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GEL CHROMATOGRAPHY OF MAGNESIUM POLYPHOSPHATE COMPLEXES

AUTOMATIC MONITORING BY AN ATOMIC ABSORPTION FLOW DETECTOR

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SUMMARY

An atomic absorption flow detector has been employed to continuously monitor the gel chromatography of magnesium polyphosphate complexes. The pH of the eluent between 4.6 and 10 greatly affects the sensitivity of detection for triphosphate, but not for Kurrol's salt, $(KPO_3)_n$, which is highly polymeric. Kurrol's salt is a useful alternative to Blue Dextran as a standard material for determining the void volume of a Sephadex G-25 column. A speculation on the binding sites and stability constants of magnesium polyphosphate complexes is presented.

INTRODUCTION

Much interest has recently centred on the use of a conventional atomic absorption spectrophotometer as a flow detector for liquid chromatography[1–10]. A great advantage of the atomic absorption flow detector (AAD) over the widely used UV and RI detectors is its extreme specificity or selectivity[3,7]. The AAD has been successfully applied to the automatic and selective monitoring of various metal ions and metal complexes in chromatographic effluents with little or no interference[1–10].

In a previous paper[8] it was shown that AAD combined with gel chromatography (Sephadex G-25) was useful for the sensitive and quantitative monitoring of various inorganic polyphosphates such as diphosphate (P_2), triphosphate (P_3), tetraphosphate (P_4) and the highly polymeric Kurrol's salt (P_n). The method is based on the formation of, for example, a magnesium triphosphate complex, Mg–P_3, during the elution of P_3 on a column pre-equilibrated and eluted with a solution of magnesium chloride whose concentration, $[Mg]_0$, is known:

$$Mg + P_3 \rightleftarrows Mg\text{–}P_3 \qquad (1)$$

The amount of Mg–P_3 formed, Q_{Mg-P_3}, can be easily determined from the peak area of Mg–P_3 obtained by continuous monitoring of the atomic absorption due to

magnesium in the effluent. The total amount of P_3 to be determined, Q_{P_3}, is then evaluated from eqn. 2 (ref. 8):

$$Q_{P_3} = \frac{([Mg]_0 K_{Mg-P_3} + 1) Q_{Mg-P_3}}{[Mg]_0 K_{Mg-P}} \quad (2)$$

K_{Mg-P_3} in eqn. 2 is the stability constant given by

$$K_{Mg-P_3} = \frac{[Mg-P_3]}{[Mg]_0 [P_3]} \quad (3)$$

where the quantities in square brackets are molar concentrations.

Quantitation of the gel chromatographic behaviour requires knowledge of some column parameters[11,12]. One of the most important of these is the void volume. This is usually determined by means of Blue Dextran (molecular weight = 2,000,000) the elution volume of which can be measured colorimetrically. Unfortunately, Blue Dextran cannot be detected by AAD. Therefore, other high-molecular-weight compounds are required that are, like Blue Dextran, completely excluded from the gel phase and which can be detected by AAD.

This paper describes the continuous monitoring by AAD of the magnesium complex of the highly polymeric Kurrol's salt[13,14], $(KPO_3)_n$, which is useful as a marker for the void volume of a Sephadex G-25 column. The effect of pH on the sensitivities of detection of polyphosphates by AAD is also described. The sensitivity of Kurrol's salt was found to be less dependent on the pH of the eluent than that of triphosphate; this can be explained in terms of the different contributions of the terminal phosphate groups of the compounds to the complexation reactions.

EXPERIMENTAL

Materials

Sephadex G-25 fine and Blue Dextran 2000 were obtained from Pharmacia (Uppsala, Sweden). Orthophosphate ($Na_3PO_4 \cdot 12H_2O$), diphosphate ($Na_4P_2O_7 \cdot 10H_2O$) and magnesium chloride were guaranteed reagents from Wako (Osaka, Japan). Triphosphate ($Na_5P_3O_{10} \cdot 6H_2O$) was purified from anhydrous triphosphate (Wako). Kurrol's salt, $(KPO_3)_n$, was prepared according to the literature[14].

The eluents used were as follows: (A) 0.1 *M* sodium chloride, (B) 0.08 *M* ammonia–0.02 *M* ammonium chloride (pH 10), (C) 0.02 *M* acetic acid–0.02 *M* sodium acetate (pH 4.6), and (D) 0.02 *M* sodium chloride. B, C and D contained $1.03 \cdot 10^{-5}$ *M* magnesium chloride.

