Application of Gel Chromatography to the Determination of the Stability Constants of a Magnesium Complex with Diphosphate

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(Rceeived July 29, 1977)

Hummel-Dreyer's method was examined for its applicability to inorganic complexes, and a modified method was proposed. A sample solution containing magnesium and diphosphate was eluted with magnesium chloride solution on a gel column. The change of magnesium concentration in an effluent was measured with an atomic absorption detector. From the peak area of the eluted complex the stability constants ($\lg \beta_1$) at $I=0.1$ and 25 °C were evaluated to be 5.57 in tetramethylammonium chloride solution and 5.06 in sodium perchlorate solution. In the modified method the total magnesium concentration in a sample solution is made to be much higher than that in an eluent. This enables one to shorten the elution time.

Hummel and Dreyer first applied gel chromatography to the investigation of complex formation between biological macromolecules and low-molecular-weight substances.[1] Since then several workers have studied the complex formation of metal ions with biological macromolecules by this method.[2-6] However, no application has been reported to the complex formation of metal ions with relatively small ligands. In the present investigation, the fundamental problems of the gel chromatographic method for the determination of stability constants of such a metal complex were examined in detail, and the original Hummel-Dreyer's method was modified to overcome some difficulties encountered therein. As ligand, diphosphate ion was chosen, because it is fairly stable against hydrolytic degradation. As metal magnesium ion was chosen, because it was shown by our previous studies[7] that the elution behavior of magnesium on a gel column could be automatically recorded with an atomic absorption spectrophotometer. The stability constants of magnesium complexes with diphosphate were determined by this method, and the values obtained were compared with those obtained by other methods.

Theoretical

A reversible complex formation between a metal ion, M, and a ligand, L, is considered. L forms successively a series of complexes, ML, M_2L, ..., and M_nL. Charges are omitted for simplicity. The stability constant for the n-th complex is defined as follows:

$$\beta_n = \frac{[M_nL]}{[M]^n[L]}. \tag{1}$$

When the sample solution containing M, L, and the complexes is applied to a gel column conditioned with an eluent containing the same metal of a known concentration, $[M]_0$, and eluted with the same eluent, the elution pattern shown schematically in Fig. 1 is obtained. The initial free metal ion concentration in the sample solution, $[M]_s$, in general, differs from $[M]_0$. However, as the sample ligand zone (zone α) migrates through the column, the free metal ion concentration at zone α, $[M]_\alpha$, approaches $[M]_0$, and eventually becomes equal to $[M]_0$. After this time, the steady state at zone α is characterized by the specific condition that $[M]_\alpha=[M]_0$. A straightforward proof of the attainment of the above condition is the appearance of a horizontal line equal to $[M]_0$ at the right hand side of zone α, as shown in Fig. 1. Equation 1 can be rewritten as

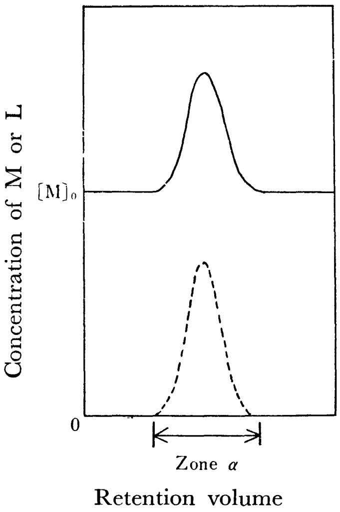

Fig. 1. Schematic elution pattern of zone α. The solid line refers to total metal ion concentration, and the broken line to total ligand concentration.

$$\beta_n = \frac{[M_nL]}{[M]_0^n[L]}. \tag{2}$$

The peak area of zone α represents the amount of bound metal which is present as complexes. The average number of bound metal ions per one ligand, $\bar{n}$, in zone α is obtained by dividing the amount of bound metal by the total amount of a ligand in zone α, which is equal to the total amount of the ligand applied to the column. $\bar{n}$ can be written as

$$\bar{n} = \frac{\sum_1^n i\beta_i[M]_0^i}{1+\sum_1^n \beta_i[M]_0^i}. \tag{3}$$

The following four prerequisites have to be fulfilled to use this method.

