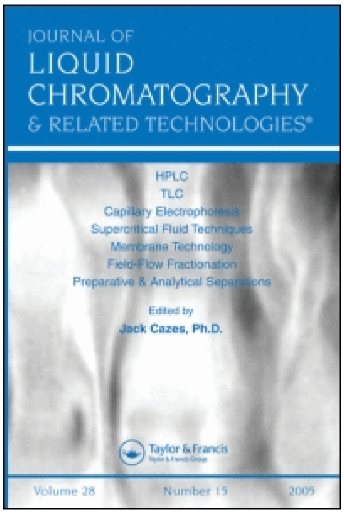

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Gel Chromatographic Evaluation of the Binding Ability of Linear Phosphate Anions to Magnesium Ions

Tohru Miyajima[a]; Shigeru Ohashi[a]
[a] Department of Chemistry, Faculty of Science, Kyushu University, Higashiku, Fukuoka, Japan

To cite this Article Miyajima, Tohru and Ohashi, Shigeru(1982) 'Gel Chromatographic Evaluation of the Binding Ability of Linear Phosphate Anions to Magnesium Ions', Journal of Liquid Chromatography & Related Technologies, 5: 9, 1787 — 1799

To link to this Article: DOI: 10.1080/01483918208067613
URL: http://dx.doi.org/10.1080/01483918208067613

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(9), 1787-1799 (1982)

GEL CHROMATOGRAPHIC EVALUATION OF THE BINDING ABILITY OF LINEAR PHOSPHATE ANIONS TO MAGNESIUM IONS

Tohru Miyajima and Shigeru Ohashi
Department of Chemistry, Faculty of Science, Kyushu University 33
Hakozaki, Higashiku, Fukuoka, 812 JAPAN

ABSTRACT

The binding ability of linear phosphate anions of various degrees of polymerization to magnesium ion has been evaluated by a zonal gel chromatographic method at pH 9.8 and 4.2. The average number of bound magnesium per one phosphate unit was determined at free magnesium concentration of 1.00×10^{-5} M in 0.1 M tetramethylammonium chloride solution at 25°C. At pH 4.2, the binding ability increased as the degrees of polymerization of the sample phosphate increased, while it decreased at pH 9.8. A gel chromatography-atomic absorption detector system was applied which allowed a rapid and continuous analysis.

INTRODUCTION

Linear phosphates (LP) of various degrees of polymerization have found widespread applications in industry because of their utility as a complexing agent. The binding characteristic of these homologous series of ligands is expected to be dependent on the numbers of phosphorus atoms which constitute the phosphate molecules. From a practical stand point, it is important to evaluate the binding abilities of various LP at a given condition.

However, since there is a considerable difficulties in the quantitative description of the ion-binding equilibria of polyelectrolyte (1), theoretical consideration has been limited mainly to relatively short LP, such as di- or triphosphate complexation.

In this work, instead of the determination of the critical equilibrium constants, a semiquantitative parameter is obtained and is compared among the LP of various degrees of polymerization, p. A zonal gel chromatographic method (2-5) has been applied, which determines the average number of bound metal ions per one phosphate unit of an LP molecule at a specified free metal concentration.

In order to automate the chromatographic procedure, an atomic absorption detector, AAD, (6-8) was applied. The fundamental problems encountered in the use of AAD as a flow detector of gel chromatography have also been examined.

THEORY

A zonal gel chromatographic method for the investigation of the binding of metal ion, M, to high-molecular-weight ligand, L, has previously been described in detail (3,5,7). A sample solution containing M, L and their complexes is applied to a gel column which is preequilibrated with an eluent containing M of a specified concentration, $[M]_0$, and is eluted with the same eluent. L and their complexes are excluded from the gel phase, while M migrates through the column with much slower velocity. By this procedure, the zone of ligand is forced to be equilibrated with the solution of M whose concentration is the predetermined value, $[M]_0$. After this zone is sufficiently equilibrated, the distribution of chemical species in this zone is characterized by the free metal concentration, $[M]_0$.