Procedure

An amount of 1 ml of a mixture of P_1, P_2, P_3 and P_n was eluted on a Sephadex G-25 column (97.5 × 1.5 cm I.D.) with eluent A. The amount of phosphate in each fraction (1.01 ml) was determined colorimetrically using a molybdenum(V)–molybdenum(VI) reagent at 830 nm (ref. 15). A mixture of P_n and blue dextran was similarly eluted. Blue Dextran was determined colorimetrically at 630 nm.

For the automatic recording by AAD, each sample solution was prepared by

dissolving the desired amounts of P_3, P_n and magnesium chloride in a solution containing the same background concentration of electrolyte as that in the eluent. 1 ml of the sample solution was injected onto a Sephadex G-25 column (89 × 1.5 cm I.D.) by use of a loop valve, and eluted using each of eluents B, C and D. The flow-rate was maintained at 1.77 ± 0.01 ml/min using a pump. The atomic absorption of the effluent at 285.2 nm was monitored by AAD (Perkin-Elmer 403) and automatically recorded at a chart speed of 5 mm/min.

RESULTS AND DISCUSSION

Elution profiles of polyphosphate ions

A mixed solution of orthophosphate, diphosphate, triphosphate and Kurrol's salt was eluted with eluent A and each fraction was colorimetrically determined for phosphorus by the heteropoly blue method[15]. As shown in Fig. 1, the elution volumes of P_1, P_2, P_3 and P_n decrease with increasing degree of polymerization, which is in accordance with earlier conclusions based on the concept of steric exclusion[15,16]. A relatively sharp peak appears for Kurrol's salt, regardless of its polydisperse character[13,14], well in advance of the elution positions of the other phosphates.

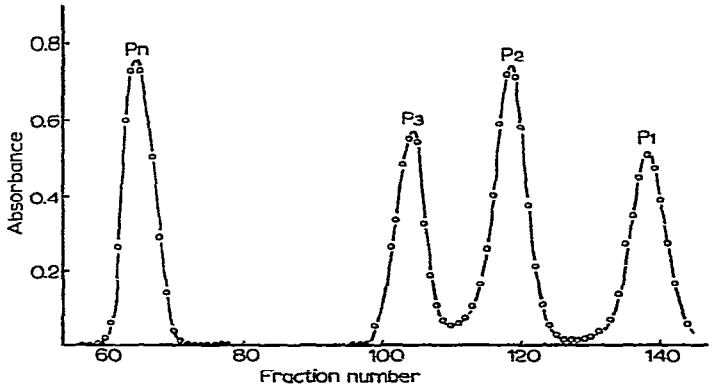


Fig. 1. An elution curve for a mixture of orthophosphate, diphosphate, triphosphate and Kurrol's salt on a Sephadex G-25 column. Eluent, 0.1 *M* NaCl. Samples: P_1, 3 μmole; P_2, 2 μmole; P_3, 1 μmole; P_n, 4 μmole (PO_3 units).

In order to compare the elution position of Kurrol's salt with that of Blue Dextran, a mixture of both compounds was also eluted with eluent A. Two peaks overlapped at the elution volume corresponding to 38% of the total column volume. This means that Kurrol's salt, as well as Blue Dextran, is completely excluded from the gel phase and can be used as a standard material for the determination of the void volume of a Sephadex G-25 column.

Flow system for AAD

In addition to a special device for the continuous detection of mercury compounds[10], there are two types of flow systems which differ in the method of introduction of the effluent into the nebulizer of the AAD. One of these systems was

developed by Manahan and co-workers[1–4] who introduced the effluent directly into the nebulizer. Because of its simplicity, this method has been widely employed not only for metal complexes[1–5] but also for organosilicon compounds[6]. A disadvantage of this flow system is the complicated dependence the of response on the flow-rate of the column effluent, which arises because the flow-rate is too low[3] to be balanced by the amount of liquid drawn by the aspirator of the AAD.

