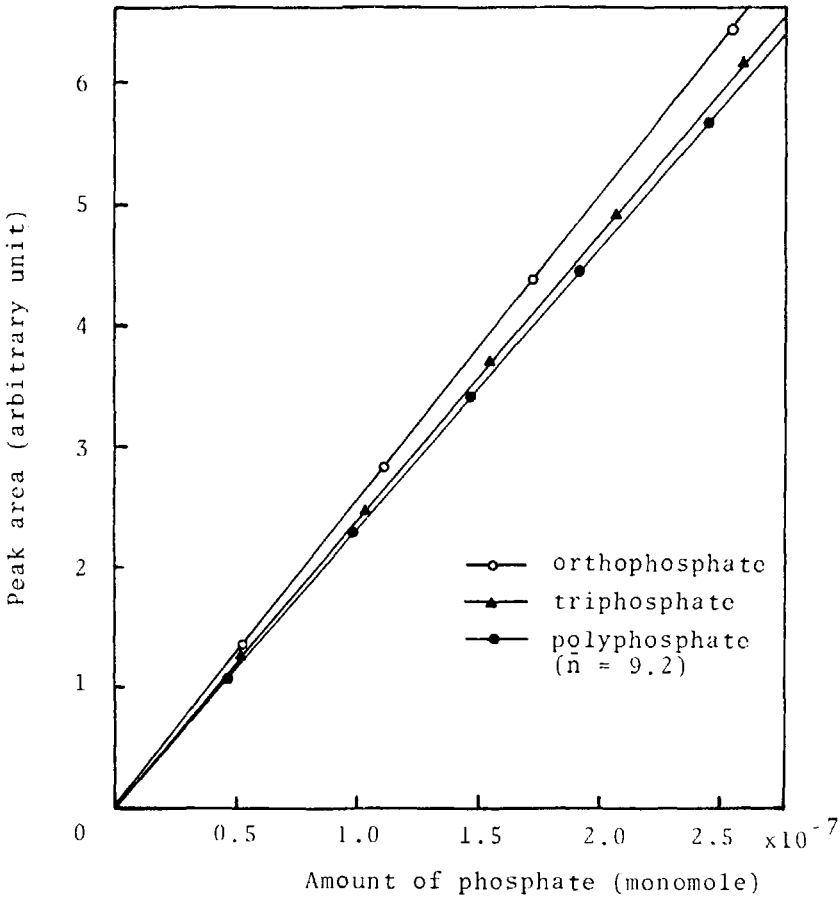

FIGURE 1.

Peak area vs. the amount of phosphate plots for ortho- and polyphosphates.

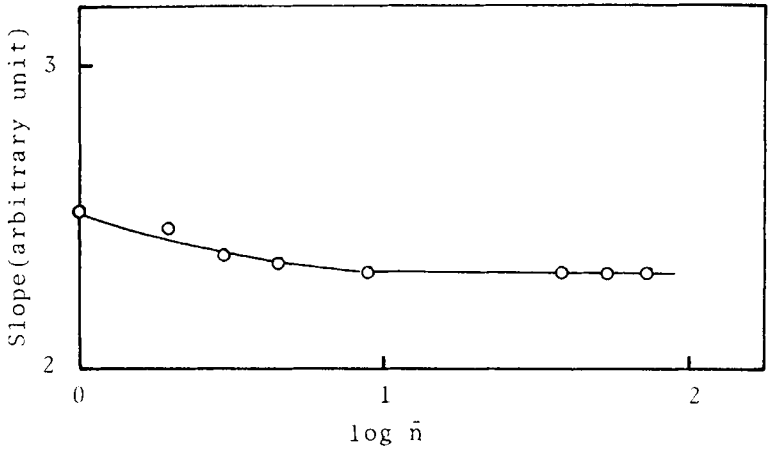

FIGURE 2.

Effect of degrees of polymerization of polyphosphates on the slope of the calibration curve.

A good linearity was obtained for the amount of polyphosphate and the peak area for samples of various degrees of polymerization (Fig. 1). Tough the sample was mixed with sulfuric acid and heated at 98°C, polyphosphates could not completely be hydrolyzed. The slope of the calibration curve was plotted against the chain length of the polyphosphate sample (Fig. 2). Since the slope did not change when the chain length is more than 10, no correction was made for the chromatograms obtained in this work.

