CHROM. 4935

GEL CHROMATOGRAPHIC BEHAVIOR OF LINEAR PHOSPHATES

YÔICHIRO UENO*, NORIMASA YOZA AND SHIGERU OHASHI
Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka (Japan)

(Received July 13th, 1970)

SUMMARY

Linear phosphates with degrees of polymerization of 1 to 13 were chromatographed on a Sephadex G-25 column with a 0.1 *M* potassium chloride solution and the following results were obtained. (1) The K_{av} values of the linear phosphates did not depend on pH of the eluents. (2) A linear relationship was observed between the K_{av} values and the logarithms of the degrees of polymerization. (3) Average degrees of polymerization of polyphosphate fractions determined by pH titration agreed approximately with those calculated from the gel chromatographic data.

INTRODUCTION

It is widely known that solute molecules are eluted from a gel chromatographic column in the decreasing order of their sizes when only the molecular-sieve effect is operative. This has stimulated many investigators[1-6] to find a relationship between the sizes and elution volumes of solute molecules. GRANATH AND KVIST[1] found that elution volumes of organic chain polymers were a linear function of the logarithms of their molecular weights and calcu' ted average molecular weights of unknown samples using this relationship. This method has also been applied satisfactorily to the estimation of molecular weights of proteins or enzymes[2].

More recently, some investigators have attempted to treat the gel chromatographic behavior of solute molecules theoretically and derived many equations on the basis of the hypothetical structures for gel matrix, *viz.*, conical pores[7], rigid rods[8], cylindrical pores[9] and a mixture[10] of cones, cylinders and crevices. Distribution coefficients of some solute molecules were successfully correlated with their sizes when there were no side effects such as adsorption. It has been demonstrated in another paper[11] that the gel chromatographic behavior of a number of oxo anions of phosphorus, under the proper conditions, is based on the molecular-sieve effect, and the contribution of the side effects is almost negligible. Therefore, a theoretical study appears to be applicable to the gel chromatographic behavior of a series of linear phosphates (polyphosphates).

* Present address: Mitsubishi Chemical Industries, Central Research Laboratories, Hisamoto-Kamoi-cho 290, Kawasaki-shi, Japan.

This paper describes the gel chromatographic behavior of linear phosphates on cross-linked dextran gel columns. The relationship between their distribution coefficients and degrees of polymerization will be briefly discussed.

Abbreviated notations as P_1, P_2, P_3, etc. are used for ortho-, di-, triphosphate, etc. in this paper.

EXPERIMENTAL

A sodium polyphosphate glass with an average degree of polymerization $\bar{p} = 10$ was prepared[12]. Polyphosphate fractions with a $\bar{p}$ of 3–13 were prepared by fractional precipitation[13] from the sodium polyphosphate glass with a $\bar{p}$ of 10. The values of $\bar{p}$ of the polyphosphate fractions were determined by pH titration[14,15] with a Hirama Automatic Titrator. Individual linear phosphates with degrees of polymerization $p = 4$ to 13 were obtained as follows. The sodium polyphosphate glass with $\bar{p} = 10$ was chromatographed on an ion-exchange column in the manner described in a separate paper[16]. The linear phosphates with p up to 13 were clearly separated from one another. A fraction at the maximum concentration of each species was concentrated and desalted to the desired extent by adding dry Sephadex G-10 gel to it. To avoid hydrolysis of the linear phosphates a gel chromatographic run was started within 1 h after they were eluted from the ion-exchange column. The concentrations of sample solutions used for gel chromatography were 3×10^{-3} to 5×10^{-3} gram atom P per l for the individual phosphates and 0.01–0.02 gram atom P per l for the polyphosphate fractions.

The eluents used in this work were 0.1 *M* potassium chloride solutions containing the suitable buffer agents recommended in the preceding paper[11]. A 0.1 *M* potassium chloride–0.01 *M* hydrochloric acid solution (pH 2) was also employed as an eluent.

The Sephadex G-25 columns were identical with those used in the preceding paper[11].