The flow system employed in this work (Fig. 2) was developed in our laboratory[7–9]. It permits water to be drawn into the AAD from an open reservoir at a flow-rate, V_b, sufficient to compensate for the "starvation of the burner". V_b varies with the variation in the flow-rate of the column effluent, V_c, but the total flow-rate, V_n ($= V_b + V_c$), into the nebulizer is kept constant. This means that the recorder response can be simply and quantitatively correlated with the amount of sample flowing into the nebulizer. A disadvantage of this system is that the effluent is diluted by a factor of $V_c/(V_b + V_c)$; however, in practice, this is not important for the detection of magnesium because of the high sensitivity.

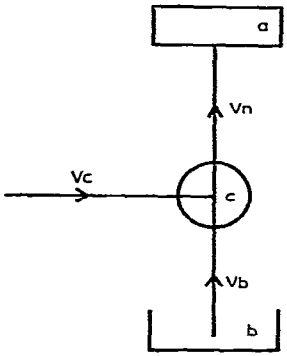


Fig. 2. The flow system of the AAD. a = Nebulizer (AAD); b = water reservoir; c = three-way connector. PTFE tubing (1 mm I.D.) was used.

Continuous monitoring of magnesium polyphosphate complexes by AAD

The gel chromatographic technique employed in this work is based on the method of Hummel and Dreyer[17] which has been increasingly applied to investigations of the interactions of metal ions with inorganic phosphates[8], humic and fulvic acids[18] and various biochemical compounds[19–21]. A characteristic advantage of this method is that the concentration of free magnesium ions, $[Mg]_0$, can be kept at a desired and predetermined value.

A Sephadex G-25 column was pre-equilibrated with eluent B (pH 10) containing magnesium ions, $[Mg]_0 = 1.0 \cdot 10^{-5}$ *M*. 1 ml of a sample solution containing triphosphate (0.1 μmole of P_3), Kurrol's salt (0.5 μmole of PO_3 units) and magnesium ions (0.5 μmole) was applied to the pre-equilibrated column and then eluted with eluent B. The absorbance at 285.2 nm, A_{285}, corresponding to the total concentration of magnesium in the effluent, was monitored by AAD and then plotted against the retention time. The resulting elution profile is shown in Fig. 3. Three peaks are formed at the elution positions of two magnesium complexes of P_n and P_3, *i.e.*, Mg–P_n and

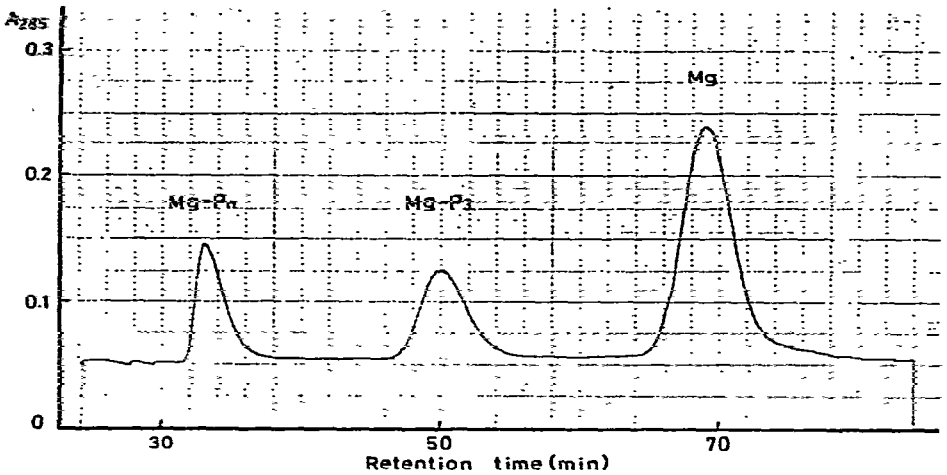


Fig. 3. An elution profile for magnesium complexes of triphosphate and Kurrol's salt and for magnesium ions on a Sephadex G-25 column at pH 10. Eluent, $[Mg]_0 = 1.03 \cdot 10^{-5}$ *M* (pH 10). Samples: P_3, 0.101 μmole; P_n, 0.495 μmole (PO_3 units); Mg^{2+}, 0.515 μmole.

Mg–P_3, and of free magnesium, Mg. The elution profile is different from the typical Hummel–Dreyer pattern[17–19] in which a negative peak should be observed for the free metal. The advantages of a positive peak for the free metal have been fully discussed[8,19–21].