RESULTS AND DISCUSSION

In the previous work (11-13), it has been expected that an eluting agent greatly affects the elution behavior of long-chain polyphosphates. In this work, therefore, 0.1 M sodium chloride solution (pH 6) was used throughout. In Fig. 3, the elution profiles are shown for various sodium phosphate glass samples. It can be seen that as the value of $(\bar{n})_{nom}$ increased the resulting peak position shifted forward, and that these glasses have a wide-

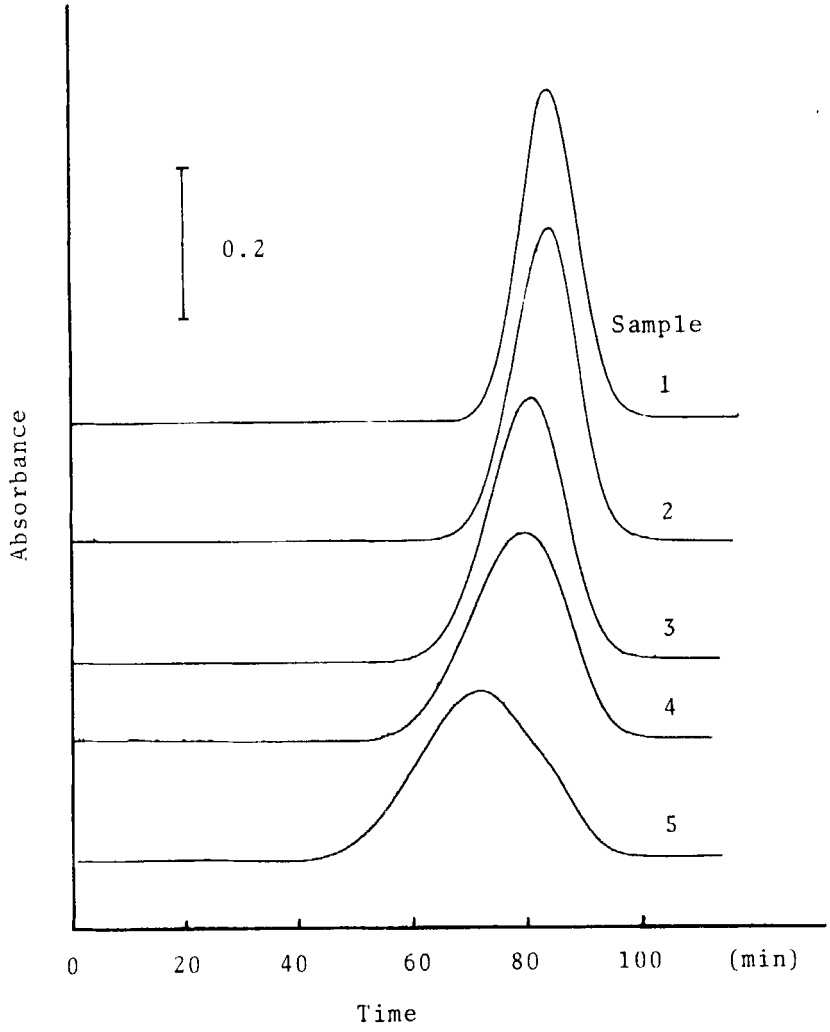

FIGURE 3-1.

Elution profiles of sodium phosphate glasses. Sample numbers are the same as those in Table 1.