RESULTS AND DISCUSSION

In the preceding paper[11] it was found that the elution curves for the oxo acids of phosphorus at sample concentrations lower than 0.01 g atom P per l are symmetrical and their elution volumes are within the total liquid volume of the column. As shown in Fig. 1, all of the elution curves for the linear phosphates with a p of 1–12 chromato-

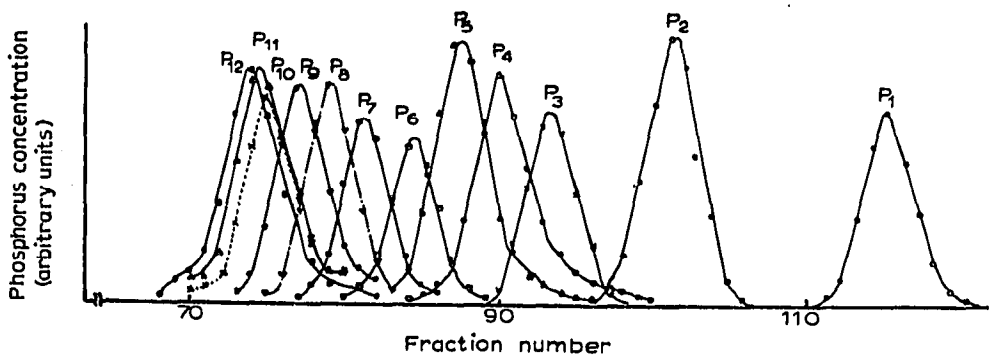

Fig. 1. Elution curves for linear phosphates. Gel, Sephadex G-25; bed volume, 150 ml; eluent 0.1 *M* KCl, pH 7.0. One fraction = 1.08 ml.

TABLE I

K_{av} AND R_s VALUES OF LINEAR PHOSPHATES AT VARIOUS ELUENT pH

Gel, Sephadex G-25; bed volume, 150 ml; eluent, 0.1 *M* KCl.

Sample	*Buffer and pH*							
	0.01 M HCl pH 2.0		*0.005 M phthalate pH 4.6*		*0.0025 M Tris pH 7.0*		*0.005 M ammonium pH 9.2*	
	K_{av}	R_s (Å)	K_{av}	R_s (Å)	K_{av}	R_s (Å)	K_{av}	R_s (Å)
P_1	0.67	4.4	0.67	4.4	0.70	4.2	0.69	4.3
P_2	0.54	5.6	0.54	5.6	0.53	5.7	0.54	5.6
P_3	0.44	6.4	0.44	6.4	0.45	6.4	0.44	6.4
P_4	0.39	6.9	0.40	6.9	0.40	6.9	0.40	6.9
P_5	0.36	7.2	0.35	7.3	0.36	7.2	0.38	7.0
P_6	0.32	7.6	0.33	7.6	0.33	7.6	0.34	7.5
P_7	0.28	8.0	0.29	8.0	0.29	8.0	0.29	8.0
P_8	0.26	8.3	0.26	8.3	0.26	8.3	0.27	8.2
P_9	0.24	8.6	0.24	8.6	0.24	8.6	0.23	8.7
P_{10}	0.21	8.9	0.21	9.0	0.21	9.0	0.21	8.9
P_{11}			0.20	9.2	0.20	9.2	0.19	9.2
P_{12}			0.18	9.4	0.20	9.2		
P_{13}			0.15	9.8				

graphed on a Sephadex G-25 column with an eluent of pH 7 are also symmetrical. The elution curves for some linear phosphates with higher p values have a relatively high background because of hydrolysis of the samples. The decomposition of the samples, however, is not so serious as to affect their elution positions because a greater part of each phosphate remained unchanged.

The K_{av} values of the linear phosphates at pH 2.0, 4.6, 7.0 and 9.2 are shown in Table I, together with their effective sizes R_s estimated according to the method described in the preceding paper[11]. The distribution coefficient K_{av} is defined by eqn. 1,

$$K_{av} = \frac{V_e - V_o}{V_t - V_o} \tag{1}$$

where V_e is the elution volume of the solute, V_t the total volume of the gel bed and V_o the void volume outside the gel particles. The R_s values of the linear phosphates increase, as expected, with increase of the degree of polymerization.

It is well known that linear phosphates with a p higher than 5 are hydrolyzed in aqueous solution to produce cyclic trimetaphosphate and smaller linear phosphates. The rate of formation of trimetaphosphate increases with increase of the acidity of solution. Therefore, it could be expected that the linear phosphates in acidic solution would have a configuration close to a six-membered cyclic structure. Deformation of this kind would be reflected in the variation in K_{av} values. As shown in Table I, however, there is no distinguishable variation in the K_{av} values of the linear phosphates in the wide pH range of the solutions.