The height of the horizontal base line corresponds to $[Mg]_0$, which is also the concentration level of free magnesium ions in the zones of Mg–P_n and Mg–P_3. On the basis of the standardized area below the base line, the peak areas of Mg–P_3 and Mg–P_n can be easily translated into the respective amounts Q_{Mg-P_3} and Q_{Mg-P_n} (ref. 19). Values of 0.089 and 0.085 μmol, respectively, were obtained. It was also confirmed that Q_{Mg-P_3} and Q_{Mg-P_n} vary according to the amounts of P_3 and P_n applied, which is in accordance with eqn. 2.

For the convenience of discussion, $\bar{n}$ is defined as the average number of magnesium ions bound to a ligand. Therefore, for a 1:1 Mg–P_3 complex, eqn. 2 can be rearranged as follows:

$$\bar{n}_{Mg-P_3} = \frac{Q_{Mg-P_3}}{Q_{P_3}} = \frac{[Mg]_0 K_{Mg-P_3}}{[Mg]_0 K_{Mg-P_3} + 1}$$

$\bar{n}_{Mg-P_3}$ is dependent on $[Mg]_0$ and K_{Mg-P_3}, but not on Q_{P_3}, the total amount of triphosphate applied.

A similar expression may be written for the complex between magnesium and Kurrol's salt, Mg–P_n:

$$\bar{n}_{Mg-P_n} = \frac{Q_{Mg-P_n}}{Q_{P_n}} \qquad (5)$$

Kurrol's salt, $(KPO_3)_n$, is composed of long chains of PO_3 units and its molecular weight is considered to be as high as 10^6 (refs. 13 and 14). Since the exact chain length

of Kurrol's salt is not known, Q_{P_n} in eqn. 5 is defined for convenience as the total number of PO_3 units of Kurrol's salt applied. Therefore, $\bar{n}_{Mg-P_n}$ means the average number of magnesium ions bound to a PO_3 unit in Kurrol's salt. From the results in Fig. 3, $\bar{n}_{Mg-P_3}$ and $\bar{n}_{Mg-P_n}$ were calculated to be 0.89 ± 0.01 and 0.17 ± 0.01, respectively.

pH dependences of $\bar{n}_{Mg-P_3}$ and $\bar{n}_{Mg-P_n}$

Since the dissociations of polyphosphoric acids are dependent on pH, it is important to examine the effect of pH on the $\bar{n}$ values or on the sensitivities to detection of P_3 and P_n by AAD. At pH 10 (Fig. 3) the sensitivity to detection of P_3 is greater than that of P_n. The results at lower pH values are discussed below.

Fig. 4 shows an elution curve at pH 4.6 for a mixture of P_3, P_n and Mg. The peak area for Mg–P_n is almost the same as that at pH 10 for the same amount of P_n. On the other hand, the peak area for Mg–P_3 has decreased in spite of the drastic increase in the amount of P_3 applied, from 0.1 μmol at pH 10 to 2 μmol at pH 4.6. When 0.1 μmol of P_3 was applied at pH 4.6 no detectable peak of Mg–P_3 was observed. The elution curves at an intermediate pH of 5.8 are also shown in Fig. 5 obtained by using eluent D, without buffering. The peak area for the Mg–P_n complex (Fig. 5a) was nearly equal to those at pH 10 and 4.6, while the sensitivity for P_3 at pH 5.8 (Fig. 5b) was intermediate between those at pH 10 and 4.6.

The overall feature of the pH dependences can be seen in Fig. 6 where $\bar{n}_{Mg-P_3}$ and $\bar{n}_{Mg-P_n}$ are plotted against the pH of the eluent. In contrast to the drastic change in $\bar{n}_{Mg-P_3}$ with increasing pH, $\bar{n}_{Mg-P_n}$ is almost unchanged. This indicates the usefulness of Kurrol's salt as a standard material for the determination of the void volume over a wide range of pH.