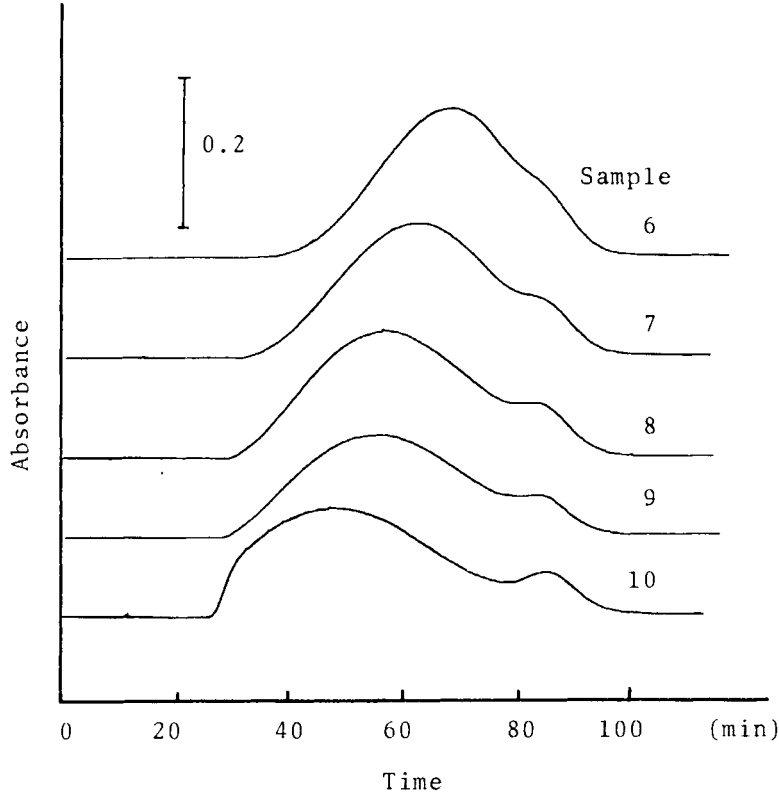

FIGURE 3-2.

Elution profiles of sodium phosphate glasses. Sample numbers are the same as those in Table 1.

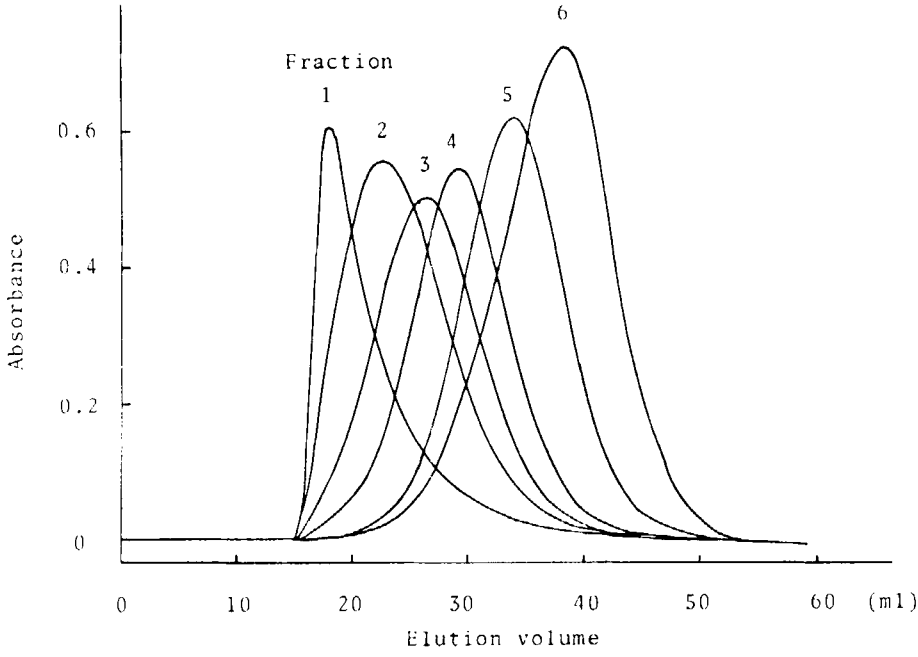

FIGURE 4.

Elution profiles of the samples obtained by acetone fractionation. Fraction numbers are the same as those in Table 2.

spread molecular weight distribution. Small peak whose retention time is approximately 85 min can be observed. These peaks correspond to cyclic phosphates, mainly trimeta- and tetrametaphosphate (2).

In Fig. 4, the elution profiles of the long-chain polyphosphate samples obtained by acetone fractionation are shown. It is evident that the sample prepared by the acetone fractionation shows much narrower dispersion than the original phosphate glass (Sample 10 in Fig. 3). Cyclic phosphates were completely removed off for fractions 1-6. Elution volumes, V_e, for these samples were deter-

