Interaction of Alkaline Earth Metal Ions with Carboxylic Acids in Aqueous Solutions studied by ^{13}C NMR Spectroscopy

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^{13}C NMR spectroscopic measurements of aqueous solutions containing alkaline earth metal chloride and a carboxylic acid have been carried out to acquire some insight into the coordination manners of various carboxylic acids to alkaline earth metal ions. The dependence of the ^{13}C NMR signal positions of the carboxylate carbons on the magnesium ion concentration in a magnesium carboxylate system is a good index to understand the coordination manner of the carboxylate ion. The upfield shift of the carboxylate carbon signal with increasing magnesium ion concentration indicates that the carboxylate ion acts as a bidentate ligand to form a ring structure. Only in the citrate systems, the existence of a relatively stable complex is evidenced in which the citrate ion is expected to act as a tridentate ligand.

Key words: ^{13}C NMR Spectroscopy, Carboxylic Acids, Alkaline Earth Metals, Coordination, Chemical Shifts.

1. Introduction

Interactions between alkaline earth metal ions and carboxylate ions in aqueous media are usually very weak. They are so weak that the changes in solution IR spectra of carboxyl groups upon interconversion from the free non-coordinating carboxylate ions (free carboxylate ions) to the carboxylate ions coordinating to alkaline earth metal ions (bound carboxylate ions) are in general hard to observe. In ^{13}C NMR spectra, individual peaks of carbons in the free carboxylate ion and those of corresponding carbons in the bound carboxylate ion are usually not observed but a single peak, whose chemical shift is the concentration-weighted average of those of the free and bound carboxylate ions. This situation is in contrast with the situation for strong metal ion-carboxylate ion interactions as exemplified by uranyl carboxylate complexes [1] and molybdenum tartrate complexes [2] in aqueous solutions, where two distinct peaks of two corresponding carbons in free and bound carboxylate ions are individually observed.

In a previous paper [3], we could obtain information on coordination manners of the acetate and lactate ions to the alkaline earth metal ions. This was made possible by ^{13}C NMR measurements of solutions containing one of the alkaline earth metal ions and either of the two carboxylate ions, coupled with isotope effects of those metals observed in cation exchange chromatography [4–7]. In ^{13}C NMR measurements, it was critically important to make measurements in the proton non-decoupling manner under the condition of the common and constant ionic strength for every NMR sample [3]. We extended the ^{13}C NMR measurements to solutions dissolving carboxylic acids other than acetic and lactic acids in order to understand systematically the interactions between alkaline earth metal ions and carboxylate ions. This paper reports the results of such experiments.

2. Experimental

2.1 Preparation of Samples for ^{13}C NMR Measurements

Alkaline earth metal ions studied in this work were magnesium, calcium, strontium and barium ions. Carboxylic acids studied were; formic acid $HCOOH$, acetic acid CH_3COOH and propionic acid CH_3CH_2COOH as monocarboxylic acids; glycollic acid $CH_2(OH)COOH$, lactic acid $CH_3CH(OH)COOH$ and 3-hydroxybutyric acid $CH_3CH(OH)CH_2COOH$ as hydroxycarboxylic acids; malonic acid $CH_2(COOH)_2$, succinic acid $HOOCCH_2CH_2COOH$ and methylsuccinic acid $HOOCCH_2CH(CH_3)COOH$ as dicarboxylic acids, malic acid $HOOCCH_2CH(OH)COOH$ as hydroxydicarboxylic acid; tartaric acid $HOOCCH(OH)CH(OH)COOH$ as dihydroxydicarboxylic acid; tricarballylic acid $HOOCCH_2CH(COOH)CH_2COOH$ as tricarboxylic acid; and citric acid $HOOCCH_2C(OH)(COOH)CH_2COOH$ and isocitric acid $HOOCCH_2CH(COOH)$-

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CH(OH)COOH as hydroxytricarboxylic acids. In addition, alkaline earth metal ion-1,3-butanediol, $CH_3CH(OH)$-$CH_2CH_2(OH)$, systems were studied to examine metal ion-hydroxyl group interactions.