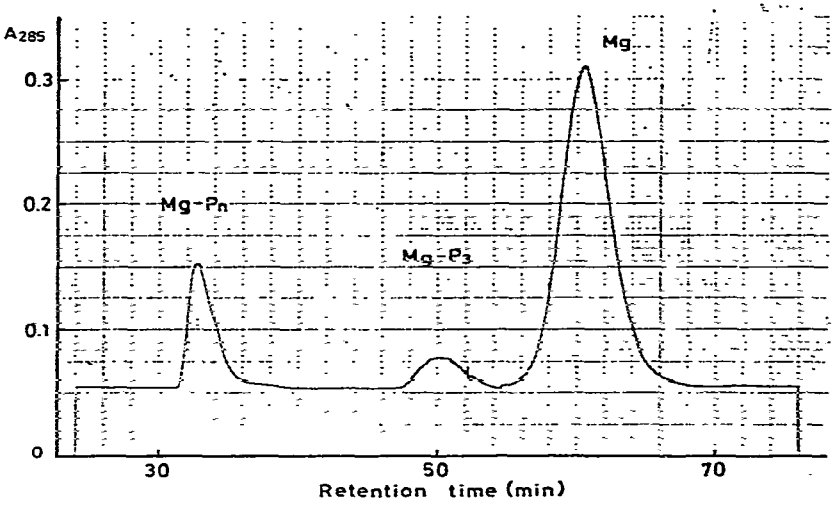


Fig. 4. An elution profile for magnesium complexes of triphosphate and Kurrol's salt and for magnesium ions on a Sephadex G-25 column. Eluent, $[Mg]_0 = 1.03 \cdot 10^{-5}$ *M* (pH 4.6). Samples: P_3, 2.02 μmole; P_n, 0.495 μmole (PO_3 units); Mg^{2+}, 0.515 μmole.

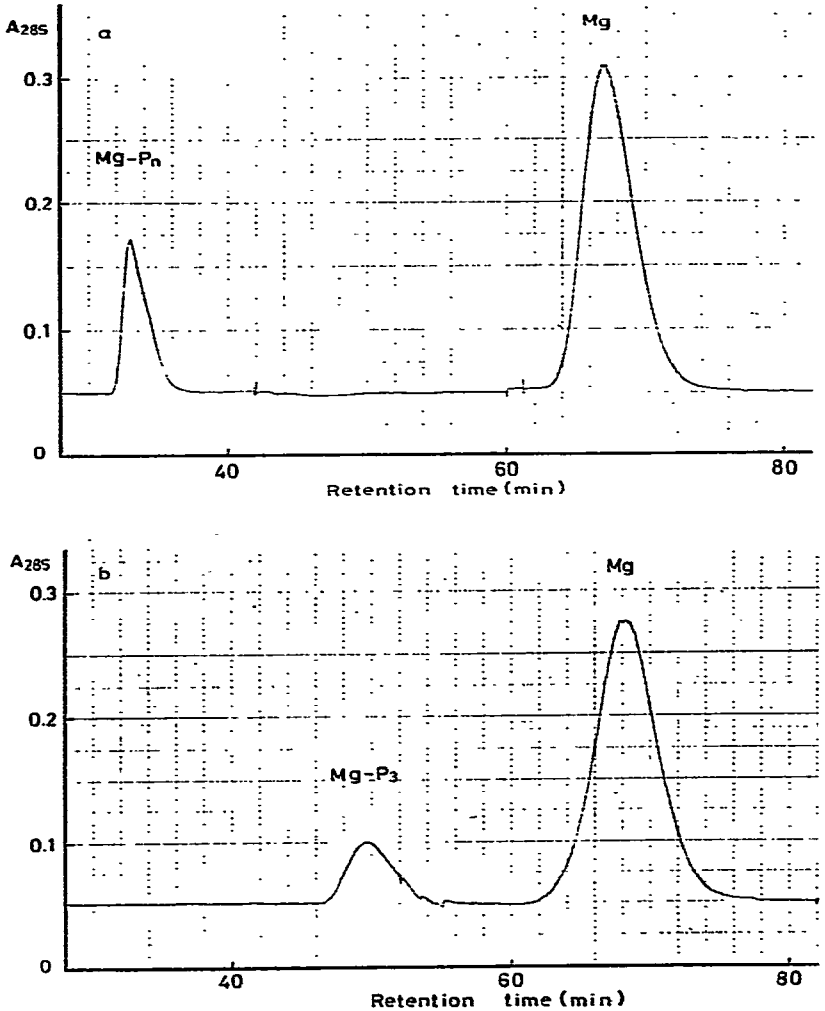


Fig. 5 (a) An elution profile for the magnesium complex of Kurrol's salt and for magnesium ions at pH *ca.* 5.8. Eluent, $[Mg]_0 = 1.03 \cdot 10^{-5}$ *M*. Samples: P_n, 0.5 μmole (PO_3 units); Mg^{2+}, 0.515 μmole. (b) An elution profile for the magnesium complex of triphosphate and for magnesium ions. Samples: P_3, 0.5 μmole; Mg^{2+}, 0.515 μmole. Other conditions as in (a).