If the sample ligand is monodisperse, the average number of bound metal ions per one ligand, $\bar{n}$, can be determined as follows;

$$\bar{n} = \frac{\sum_{1}^{n} i\beta_i [M]_0^i}{1 + \sum_{1}^{n} \beta_i [M]_0^i} \qquad (1)$$

where β_i is the overall stability constant of the complex M_iL. In case where a mixture of LP is used as a ligand, $\bar{n}$ defined in eqn. (1) cannot be calculated. Instead, the average number of bound metal ions per one phosphate unit, $\bar{m}$, has been determined. As with $\bar{n}$, $\bar{m}$ is a function of $[M]_0$. When $[M]_0$ is kept constant, constant $\bar{m}$ value is expected to be obtained. The value of $\bar{m}$ can be calculated by dividing the amount of bound metal ion, N_M by the total amount of PO_3 unit, N_P, which is applied to the column.

EXPERIMENTAL

Chemicals

Sodium triphosphate hexahydrate (9) and ammonium tetraphosphate hexahydrate (10) have been prepared according to the literatures. Sodium phosphate glasses (a mixture of $Na_{p+2}P_pO_{3p+1}$) with average degrees of polymerization, $\bar{p}$, of 6.4, 9.2, 13.7, 22.2 were prepared according to the literatures (11,12). In order to prepare samples of LP of higher $\bar{p}$ value, a sodium phosphate glass whose $\bar{p}$ value was 75.8 was fractionated by solubility fractionation using acetone. The $\bar{p}$ values of the fractions were 36, 50, 85, 101 and 127. Molecular weight distribution analysis of these sample phosphates was carried out by a Sephadex G-100 column (12). All the sample solutions of LP were standardized colorimetrically with a molybdenum (V)-molybdenum (VI) reagent. All other chemicals were of analytical grade.

Elution experiments

A Kyowa KHU-W-188 reciprocating pump was used throughout. A column of 700x8 mm I.D. Pyrex tubing was packed with Sephadex

G-15 (Pharmacia). The temperature of the column was kept at 25.0 ± 0.1°C. The column was first conditioned with an eluent containing 1.00×10^{-5} M magnesium chloride, 0.1 M tetramethylammonium chloride and buffer agent of desired concentrations. Two types of buffer agent were used. One type was 2×10^{-3} M NH_3 - 10^{-3} M NH_4Cl (pH 9.8), and another type was 2×10^{-3} M CH_3COOH - 10^{-3} M CH_3COONa (pH 4.2). The sample solution contained magnesium chloride and sample phosphate in addition to tetramethylammonium chloride and buffer agent of the same concentrations as those in the eluent. A 0.6-ml portion of a sample solution was fed onto the column with a loop injector. The elution flow rate was kept at 2.18 ml/min. The effluent was introduced into the AAD (Perkin-Elmer 403) for the automatic recording of the absorbance of magnesium at 285.2 nm. No inhibition effect of polyphosphates on the atomization of magnesium has been observed (3,7,14). Elution patterns as shown in Figs. 5 and 8 were obtained. The horizontal lines at the rear edge of the complex peak promises that the sample ligand zone was sufficiently equilibrated with magnesium solution of 1.00×10^{-5} M.

RESULTS AND DISCUSSION

Atomic Absorption Detector (AAD)

Usually the column effluent flow rate, v_c, is much less than the nebulizer aspiration rate, v_c, which is adjusted to maximize sensitivity. In order to balance the flow, water was drawn into the AAD from an open reservoir through a three-way connector at a flow rate, v_b (Fig. 1). The value of v_b varies with the variation in v_c, keeping the relation, $v_n = v_b + v_c$. This system gives a stable stream of liquid to the AAD which produces a stable flame. One disadvantage of this flow system is the fact that the effluent is always diluted by a factor of v_c/v_n (8,14).

The diluting ratio, R, has been determined without the gel column in the following way. First, water was introduced to the flow system at a flow rate, v_c, and 1.00×10^{-5} M magnesium chloride

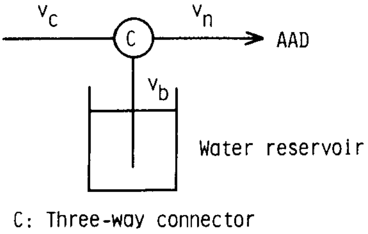

FIGURE 1.