Each solution for ^{13}C NMR measurements (NMR sample solution), which contained one of the alkaline earth metal ions and one of the carboxylic acids at predetermined concentrations, was prepared by dissolving measured amounts of the metal chloride and the acid into distilled water. The ionic strength of the sample solution was then adjusted at 0.60 M (M = mol/dm^3) by HCl or NaOH solution. The concentration of the metal ion was 0.0, 0.050, and 0.10 M in most cases, and that of the carboxylic acid was chosen so that the ratio of the metal concentration to the acid concentration decreased with the increase in the metal ion concentration at the given pH. Consequently, a higher metal ion concentration corresponded to a larger percentage of bound carboxylate ions and a smaller percentage of free carboxylate ions in the system.

The pH values of NMR sample solutions at which ^{13}C NMR measurements were made are tabulated in the second "pH" column of Table 1. The pH value was chosen so that the molecular carboxylic acid or the (hydrogen)carboxylate anion with the specified charge in the third "Charge" column of the table is the predominant species at that pH value, judging from the dissociation constant value(s) of the carboxylic acid and the values of complex formation constants between the carboxylic acid and alkaline earth metal ions [8]. For instance, the major chemical form of tricarballylic acid expected for the magnesium system with the magnesium ion concentration of 0.10 M and the pH value of 3.8 [9] is 6% MgHL (L^{3-} = tricarballylate ion), 12% MgH_2L^+, 23% H_3L, 49% H_2L^-, and 9% HL^{2-} at pH = 3.8. Thus, dihydrogentricarballylate ion (LH_2^-) accounts for 61% of all the chemical forms of tricarballylic acid, and consequently, "–1" is written in the third column at pH = "3.8" in the second column.

2.2 ^{13}C NMR Measurements

The NMR probe unit used was composed of a 10 mm o.d. glass probe and a 5 mm o.d. glass probe. 1,4-dioxane, used as external reference whose chemical shift δ was 67.8 ppm dissolved in about 1 cm^3 of D_2O used as a lock, was placed in the 5 mm o.d. probe. This probe was capped, inserted into the 10 mm o.d. probe and fixed with Teflon fixing kits. 3–4 cm^3 of an NMR sample solution was placed into the 10 mm o.d. probe.

Table 1. Summary of changes in ^{13}C NMR chemical shifts with increasing metal ion concentrations.

Carboxylic acid	pH	Charge	Carbon	Shift			
				Mg	Ca	Sr	Ba
Formic acid HCOOH	7.0	–1		→	↘	↘	↘
Acetic acid ${}^{a}CH_3{}^{b}COOH$	3.0	0	a	→	→		
			b	↘	↘		
	7.0	–1	a	↗	↗	→	↗
			b	↘	↘	↘	↘
Propionic acid ${}^{a}CH_3{}^{b}CH_2{}^{c}COOH$	3.0	0	a	→	→		
			b	→	→		
			c	↘	↘		
	7.0	–1	a	↗	↗	↗	↗
			b	↗	↗	↗	↗
			c	↘	↘	↘	↘
Glycolic acid ${}^{a}CH_2(OH){}^{b}COOH$	7.0	–1	a	↗		↘	↘
			b	↗		↘	↘
Lactic acid ${}^{a}CH_3{}^{b}CH(OH){}^{c}COOH$	1.5	0	a	↗	→		
			b	↘	↘		
			c	↘	↘		
	7.0	–1	a	↗	↘	↘	↘
			b	↗	↘	↘	↘
			c	↗	↘	↘	↘
3-hydroxybutyric acid ${}^{a}CH_3{}^{b}CH(OH){}^{c}CH_2{}^{d}COOH$	7.0	–1	a	↗	→	↗	↗
			b	→	→	→	→
			c	↗	↗	↗	↗
			d	↘	↘	↘	↘
Malonic acid ${}^{a}CH_2({}^{b}COOH)_2$	4.2	–1	a	↘			
			b	↘			
	7.0	–2	a	↗			
			b	↗			
Succinic acid $({}^{a}CH_2{}^{b}COOH)_2$	4.6	–1	a	↘	↘		
			b	↘	↘		
	7.0	–2	a	↗	↗		
			b	↘	↘		
Methylsuccinic acid $HOO{}^{a}C{}^{b}CH_2$- ${}^{c}CH({}^{d}CH_3){}^{e}COOH$	4.6	–1	a	↘			
			b	↘			
			c	↘			
			d	↘			
			e	↘			
	7.0	–2	a	↘			
			b	↗			
			c	→			
			d	→			
			e	↘			
Malic acid $HOO{}^{a}C{}^{b}CH_2$- ${}^{c}CH(OH){}^{d}COOH$	4.0	–1	a	↘	↘	↘	↘
			b	→	→	→	→
			c	↘	↘	↘	↘
			d	↘	↘	↘	↘
	7.0	–2	a	↗	→	→	→
			b	↗	↗	↗	↗
			c	↗	↗	↗	↗
			d	↗	↘	↘	↘
Tartaric acid $({}^{a}CH(OH){}^{b}COOH)_2$	5.5	–2	a	→			
			b	↗			