1. The size of the ligand should be much larger than that of the metal ion.
2. The peaks due to the ligand and the complexes should not be separated from one another. This can be achieved when the complex equilibria are established

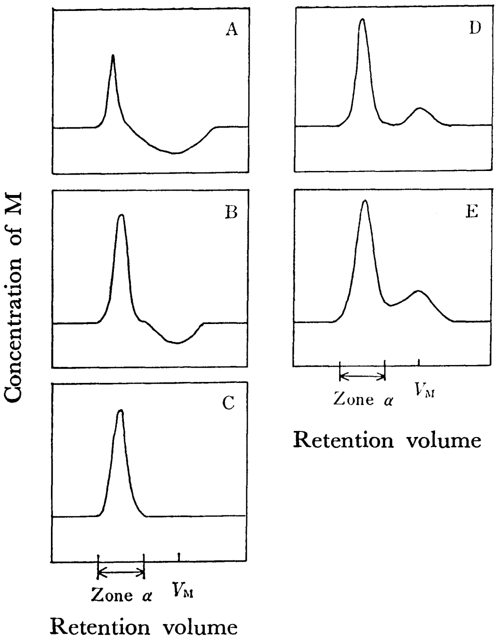

Fig. 2. Typical elution patterns of equilibrium systems of metal and ligand.

A; $[M]_s \ll [M]_0$ $[M]_\alpha \neq [M]_0$
B; $[M]_s < [M]_0$
C; $[M]_s = [M]_0$ $[M]_\alpha = [M]_0$
D; $[M]_s > [M]_0$
E; $[M]_s \gg [M]_0$ $[M]_\alpha \neq [M]_0$

so fast that this separation is prevented.

3. The condition of the steady state, *i.e.*, $[M]_\alpha = [M]_0$, should be establsihed before zone α leaves the column.

4. The ionic strength of the eluent should be kept sufficiently high to prevent the Donnan redistribution. In other words $[M]_\alpha$ should be equal to $[M]_0$.

Figure 2 shows the schematic elution patterns of the metal ion on the assumption that the total ligand concentration in a sample solution, $[L]_t$, and $[M]_0$ are kept constant and that the total metal ion concentration in a sample, $[M]_t$, is varied. If, incidentally, $[M]_s$ is equal to $[M]_0$, an elution pattern such as that shown in Fig. 2C is obtained. Here, only the broadening of zone α occurs. When the difference between $[M]_s$ and $[M]_0$ is relatively small, a pattern like Figs. 2B or 2D is obtained. At the retention volume of the free metal ion, V_M, a negative or positive peak appears. In the three cases mentioned above, the peak areas of zone α are equal to one another. This indicates that the steady state mentioned above is established . However, when $[M]_s$ is much lower or higher than $[M]_0$, the steady state cannot be established until zone α leaves the column (Fig. 2A or 2E). The peak area of zone α in Fig. 2A is smaller than those in Figs. 2B, 2C, and 2D, and that in Fig. 2E is larger.

According to Hummel-Dreyer's method, a sample solution is prepared by dissolving the ligand in an eluent containing metal ions of $[M]_0$. The procedure is particular in that $[M]_t$ is equal to $[M]_0$. The expected elution pattern will be like that in Figs. 2A or 2B. Hummel-Dreyer's method has the disadvantage that, if the free metal ion is significantly depleted by the complex formation, there is no appearance of a horizontal line between a positive and a negative peak, as shown in Fig. 2A. In this case a longer column should be used to separate the two peaks completely. This requires a longer time for elution. In order to overcome this difficulty, a sample solution should be prepared so as to contain metal ions of $[M]_t$ much higher than $[M]_0$.

Experimental

Chemicals. A stock solution of magnesium chloride was standardized volumetrically with EDTA, using Eriochrome Black T as an indicator. A stock solution of sodium diphosphate prepared from analytical grade tetrasodium diphosphate decahydrate was standardized colorimetrically with a molybdenum(V)-molybdenum(VI) reagent.[8] Tetramethylammonium chloride obtained from Tokyo Kasei Kogyo Co. was used without further purification as a background electrolyte.

High Speed Gel Chromatographic Device with an Atomic Absorption Detector. A column of 8×700 mm Pyrex tubing was packed with Sephadex G-15 (Pharmacia). The temperature of the column was kept at 25.0±0.1 °C. A JLC-P2 pumping system (JEOL) was used. A Model 403 atomic absorption spectrophotometer (Perkin Elmer) was used as a detector. The details of the present device were described elsewhere.[9—11]

Elution Experiments. The column was first conditioned with an eluent containing magnesium chloride of a desired concentration, 0.1 mol dm^{-3} tetramethylammonium chloride, and 5×10^{-3} mol dm^{-3} ammonia–10^{-3} mol dm^{-3} ammonium chloride as a buffer of pH 10. At this pH, the diphosphate ion is considered to be almost deprotonated. Sample solutions contained magnesium chloride and sodium diphosphate in varying concentrations, in addition to tetramethylammonium chloride, ammonia, and ammonium chloride of the same concentrations as those in the eluent. A 1.00 cm^3 portion of a sample solution was fed onto the column with an injection valve. The elution flow rate was kept at 1.71 cm^3 min^{-1}. The effluent was introduced into the detector for the automatic recording of the absorbance of magnesium at 285.2 nm. An effluent fraction of 2—6 min and that of 6—10 min were collected manually. The magnesium concentrations in these two fractions were determined by means of atomic absorption spectrophotometry.