Many investigators have found linear relationships between elution volumes of solute molecules and logarithms of their molecular weights or degrees of polymerization. As can be seen in Fig. 2, there is a similar relationship between the K_{av} values

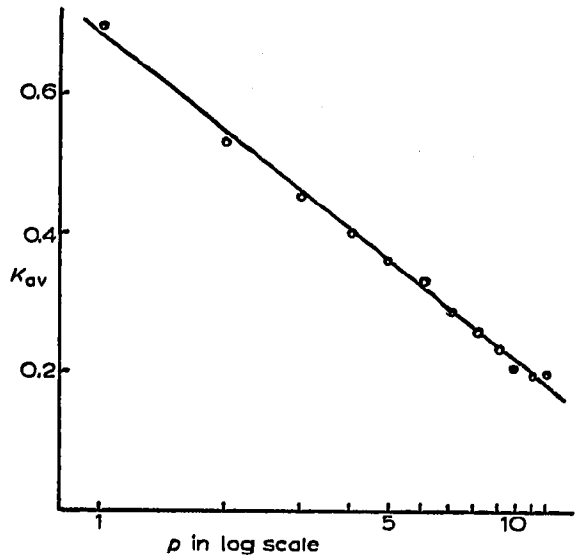

Fig. 2. Relationship between K_{av} and p. Gel, Sephadex G-25; bed volume, 150 ml; eluent, 0.1 M KCl, pH 7.0.

and the logarithms of the p values of the linear phosphates. Although this relationship is quite empirical, it is useful for estimating the molecular weights or distribution of the linear phosphates.

In order to test the applicability of the above relationship the following experiments were carried out. Several polyphosphate fractions, the $\bar{p}$ of which had preliminarily been determined by conventional pH titration, were chromatographed on a Sephadex G-25 column with a 0.1 M potassium chloride solution adjusted to pH 7.0. As an example, the elution curves for polyphosphates with $\bar{p}$ values of 3–13 are

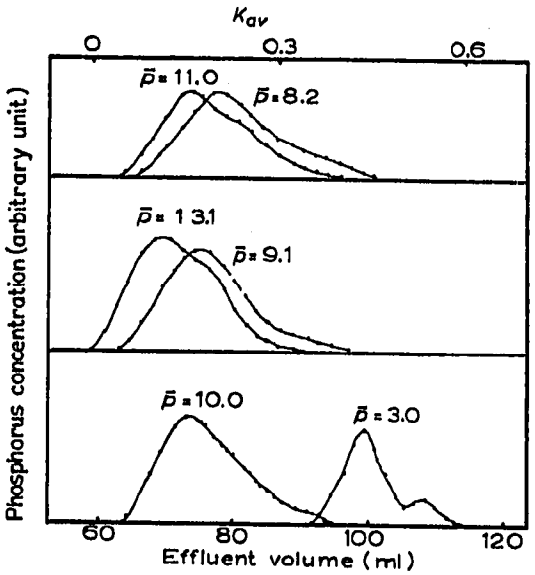

Fig. 3. Elution curves for some polyphosphate fractions. Gel, Sephadex G-25; bed volume, 150 ml; eluent, 0.1 M KCl, pH 7.0.

illustrated in Fig. 3. The $\bar{p}$ of a sample can be calculated from such an elution curve by eqn. 2,

$$\bar{p} = \frac{\Sigma x_i}{\Sigma(x_i/\bar{p}_i)} \quad (2)$$

where x_i denotes the content of phosphorus in the i-th fraction of the effluent and $\bar{p}_i$ is the average degree of polymerization of the linear phosphates in the i-th fraction, which can be determined from the linear relationship in Fig. 2. The $\bar{p}$ values of the

TABLE II

AVERAGE DEGREES OF POLYMERIZATION OF SODIUM POLYPHOSPHATE FRACTIONS DETERMINED BY GEL CHROMATOGRAPHY AND pH TITRATION

Gel, Sephadex G-25; bed volume, 150 ml; eluent, 0.1 *M* KCl (pH 7.0).

Gel chromatography on Sephadex G-25	*pH titration*
3.2	3.0
8.4	8.2
9.3	9.1
9.5	10.1
12.0	11.0
14.0	13.1

polyphosphate fractions calculated in this manner are listed in Table II. Good agreement is observed between the $\bar{p}$ values determined by both methods.

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