Table 1. (continued).

Carboxylic acid	pH	Charge	Carbon	Shift			
				Mg	Ca	Sr	Ba
Tricarballylic acid $HOO^aC^bCH_2$- $^cCH(^dCOOH)^bCH_2{}^aCOOH$	3.8	−1	a	↘↗	↘↗	↘→	↘→
			b	↘↗	↘↗	↘→	↘→
			c	↘↗	↘↗	↘→	↘→
			d	↘↗	↘↗	↘→	↘→
	5.1	−2	a	↘	↘	↘	↘
			b	↘	↘	↘	↘
			c	↘	↘	↘	↘
			d	↘	↘	↘	↘
	7.0	−3	a	↘	↘	↘	↘
			b	→	→	→	→
			c	↗	↗	↗	↗
			d	↘	↘	↘	↘
Citric acid $HOO^aC^bCH_2{}^cC(OH)$- $(^dCOOH)^bCH_2{}^aCOOH$	3.8	−1	a	↘	↘	↘	↘
			b	→	→	→	→
			c	↘	↘	↘	↘
			d	↘	↘	↘	↘
	5.2	−2	a	↘	↘	↘	↘
			b	↗	→	↘	↘
			c	↘	↘	↘	↘
			d	↘	↘	↘	↘
	7.0	−3	a	↗	→	→	→
			b	↗	↗	↗	→
			c	↘	↘	↘	↘
			d	↗	↘	↘	↘
Isocitric acid $HOO^aC^bCH_2{}^cCH(^dCOOH)$- $^eCH(OH)^f(COOH)$	3.6	−1	a	→	↘		
			b	→	→		
			c	→	→		
			d	→	→		
			e	→	↘		
			f	→	↘		
	5.0	−2	a	↘	↘		
			b	↘	↘		
			c	↗	↘		
			d	↘	↘		
			e	↗	↘		
			f	↘	↘		
	7.0	−3	a	↗	↘		
			b	↗	↗		
			c	↗	↗		
			d	↗	↘		
			e	↗	↗		
			f	↗	↘		
1,3-butanediol $^aCH_3{}^bCH(OH)^cCH_2{}^dCH_2(OH)$	7.0	0	a	→	→	→	→
			b	→	→	→	→
			c	→	→	→	→
			d	→	→	→	→

The NMR spectrometer was a JEOL JNM-GX270 operated at 67.8 MHz and 300 ± 0.5 K. The number of pulses accumulated for a measurement was 500–20,000, and the repetition time was 3.9 μs. The measurements were made in the proton non-decoupling manner as before [3]. Consequently, ^{13}C NMR signals were usually observed as multiplets. The peak position of a multiplet was determined by algebraically averaging the positions of the individual lines of the multiplet.