Results and Discussion

The Effects of M and L Concentrations in a Sample Solution. The effects of $[M]_t$ and $[L]_t$ on the peak area of zone α were investigated for the complex formation of magnesium with diphosphate. The notations $[Mg]_0$, $[Mg]_t$, $[Mg]_s$, $[Mg]_\alpha$, and $[P]_t$ will be used hereafter. These correspond to $[M]_0$, $[M]_t$, $[M]_s$, $[M]_\alpha$, and $[L]_t$ which are defined in the "Theoretical" section. The elution patterns shown in Figs. 2A, 2B, *etc.*, will be called type A, type B, *etc.*

Figure 3 shows the peak areas of zone α for various $[Mg]_t$ and $[P]_t$. $[Mg]_0$ was fixed at 3.06×10^{-6} mol·dm^{-3}. In the region on the left hand side of line X, the elution patterns of type A were obtained. This means that $[Mg]_s \ll [Mg]_0$. In the region on the right hand side of line Y, the elution patterns of type E were obtained. This means that $[Mg]_s \gg [Mg]_0$. In the

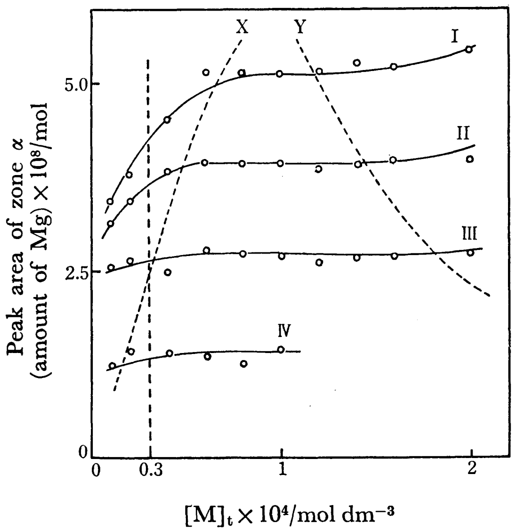

Fig. 3. Effects of $[Mg]_t$ and $[P]_t$ on the peak area of zone α.
$[Mg]_0=3.06\times10^{-6}$ mol dm^{-3}.
$[P]_t\times10^5$ /mol dm^{-3}; I: 9.90, II: 7.43, III: 4.95, IV: 2.48.

region between lines X and Y, the elution patterns of type B, C, or D were obtained. In these cases the steady state was established, *i.e.*, $[Mg]_\alpha=[Mg]_0$.

As shown in Fig. 3, for example, when $[Mg]_t=0.3\times10^{-4}$ mol dm^{-3}, the elution pattern of type B, C, or D will be obtained in case IV, but that of type A in cases I, II, and III. This indicates that, because $[P]_t$ is lower, the time for attainment of the condition that $[Mg]_\alpha=[Mg]_0$ is shorter. However, too low a value of $[P]_t$ leads to a small peak area of zone α, giving rise to a large error in the determination of $\bar{n}$. According to the original Hummel-Dreyer's method, $[Mg]_t$ is equal to $[Mg]_0$, *i.e.*, 3.06×10^{-6} mol dm^{-3}. In this case a much longer column should be used for the present purpose. Since in the modified method $[Mg]_t$ is made to be much higher than $[Mg]_0$, it is possible to use a shorter column.

The amount of bound magnesium thus obtained was plotted against the amount of total diphosphate. The resulting straight line is good evidence for the absence of an ML_2 type complex. The slope, $\bar{n}$, at $[Mg]_0=3.06\times10^{-6}$ mol dm^{-3} was 0.52.

The Effect of a Background Electrolyte. As mentioned above, it is important to confirm prerequisite 4. It should be noted that the anions of a background electrolyte in an eluent are excluded by the Donnan effect from zone α.[12] This in turn may result in the variation of magnesium ion concentration in zone α.

