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Note

Applicability of Toyopearl packing to the gel chromatography of inorganic linear and cyclic phosphates

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Though soft gels such as cross-linked dextrans have been widely used in the characterization of inorganic linear and cyclic phosphates[1–4], they have a drawback in that they cannot tolerate a high mobile-phase velocity and hence require elution times of many hours. Semi-rigid hydrophilic porous polymer packing materials, Toyopearl (Toyo Soda, Tokyo, Japan), have recently been applied to the analyses of various water-soluble polymers[5]. In this study, we applied Toyopearl HW-55F to the characterization of inorganic linear and cyclic phosphates. The molecular weight distribution profile of a long-chain polyphosphate mixture can be obtained within 30 min. A linear relationship was obtained between the elution volumes and the logarithms of the average chain lengths.

EXPERIMENTAL

Materials

Analytical grade disodium monohydrogen orthophosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and tetrasodium diphosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$) were used without further purification. Sodium triphosphate hexahydrate ($Na_5P_3O_{10} \cdot 6H_2O$) was recrystallized from water–ethanol solvent. Trisodium trimetaphosphate hexahydrate ($Na_3P_3O_9 \cdot 6H_2O$)[6], tetrasodium tetrametaphosphate tetrahydrate ($Na_4P_4O_{12} \cdot 4H_2O$)[6], sodium phosphate glass, $(NaPO_3)_n$[7], and Kurrol's salt, $(KPO_3)_n$[8], were prepared according to the literatures. Fractionated samples were obtained by a solubility fractionation with acetone[9]. The average chain length, $\bar{n}$, of long-chain polyphosphate mixtures were determined by an end-group titration method[10].

Procedure for packing

Toyopearl packings are produced by polymerization of hydrophilic vinylmonomers. The gel was packed according to the literature[11]. Toyopearl HW-55 F (Lot No. 55115 M-F) gel was suspended in distilled water and allowed to stand. The fine particles in the supernatant were removed by decantation. The gel was washed with 0.1 *M* sodium chloride solution. A slurry of 45 % gel by volume was prepared in 0.1 *M* sodium chloride solution. The resultant slurry was poured into a slurry reservoir (60

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× 1.5 cm I.D.) and the analytical column (30 × 1.5 cm I.D.) mounted vertically. The packing velocity was maintained constant (*ca.* 2 ml/min). After passing three column volumes of 0.1 *M* sodium chloride solution, the analytical column was disconnected from the reservoir column.

Procedure for elution

A Kyowa KHU-W-188 reciprocating pump was used throughout. A 0.1 *M* sodium chloride solution was used as an eluent. Then 0.25-ml portions of sample solutions which contained each phosphate as well as sodium chloride (0.1 *M*) were applied to the column. The total phosphate concentration of an effluent was monitored with AutoAnalyzer II (Technicon, Tarrytown, NY, U.S.A.). The details of the applicability of the AutoAnalyzer II as a post column detector has been described previously[12]. During the chromatographic run, care has been taken lest the pressure should exceed 5 kg/cm^2.

RESULTS AND DISCUSSION

In Fig. 1, the elution profile of the mixture of Kurrol's salt, P_k, whose $\bar{n}$ value is estimated to be several ten thousands, and orthophosphate, P_1, is shown. The sample concentration dependence of both phosphate samples was examined in the range 10^{-4}–10^{-3} *M* (as P). Because the effect of sample concentration on elution volume could not be observed, it was concluded that inorganic phosphate samples do not adsorb on the gel support. Assuming that P_k molecules cannot permeate the gel phase, the void volume of the column, V_0, was determined to be *ca.* 18 ml (34% of the total column volume).

The efficiency of the column was measured with P_1 as the solute by the equation $N = 16\ (t_R/t_W)^2$, where N is the number of theoretical plates, t_R is the peak retention time and t_W is the band width. The value of t_W was determined by drawing tangents to each side of the band to extend to touch the baseline. When the elution flow-rate was 1.76 ml/min, the N value was *ca.* 1000. The N value increased as the