2.3 Measurements of IR Spectra

The IR spectrum of each NMR sample solution was taken by the attenuated total reflection method. The Perkin Elmer FTIR 1650 spectrometer was equipped with a cell for solution samples whose windows were made of ZnSe (Spectra Tech Inc. ATR-H-1403).

3. Results and Discussion

As an example of ^{13}C NMR spectra, in Fig. 1 is shown that of the calcium malate system with pH 4.0, 0.050 M calcium chloride and 0.45 M malic acid. At this pH value, the expected major species of the malic acid is the monovalent anion (hydrogenmalate ion), the amounts of the divalent anion and the neutral malic acid molecule being non-negligible. As is seen in Fig. 1, only four ^{13}C NMR signals are observed other than the signals of the reference [10]; no distinct ^{13}C NMR signals corresponding to neutral malic acid, free and bound hydrogenmalate ions and free and bound malate ions are observed for neither of the four kinds of carbons. This indicates the fast interchange among those chemical species of the malic acid. This situation also holds for the other alkaline earth metal ion-malic acid solutions and for the other carboxylic acids examined, with the only exception of the citrate systems, in which new ^{13}C NMR signals corresponding to relatively stable alkaline earth metal citrate complexes are observed. The citrate systems will be discussed independently later.

Each ^{13}C NMR signal shifts upfield or downfield or shifts little with increasing metal ion concentration within experimental uncertainty, with some exceptions. Trends in changes of ^{13}C NMR peak positions with increasing metal ion concentrations are summarized qualitatively in the "Shift" column in Table 1. The symbols ↘, ↗ and →, respectively, denote that the signal shifts downfield, shifts upfield and shifts little with increasing metal ion concentrations. The symbols ↘→ and ↘↗ denote that the signal shows first a downfield shift then little change and the signal shows first a downfield shift and then an upfield shift, respectively, with increasing metal ion concentration. Trends in the strontium and barium systems ("Sr" and "Ba" columns under "Shift" in Table 1) are in general what can be expected from the trend in the calcium systems. The trend in the magnesium system is, however, sometimes different from others, as has been observed in the previous paper [3].

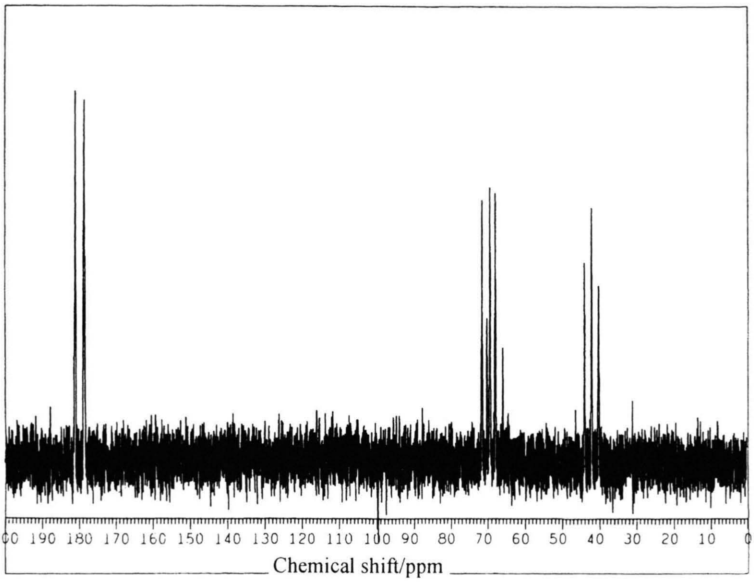

Fig. 1. A ^{13}C NMR spectrum of the calcium malate system. The NMR sample solution contains 0.050 M calcium chloride and 0.45 M malic acid and pH and the ionic strength are adjusted at 4.0 and 0.6 M, respectively. 1,4-dioxane is used as the external reference. Peak assignments are given in the text.