The magnitude of this effect was calculated according to the equation presented by Jordan *et al.*[13] in the case of the highest concentration of the fully deprotonated ligand used in this study, *i.e.*, 10^{-4} mol dm^{-3} of $P_2O_7{}^{4-}$. Even in this limiting case, the concentrations of free magnesium ions in the mobile and the stationary phases differ from each other by less than 0.5%.

A Source Material of Ligand. As a source material of diphosphate we used its sodium salt, but not its tetramethylammonium salt. The sodium ions in a sample solution are expected to be removed from zone α while this zone migrates through the column. In order to check this point, the sodium emission spectra of an effluent were examined. The peak of the sodium ions was found at 16 min and was completely separate from zone α (6—10 min, see Fig. 4). Therefore, it is concluded that we need not take into account the ion-pair formation of sodium ions with diphosphate ions. Consequently, we need not necessarily use a tetramethylammonium salt of diphosphate, which is usually employed for the determination of stability constants. This is the most remarkable characteristic of the gel chromatographic method.

Calculation of the Stability Constant. In Fig.4, the representative elution patterns for the magnesium–diphosphate system are shown. The positive peak at 7.7 min corresponds to the complexes formed (zone α), and the positive or the negative peak in the range of 12—17 min corresponds to free magnesium ions. When the negative peak appears, it is not clear whether zone α has reached the steady state or not, because of the overlapping of the positive peak at 7.7 min and the negative peak. On the contrary, zone α reaches the steady state when there appears a horizontal line between the rear edge of zone α and the front edge of a positive peak of free magnesium ions.

The amounts of bound magnesium can be calculated directly from the peak area of complexes.[11] In

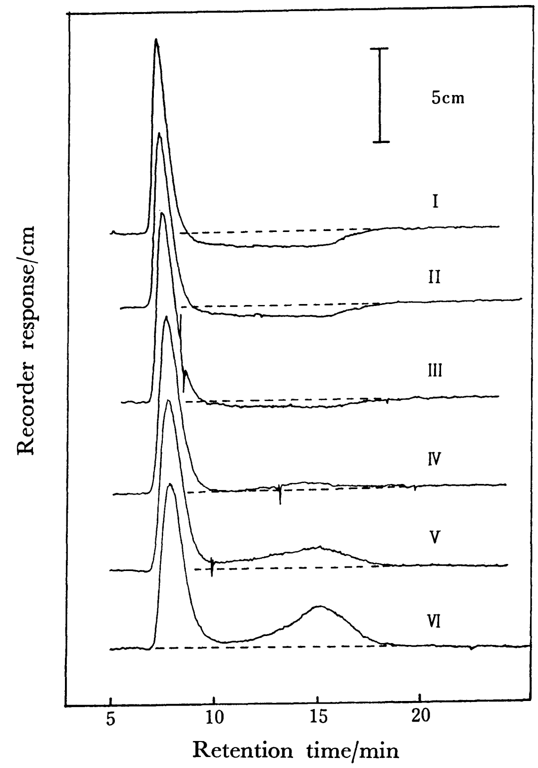

Fig. 4. Representative elution patterns of the Mg–P_2O_7 system.
$[Mg]_0=3.06\times10^{-6}$ mol dm^{-3}, $[P]_t=1.02\times10^{-4}$ mol·dm^{-3}.
$[Mg]_t\times10^5$ /mol dm^{-3}; I: 0.977, II: 1.95, III: 3.91, IV: 5.96, V: 7.92, VI: 9.77.

Table 1. The data of the gel chromatographic analysis for the Mg-P_2O_7 system ($[P]_t = 0.828 \times 10^{-4}$ mol dm^{-3})

$[Mg]_0 \times 10^7$ / mol dm^{-3}	$[Mg]_t \times 10^5$ / mol dm^{-3}	$\bar{n}$
3.21	0.988	0.11
3.93	0.988	0.12
4.94	0.988	0.14
6.22	0.988	0.18
7.88	0.988	0.22
9.88	1.98	0.28
12.5	1.98	0.32
15.6	1.98	0.37
19.8	1.98	0.42
24.7	1.98	0.50
31.2	3.95	0.54
39.3	5.93	0.61
49.5	5.93	0.66
62.3	5.93	0.70
78.4	5.93	0.75
98.8	5.93	0.77
125	7.90	0.83
156	7.90	0.86
198	7.90	0.88