Speculation on the binding sites and stability constants

Triphosphoric acid dissociates at pH 10 to give predominantly the non-protonated species, $P_3O_{10}^{5-}$, while at pH 4.6 the formation of $H_2P_3O_{10}^{3-}$, due to the partial protonation of two terminal phosphate groups, is considered to be predominant[13]. A binding model for the magnesium triphosphate complex (Fig. 7a), suggested by Watters and co-workers[22,23], seems to provide a reasonable interpretation of the pH dependence of the formation of $Mg–P_3$. If hydrogen ions compete with magnesium ions for binding with the terminal phosphate groups, it is expected that a decrease in pH of the eluent will result in a decrease in $\bar{n}_{Mg-P_3}$.

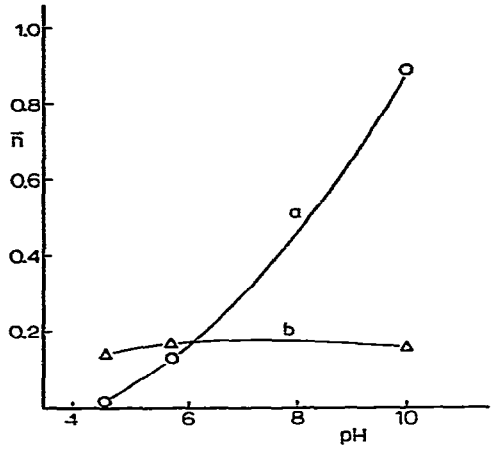
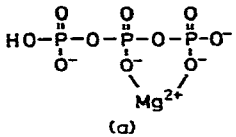


Fig. 6. The effect of pH on $\bar{n}$ for magnesium complexes of triphosphate (a) and Kurrol's salt (b).

Competition between hydrogen ions and magnesium ions for the terminal phosphate groups is also likely in Kurrol's salt. Therefore, if only the terminal phosphate groups are concerned with the complex formation, the formation of the Mg–P_n complex is also expected to be dependent on the pH of the eluent. However, this is not consistent with the experimental finding that $\bar{n}_{Mg-P_n}$ is practically independent of the pH of the eluent. The discrepancy can be explained by considering that the predominant complexing sites for magnesium (Fig. 7b) in P_n are the central phosphate groups which are unlikely to be protonated in the range pH 4.6–10. Assuming that the molecular weight of Kurrol's salt[14], $(KPO_3)_n$, is 10^6, the number of magnesium ions bound to a P_n molecule (not a PO_3 unit) is calculated to be as high as 1440 from the $\bar{n}_{Mg-P_n}$ value (0.17). This means that, at most, 0.2% of the magnesium is bound to the terminal phosphate groups.

An approximate value of the conditional stability constant[24] for the Mg–P_3 complex at pH 10 can be estimated from eqn. 4: $\log K_{Mg-P_3} = 5.9$. This value is somewhat greater than that for the magnesium diphosphate complex, $\log K_{Mg-P_2} = 5.6$ (ref. 8). The stability constant for the magnesium complex of Kurrol's salt is not



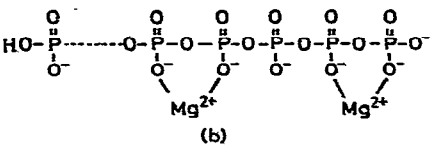


Fig. 7. Binding models for magnesium complexes of triphosphate (a) and Kurrol's salt (b).

readily determined because of the difficulty in defining the concentrations of the species involved which exhibit a complicated relation with the conformational factors of the highly polymeric ligands[25]. However, it is important to note the stoichiometry of the $Mg–P_n$ complex, *i.e.*, about six PO_3 units per magnesium. This value is unchanged even when $[Mg]_0$ is increased from $1.0 \cdot 10^{-5}$ *M* to $1.5 \cdot 10^{-5}$ *M*, which suggests that the complexing sites of the ligands are saturated. Further investigation is in progress to correlate the stoichiometric data with the conformational properties of Kurrol's salt.

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