3.1 Interaction of Alkaline Earth Metal Ions with Monocarboxylate Ions

Formic, acetic and propionic acids are monocarboxylic acids. The ^{13}C NMR study [3] and isotope effect studies [4–7] of the acetate systems suggested that the acetate ion acts as a monodentate ligand coordinating to an alkaline earth metal ion using the single bonded oxygen of the carboxylate group as shown in Fig. 2 (Structure I). They also suggested that the magnesium ion behaves as if it were larger than the barium ion due to the hydration effect (Fig. 2, Structure II). In Fig. 3, we plot the chemical shifts of carboxylate carbon signals of the formate ion (Fig. 3a), the acetate ion (Fig. 3b) and the propionate ion (Fig. 3c) at pH = 7.0 against alkaline earth metal ion concentrations. At this pH value, practically no neutral carboxylic acid molecule exists in any system.

Metal ion concentration dependence of the peak positions of the carboxylate carbon signals of formate and propionate ions is similar to that of the acetate ion, which indicates that they act as monodentate ligands like the acetate ion. Thus, it may be possible to state generally that monocarboxylate ions are monodentate ligands when they coordinate to alkaline earth metal ions in aqueous solutions.

The metal ion concentration dependences of the change in chemical shifts in the formate system are small, compared with the corresponding dependences in the acetate and propionate systems. This may be attributable to the property of formic acid as an aldehyde, which acetic and propionic acids lack. We also notice in Fig. 3 that the metal ion concentration dependences in the acetate and propionate systems are very similar to

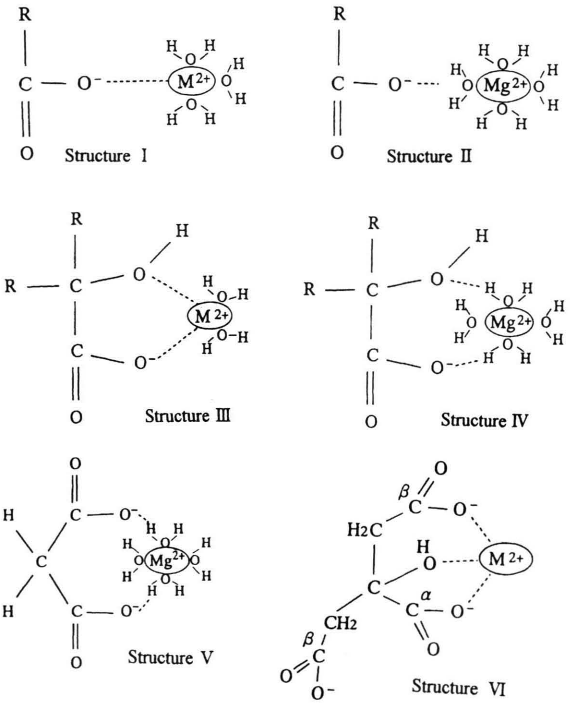

Fig. 2. Schematic drawings of possible coordination manner of carboxylate ions to alkaline earth metal ions in aqueous solutions.