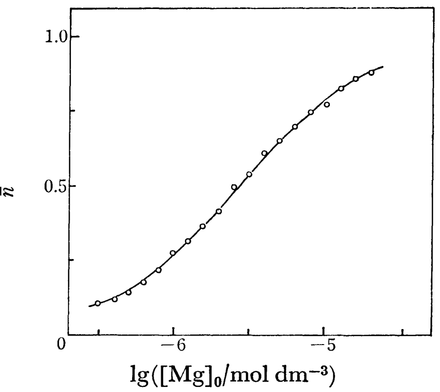

Fig. 5. Plots of $\bar{n}$ *vs.* lg $[Mg]_0$.
$[P]_t = 0.828 \times 10^{-4}$ mol dm^{-3}.
The solid line is calculated from Eq. 3, when only the formation of an ML type complex is considered, and lg $\beta_1 = 5.57$.

this work, however, an effluent was fractionated as follows in order to determine $\bar{n}$ more precisely. The amount of bound magnesium was calculated from the difference between the total amount of magnesium in zone α (fraction of 6—10 min) and the amount of free magnesium ions in the preceding zone (fraction of 2—6 min). $\bar{n}$ obtained is tabulated in Table 1. The elution patterns obtained in these experiments were types B, C, and D. In Fig. 5 the plots of $\bar{n}$ *versus* lg $[Mg]_0$ are shown.

Since $\bar{n}$ is between 0.1 and 0.9, only the complexes of ML and M_2L type are taken into consideration. Equation 3 can be written as

$$\bar{n} = \frac{\beta_1[Mg]_0 + 2\beta_2[Mg]_0^2}{1 + \beta_1[Mg]_0 + \beta_2[Mg]_0^2}. \tag{4}$$

Equation 4 can be rearranged into

$$\frac{\bar{n}}{2-\bar{n}} \cdot \frac{1}{[Mg]_0^2} = \beta_2 + \beta_1 \frac{1-\bar{n}}{2-\bar{n}} \cdot \frac{1}{[Mg]_0}. \tag{5}$$

By plotting $\bar{n}/(2-\bar{n})[Mg]_0^2$ *versus* $(1-\bar{n})/(2-\bar{n})[Mg]_0$, we can obtain β_1 from the slope and β_2 from the intercept. By using the least squares method, β_1 was found to be 3.7×10^5 (lg $\beta_1 = 5.57$). Since the intercept was found to be nearly zero, it was concluded that the formation of the complex ML was predominant under these experimental conditions. The experimental plots in Fig. 5 lie close to the theoretical curve calculated from the value of lg β_1, 5.57.

The side reaction coefficient of ammonium ions for diphosphate ions is estimated to be nearly unity, because the stability constants of ion-pairs of ammonium ions are in general lower than those of potassium ions (lg β_1 for diphosphate, 0.80 at $I = 1$ and at 25 °C),[14] and $[NH_4^+] = 10^{-3}$ mol dm^{-3}.

The Stability Constants Obtained by Various Methods. For the purposes of comparison, the stability constant of an ML type complex of magnesium and diphosphate in sodium perchlorate solution was determined by both the gel chromatographic method and the ion-exchange method. For the latter, Schubert's method was employed.[15]

Table 2. Stability constants of MgP_2O_7 complex

lg (β_1/ mol^{-1} dm^3)	Method[a]	Ionic strength / mol dm^{-3}	Supporting electrolyte	Temp °C	Ref.
5.42	A	1	Me_4NBr	25	16
5.41	A	1	Me_4NCl	25	17
5.57	B	0.1	Me_4NCl	25	This work
4.7	A	0.1	$NaNO_3$	25	18
5.18	C	0.1	$NaClO_4$	21	This work
5.06	B	0.1	$NaClO_4$	25	This work

a) A: pH titration method; B: gel chromatographic method; C: cation-exchange method.

The stability constants of a magnesium–diphosphate complex thus obtained as well as those reported by other workers are listed in Table 2. The stability constants obtained by both the pH titration method and the gel chromatographic method agree fairly well with each other, even though the two methods are based on quite different principles. The stability constant obtained by the gel chromatographic method is somewhat larger than those obtained by the pH titration method. One of the possible reasons for this discrepancy may be due to the difference in the ionic strengths. The stability constants of the complex in sodium perchlorate solution obtained by both the ion-exchange method and the gel chromatographic method accord fairly well with each other. However, the stability constants of the complex in sodium nitrate solution obtained by the pH titration method is considered to be a little too low.

It has been shown by the present investigation that the gel chromatographic method is applicable even in the field of inorganic complex chemistry.

The authors wish to express their appreciation to Dr. Norimasa Yoza for his interest in this work and for many helpful suggestions.

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