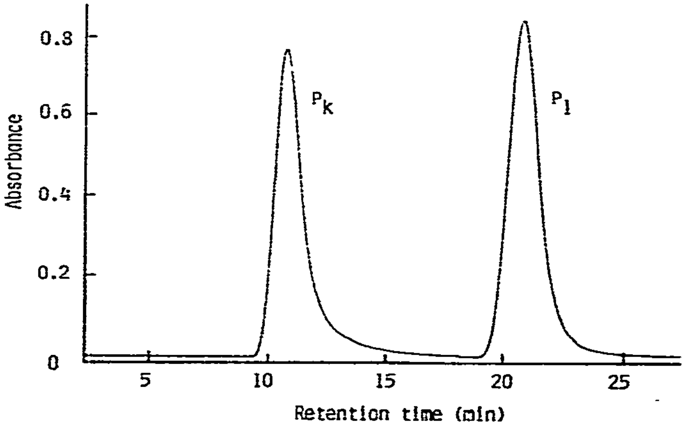
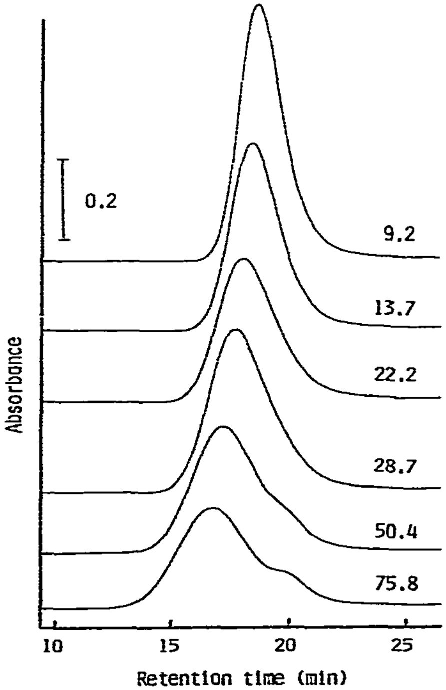


Fig. 1. Elution profiles of P_k and P_1. The concentration of each phosphate sample was 10^{-3} *M* (as P).

flow-rate decreased. However, in order to shorten the analysis time, the elutions were carried out at 1.76 ml/min.

In Fig. 2, the elution profiles are shown for various sodium phosphate glass samples. It can be seen that as the value of $\bar{n}$ increased the resulting peak position shifted forward, and that these glasses have a widespread molecular weight distribution. The small peak whose retention time is *ca.* 20 min is attributable to cyclic phosphates, mainly trimeta- and tetrametaphosphates[9].

In order to calibrate the column, monodisperse samples of di-, tri-, trimeta- and tetrametaphosphate together with polydisperse samples of long-chain polyphosphates have also been eluted. The elution volumes, V_e, of various inorganic polyphosphate samples are plotted against the logarithms of chain length, n or $\bar{n}$ (Fig. 3). An approximately linear relationship was obtained. The slope of the straight line was found to be much less than those obtained with Sephadex G-50 or G-100 under the same experimental conditions[3]. The pore size distribution of Toyopearl HW-55F is estimated to be greater than those of Sephadex G-50 or G-100. From Fig. 3, it can be seen that this packing material seems useful for the characterization of the long-chain polyphosphates whose $\bar{n}$ value is in the range of several hundreds and several thousands, which has been unaccessible by the conventional end-group titration method.



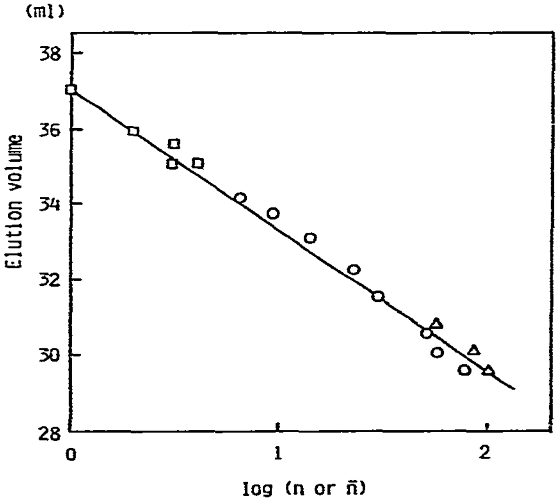


Fig. 2. Elution profiles of sodium phosphate glasses. Numbers at the rear edge of the peaks refer to the sample $\bar{n}$ values. The concentration of each phosphate sample was 10^{-3} *M* (as P).

Fig. 3. Plots of elution volume *vs.* log n or $\bar{n}$. □, Monodisperse phosphate sample; ○, sodium phosphate glass sample; △, fractionated phosphate sample.

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