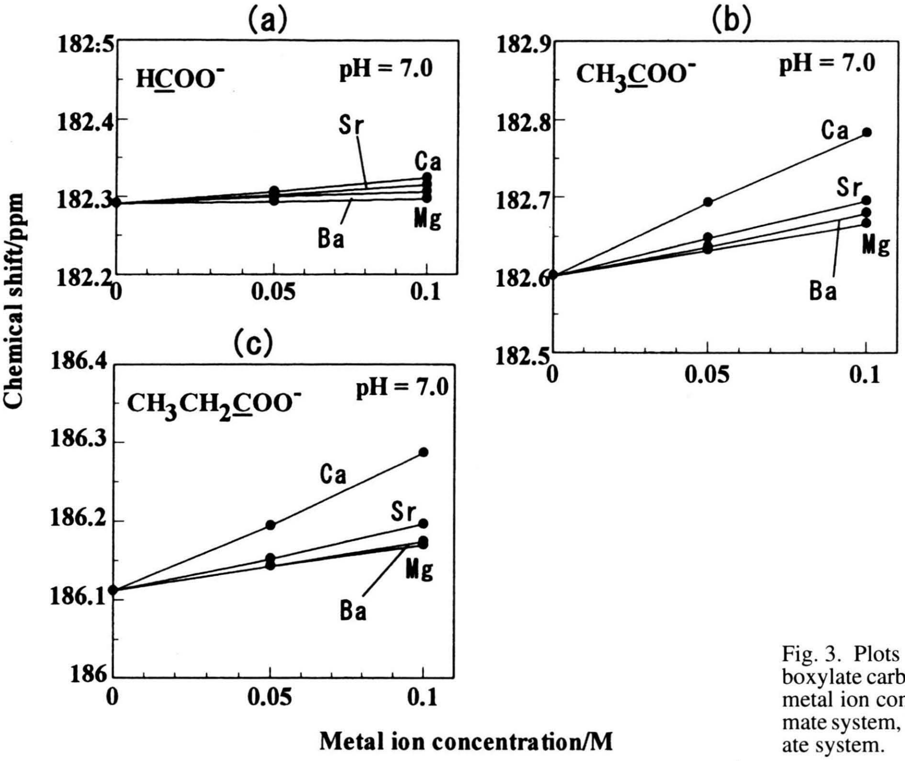

Fig. 3. Plots of chemical shifts of the carboxylate carbon signal against alkaline earth metal ion concentration at pH = 7.0. (a) formate system, (b) acetate system; (c) propionate system.

each other. This indicates that the influence of alkyl groups (CH_3- vs. CH_3CH_2-) on the interaction between the alkaline earth metal ion and the carboxylate group of monocarboxylate ions is small.

3.2 Interaction of Alkaline Earth Metal Ions with Hydroxycarboxylate Ions

Glycollic and lactic acids are α-hydroxycarboxylic acids (the carboxyl group is at α position against the hydroxyl group), and 3-hydroxybutyric acid is a β-hydroxycarboxylic acid. ^{13}C NMR and isotope effects studies in alkaline earth metal lactate systems [3–7] suggested that the lactate ion coordinates to alkaline earth metal ions using both the carboxylate group and the hydroxyl group to form a five member ring, as is shown in Fig. 2 (Structure III). They also suggested that the magnesium ion does not interact directly with lactate ions but indirectly through hydrating waters, as schematically drawn in Fig. 2 (Structure IV).

In Fig. 4 we plot the chemical shifts of carboxylate carbon signals (Fig. 4a) and methylene carbon ($\underline{C}H_2(OH)COO^-$) signals (Fig. 4b) of the glycolate ion at pH = 7.0 against the alkaline earth metal ion concentration. Those plots are very similar to the corresponding plots of the lactate ion in Figs. 4(c) and 4(d), suggesting that the coordination manner of the glycollate ion is the same as that of the lactate ion. That is, the glycolate ion is a bidentate ligand and coordinates to alkaline earth metal ions using both the carboxylate group and the hydroxyl group to form a five member ring. The most striking feature of the α-hydroxycarboxylate systems is the upfield change of the carboxylate carbon signal in the magnesium system with increasing magnesium ion concentration (Figs. 4(a) and 4(c)).

In Figs. 4(e) and 4(f), we plot the chemical shift of carboxylate carbon signals (Fig. 4(e)) and methine carbon ($\underline{C}H(OH)$) signals (Fig. 4(f)) of the 3-hydroxybutyrate ion at pH = 7.0 against the alkaline earth metal ion concentration. Contrary to the cases of the magnesium α-hydroxycarboxylate systems above, the carboxylate carbon signal of the magnesium 3-hydroxybutyrate system shows a downfield shift with increasing magnesium ion concentration. This behavior of the carboxylate carbon signal is similar to that of the carboxylate carbon signal in the magnesium monocarboxylate systems and indicates that the 3-hydroxybutyrate ion acts as a monodentate ligand in the coordination to alkaline earth metal ions.