Flow system of the AAD.

solution was drawn from the compensating reservoir (Experiment A). The absorbance of magnesium was measured at various flow rates, and was plotted against v_c (Fig. 2). Next, these two liquids were exchanged, i.e., 1.00×10^{-5} M magnesium chloride solution was introduced to the system at the flow rate of v_c, and water was drawn from the reservoir (Experiment B). The absorbance obtained was also plotted against v_c (Fig. 2). As can be seen from Fig. 2, the sum of the two absorbances at a specific v_c value was constant. This result ensures that v_n is independent of v_c, and indicates that the recorder response can be quantitatively correlated to the amount of sample introduced to the nebulizer. The value of R at a specific v_c value can be calculated by the following equation.

$$R = \frac{(\mathrm{Abs})_A}{(\mathrm{Abs})_A + (\mathrm{Abs})_B} \qquad (2)$$

where suffixes "A" and "B" represent the experiment "A" and "B", respectively. The calculated R value is plotted against v_c (Fig. 3). When v_c is 2.18 ml/min the column effluent is expected to be diluted by a factor of ca. 0.3.

In Fig. 4, the calibration graphs for magnesium chloride obtained at various pump flow rates are shown. It can be seen

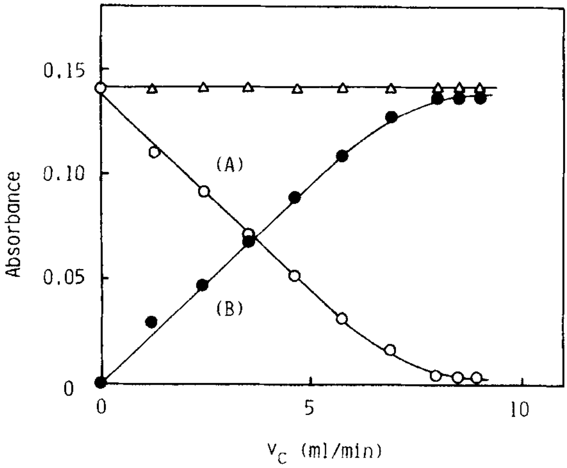

FIGURE 2.

Plots of absorbance of magnesium vs. v_c.

○ : $(Abs)_A$, ● : $(Abs)_B$, △ : $(Abs)_A + (Abs)_B$.

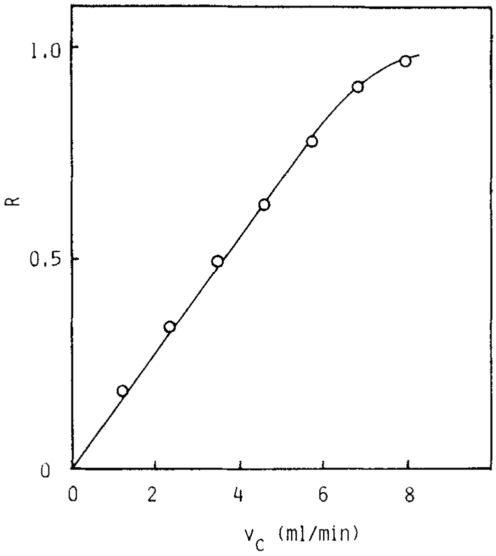

FIGURE 3.

Plots of R vs. v_c.

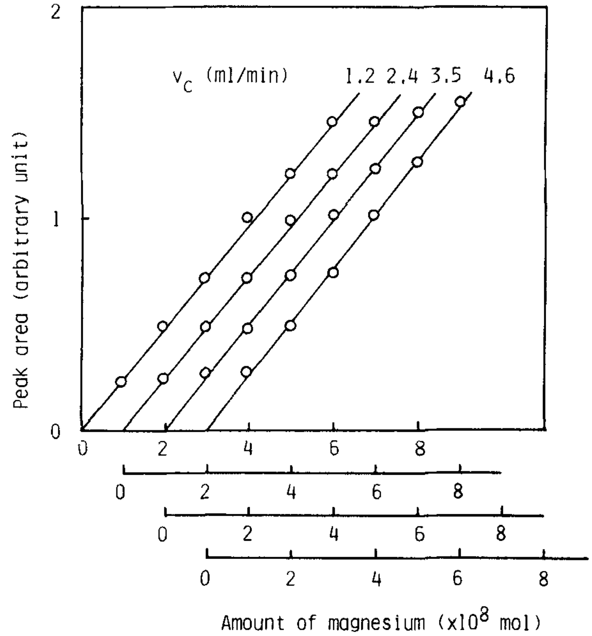

FIGURE 4.