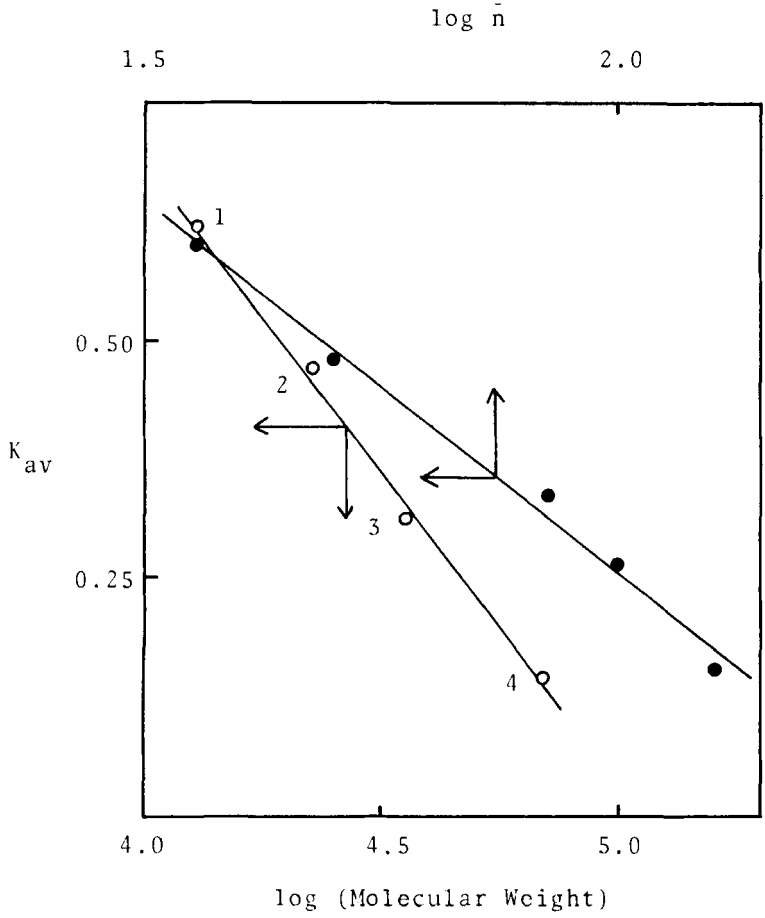

FIGURE 5.

Plots of distribution coefficient (K_{av}) vs. log $\bar{n}$ or log (Molecular Weight) for linear phosphates or proteins.
V_0 was determined by Blue Dextran 2000. 20 mg of protein sample was dissolved into 10 ml of the eluent. A 0.5 ml portion of the sample solution was applied to the column.
1) Cytochrome C, 2) α-Chymotrypsin, 3) Pepsin, 4) Bovine serum albumin

mined from the peak positions of these samples. Distribution coefficients, K_{av}, were calculated according to eqn.(2).

$$K_{av} = (V_e - V_0)/(V_t - V_0) \quad (2)$$

where V_0 is the void volume and V_t is the total bed volume.

In order to calibrate this column, several proteins were chromatographed with 0.1 M sodium chloride solution (pH 6). A UV detector (228 nm) was used in order to monitor these proteins. As has been pointed out by Whitaker (14), a linear relationship was obtained between the K_{av} values and the logarithms of the molecular weights of the proteins (Fig. 5). For long-chain polyphosphate samples, the values of K_{av} were also plotted against the logarithms of the average chain lengths (Fig. 5). As with the proteins system, a linear relationship was obtained. The relationship may be useful for calculating the distribution of the chain length of sodium phosphate glasses.

As can be seen from Figs. 3 and 4, a rough evaluation of the molecular weight distribution profile of the long-chain polyphosphate mixture could be made within 2 h by the gel chromatography-AutoAnalyzer system. This system is quite useful for the characterization of long-chain polyphosphates.

ACKNOWLEDGMENTS

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REFERENCES

1. Van Wazer, J. R., J. Am. Chem. Soc., 72, 644(1950).
2. Van Wazer, J. R., J. Am. Chem. Soc., 72, 647(1950).
3. Van Wazer, J. R., Griffith, E. J. and McCullough, J. F., Anal. Chem., 26, 1755(1954).
4. Yamaguchi, H., Nakamura, T., Hirai, Y. and Ohashi, S., J. Chromatogr., 172, 131(1979).
5. Hirai, Y., Yoza, N. and Ohashi, S., J. Liq. Chromatogr., 2, 677 (1979).

6. Yoza, N., Ito, K., Hirai, Y. and Ohashi, S., J. Chromatogr., 196, 471(1980).

7. Ueno, Y., Yoza, N. and Ohashi, S., J. Chromatogr., 52, 469, 481(1970)

8. Felter, S., Dirchimer, G. and Ebel, J. P., J. Chromatogr., 35, 207(1968).

9. Conde, F. L. and Prat, L., Anal Chim. Acta, 16, 473(1957).

10. Yoza, N., Ishibashi, K. and Ohashi, S., J. Chromatogr., 134, 497(1978).

11. Miyajima, T., Ibaraki, M., Yoza, N. and Ohashi, S., J. Liq. Chromatogr., 4, 259(1981).

12. Miyajima, T., Yoshikawa, Y., Yoza, N. and Ohashi, S., J. Liq. Chromatogr., submitted for publication.

13. Cooper, A. R. and Van Derveer, D. S. J. Liq. Chromatogr., 1, 693 (1978).

14. Whitaker, J. R., Anal. Chem., 35, 1950(1963).