Separation of inorganic phosphates by molecular-sieve chromatography

Chromatographic separation of macromolecules with Sephadex, which is a cross-linked polysaccharide, has recently been developed in the field of biochemistry. The basic principle of this method has been explained in terms of the molecular sieving effect in the gel phase[1-4]. As shown in eqn. (1) the effluent peak volume V_e of a given solute in the column operation is related to the void volume V_o of a solvent in the gel bed and the internal volume V_i of the gel phase available for the solvent. K_d is defined as a fraction of the internal volume available for the solute.

$$V_e = V_o + K_d V_i \qquad (1)$$

The present work describes the application of this method to the separation of inorganic phosphates. An elution curve for a series of polyphosphates using a column of Sephadex G-25 (fine) and 0.1 M potassium chloride solution as eluent is shown in Fig. 1. The amount of phosphate and tritiated water (THO) in each 1 ml fraction was determined by colorimetry and with a liquid scintillation counter, respectively.

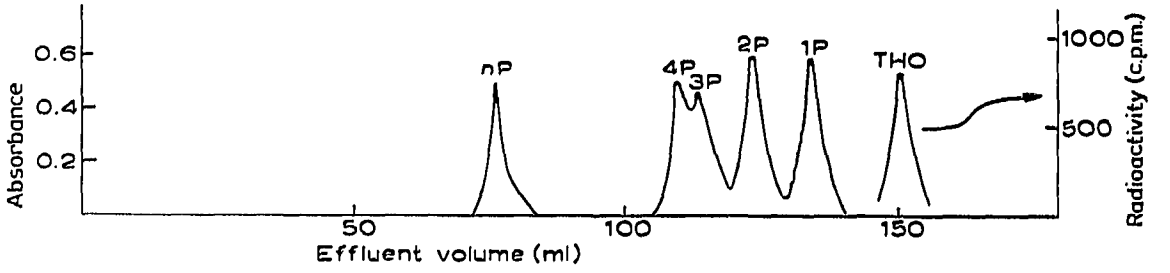

Fig. 1. Elution curve of inorganic phosphates and tritiated water. Column bed: 1.5 × 98 cm; flow rate: 18 ml/h. THO = tritiated water; 1 P = orthophosphate; 2 P = diphosphate; 3 P = triphosphate; nP = Kurrol's salt.

The effluent peak volume of a Kurrol's salt[5] $(KPO_3)_n$ was the same as that of Blue Dextran (mol. wt. = 2,000,000) which is considered to be completely excluded from the gel phase. Peak positions of the other phosphates were in the order of decreasing molecular weights. K_d values of phosphates were calculated according to eqn. (1) in which V_o and V_i were determined by the use of Blue Dextran and tritiated water as standard materials of K_d = 0 and 1.

K_d values were also determined by a batch method, eqn. (2), which is based on the assumption that the solute concentration within the gel pore is essentially the same as that in the external liquid phase. Then,

$$K_d = 1 - \frac{V_o' + V_i'}{V_i'} \frac{C_2 - C_1}{C_2} \qquad (2)$$

where V_o' is the void volume of the solvent and V_i' is the internal volume. C_2 is the concentration of a solute in equilibrium with a certain amount of Sephadex and C_1 is the concentration of it when no Sephadex is added. In the present work, 0.1 M potassium chloride solution was used as solvent and 3 g of dry Sephadex was equilibrated with 30 ml = $V_o' + V_i'$) of the solvent containing a known concentration

(= C_1) of each phosphate. To determine V_i' in eqn. (2) Kurrol's salt was used as a standard material of $K_d = 0$.

As shown in Fig. 2 there is no marked difference between K_d values of each phosphate obtained by the column and batch methods. This fact supports that the separation mechanism of phosphates in the column of Sephadex may be ascribed to the molecular sieving effect.

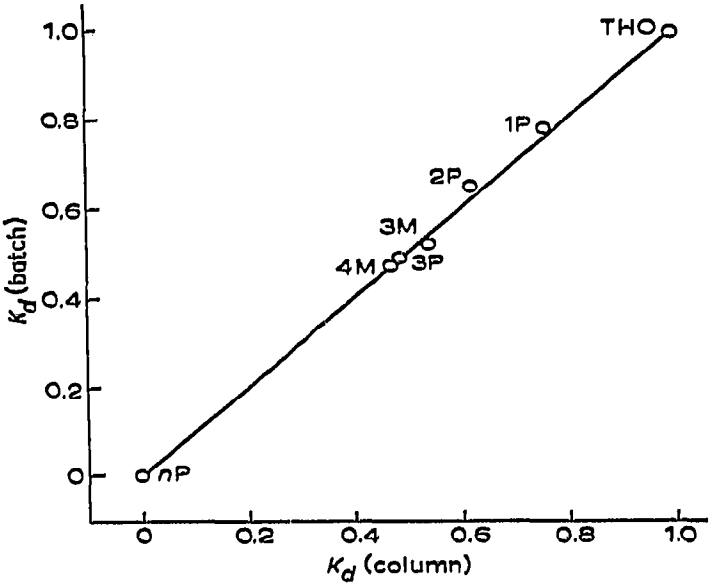

Fig. 2. Comparison between K_d values of phosphates obtained by the column and batch methods. 3M = trimetaphosphate; 4M = tetrametaphosphate. For other abbreviations, see legend Fig. 1.

Details concerning the behavior of various polyphosphates and the correlation between K_d values and molecular weights of phosphates will be discussed later.

Department of Chemistry, Faculty of Science,
Kyushu University, Fukuoka (Japan)

SHIGERU OHASHI
NORIMASA YOZA
YÔICHIRO UENO

1 G. K. ACKERS, *Biochemistry*, 3 (1964) 723.
2 T. C. LAURENT AND J. KILLANDER, *J. Chromatog.*, 14 (1964) 317.
3 J. PORATH, *Metab. Clin. Exptl.*, 13 (1964) 1004.
4 A. TISELIUS, J. PORATH AND P.-A. ALBERTSON, *Science*, 141 (1963) 13.
5 H. MALMGREEN AND O. LAMM, *Z. Anorg. Chem.*, 252 (1944) 256.

Received March 18th, 1966