Calibration graph for magnesium.
The value of v_c is shown in the figure.

that the plots give a good linearity. As expected, the slopes of the calibration graphs for various pump flow rates are almost consistent with each other.

Calculation of $\bar{m}$

The representative elution patterns obtained for the magnesium-triphosphate system are shown in Fig. 5. The positive peak corresponds to the complex and the negative peak reflects the deficiency of the free magnesium. The horizontal line at the rear edge of the complex peak assures that the sample ligand zone is sufficiently equilibrated. The area of the complex peak increased

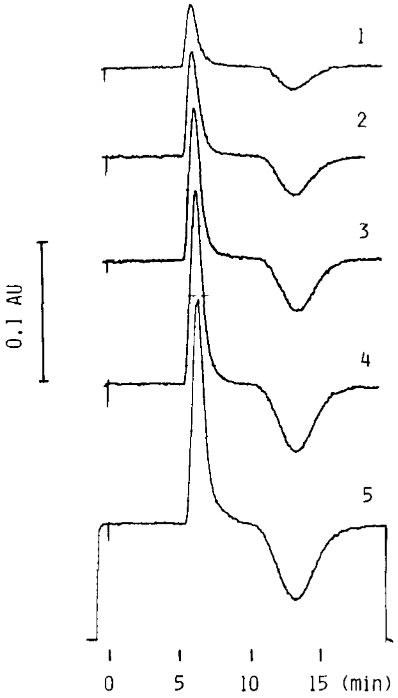

FIGURE 5.

Representative elution patterns of the Mg-P_3O_{10} system. $[M]_0 = 1.00 \times 10^{-5}$ M, pH 9.8. $N_P \times 10^9$ mol 1):0.37, 2):0.65, 3):0.90, 4):1.20, 5):1.46.

with an increase in the amount of triphosphate in the sample solution. N_M calculated from the peak area is plotted against N_P (Fig. 6). N_M increase is proportional to N_P. The slope of this straight line is the value of $\bar{m}$. As expected, a constant $\bar{m}$ value is obtained when free metal concentration is kept constant. In order to check the effect of flow rate on $\bar{m}$, the elution flow rate was varied between 1.3 and 3.5 ml/min. However, no change in $\bar{m}$ value could be detected at all.

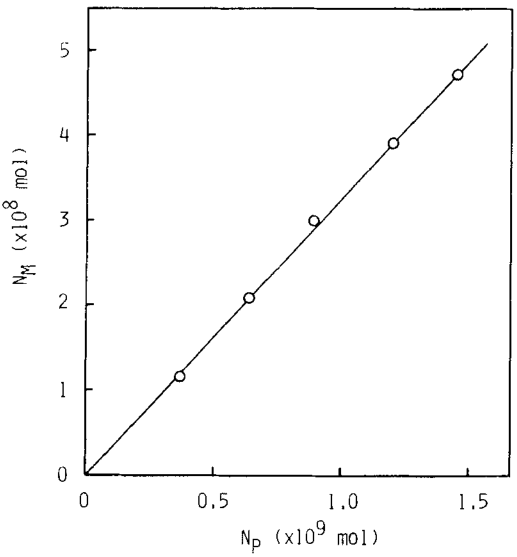

FIGURE 6.

Plots of N_M vs. N_P for the Mg-P_3O_{10} system. $[M]_0 = 1.00 \times 10^{-5}$ M, pH 9.8.

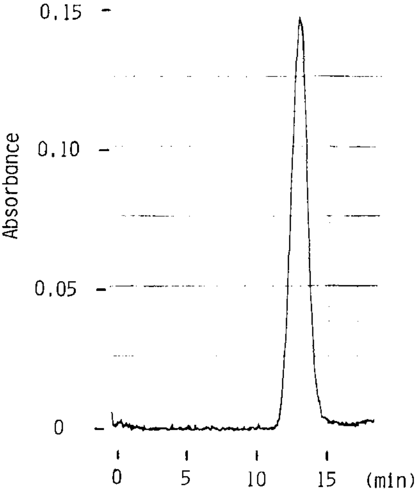

FIGURE 7.

Elution behavior of sodium ions in the Mg-P_3O_{10} system. Absorbance of sodium at 589.0 nm was monitored by the AAD. $[M]_0 = 1.00 \times 10^{-5}$ M, $N_P = 1.46 \times 10^{-9}$ mol, pH 9.8.

As source materials of LP, their sodium or ammonium salts have been used. The elution behavior of sodium ions in the magnesium-triphosphate system was monitored by the AAD (Fig. 7). Since the sodium ions are completely separated from sample ligand zone, it can be concluded that the ion-pair formation of sodium ions with phosphate anions need not to be taken into account in the calculation of $\bar{m}$. In Fig. 8, an example of the continuous analysis of magnesium-LP binding by the gel chromatography-AAD system is shown. Sample solutions could be applied to the column continuously every ten minutes.

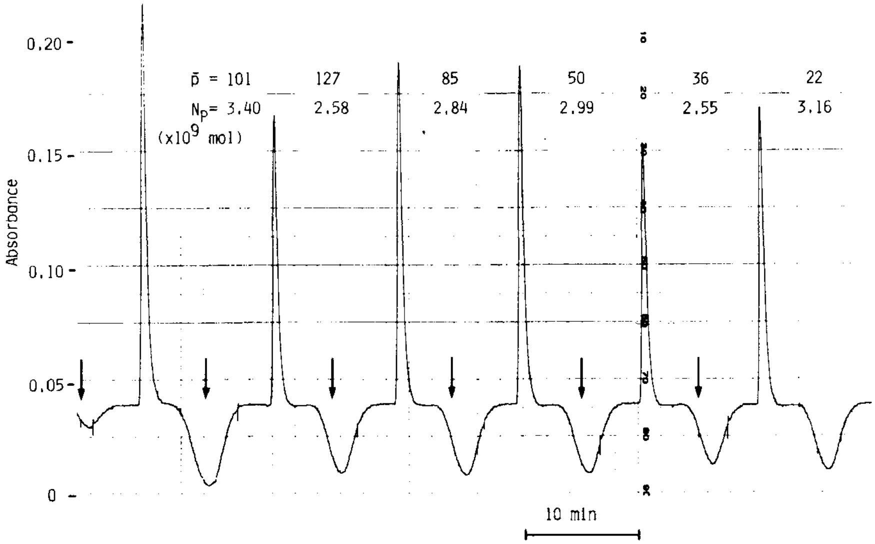

FIGURE 8.

Representative elution patterns of the Mg-LP systems (pH 4.2). The average degrees of polymerization, $\bar{p}$, and the N_p values of the samples are shown in the figure. Arrows show the time of sample injection.

Comparison of $\bar{m}$

The values of $\bar{m}$ thus obtained are plotted against the logarithms of p or $\bar{p}$ (Fig. 9). It can be seen that the binding characteristic of the LP anions is greatly dependent on the degrees of polymerization of the sample phosphate. It was found that at pH 9.8, di- or triphosphate has greater binding ability than the long LP, though at pH 4.2 an opposite trend is observed.

LP anions are composed of two types of phosphate units, i.e., end and middle ones which bear negative charge of two and one minus, respectively. At pH 9.8, where all the sample phosphates are considered to be almost completely dissociated, the average negative charge on one phosphate unit decreases with an increase in the degrees of polymerization. This may be the reason for the decrease in $\bar{m}$ value

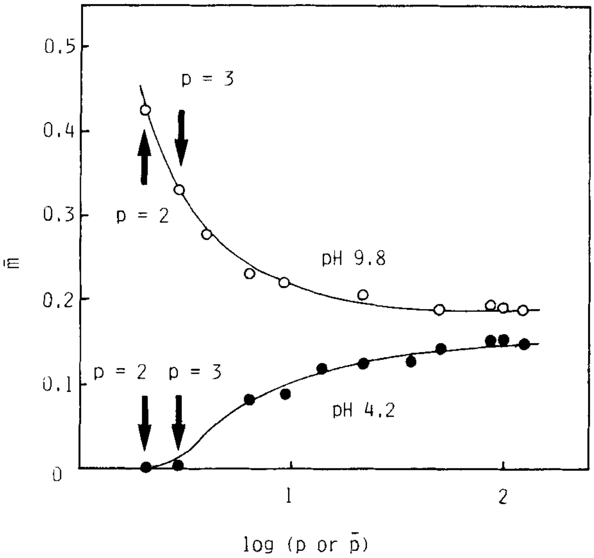

FIGURE 9.

Plots of $\bar{m}$ vs. log (p or $\bar{p}$).

with an increase in p or $\bar{p}$ at pH 9.8. However, at pH 4.2, each units of LP anions are considered to be almost monoprotonated. Under this condition, the contribution of end unit to the binding of metal ions greatly decreases, which results in the great decrease in the $\bar{m}$ values of short LP systems. Since the longer LP are composed mainly of middle units which are almost dissociated even at pH 4.2, the $\bar{m}$ values approach to those obtained at pH 9.8, when $\bar{p}$ increases.

It should be pointed out that the $\bar{m}$ values of the long LP are much greater than diphosphate at pH 4.2. At pH 4.2, diphosphate anions are considered to be in the form of $H_2P_2O_7^{2-}$ (15). Results obtained at pH 4.2 indicates that LP anion whose p value is 100 can bind magnesium much strongly than the group of fifty molecules of $H_2P_2O_7^{2-}$ can. This phenomenon should be attributed to the trapping of magnesium ions by the strong electric field on the long LP molecule formed by the crowded negative charge and/or some specific complex formation.

As has been pointed out in the section of "Theory", $\bar{m}$ value is a function of $[M]_0$. Therefore, in order to clarify the polymer-metal ion binding, it is necessary to determine $\bar{m}$ values under various free metal concentrations (16). Determination of $\bar{m}$ values at a specific free metal concentration, however, gives useful information for the comparison of the binding ability of these homologous series of ligands. The zonal gel chromatographic method is well suited for this purpose. A combination of gel chromatography with an AAD provides a rapid and continuous analysis technique for these polymer-metal ion interaction study.

ACKNOWLEDGEMENT

The present work was partially supported by a Grant-in-Aid for Scientific Research Nos. 574224 and 56104004 from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Marinsky, J. A., Coord. Chem. Rev., 19, 125(1976).
2. Hummel, J. P. and Dreyer, W. J., Biochem. Biophys. Acta, 63, 532(1962).
3. Miyajima, T. and Ohashi, S., Bull. Chem. Soc. Jpn., 51, 2543(1978).
4. Wood, G. C. and Cooper, P. F., Chromatogr. Rev., 12, 88(1970).
5. Yoza, N., J. Chem. Educ., 54, 284(1977).
6. Yoza, N. and Ohashi, S., Anal. Lett., 6, 595(1973).
7. Yoza, N., Kouchiyama, K. and Ohashi, S., At. Absorpt. Newsl., 18, 39(1979).
8. Yoza, N., Aoyagi, Y., Ohashi, S. and Tateda, A., Anal. Chim. Acta, 111, 163(1979).
9. Watters, J. I., Loughran, E. D. and Lambert, S. M., J. Am. Chem. Soc., 78, 4855(1956).
10. Griffith, E. J., J. Inorg. Nucl. Chem., 26, 1381(1964).
11. Van Wazer, J. R., J. Am. Chem. Soc., 72, 644(1950).
12. Miyajima, T., Yamauchi, K. and Ohashi, S., J. Liq. Chromatogr., 4, 1891(1981).
13. Van Wazer, J. R., J. Am. Chem. Soc., 72, 647(1950).
14. Kouchiyama, K., Yoza, N. and Ohashi, S., J. Chromatogr., 147, 271(1978).
15. McNabb, W. M., Hazel, J. F. and Baxter, R. A., J. Inorg. Nucl. Chem., 30, 1585(1968).
16. Onaka, T.,Miyajima, T. and Ohashi, S., J. Inorg. Nucl. Chem., 43, 3323(1981).