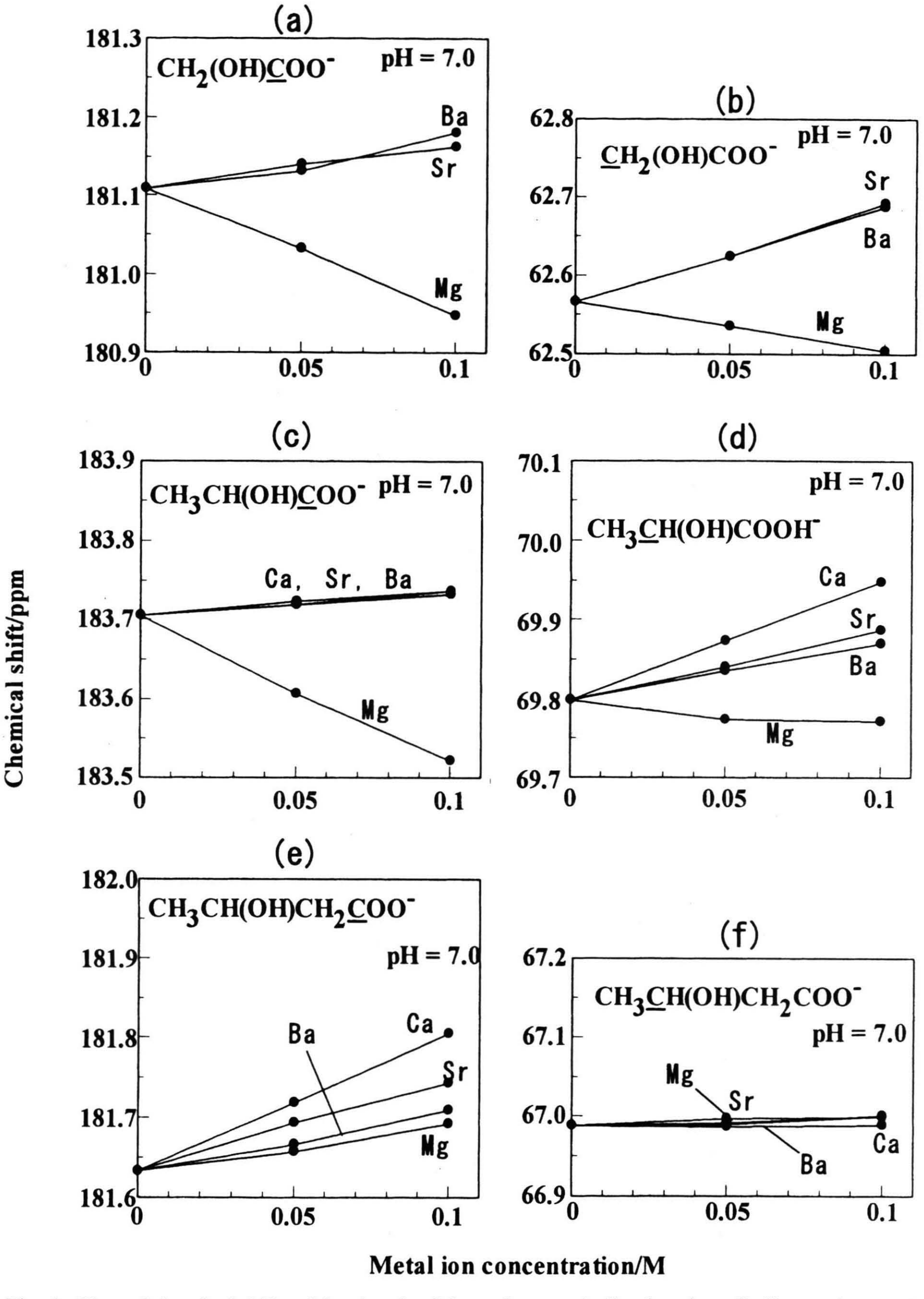

Fig. 4. Plots of chemical shifts of the signals of the carbons underlined against alkaline earth metal ion concentrations at pH = 7.0. (a) and (b) glycollate system; (c) and (d) lactate system; (e) and (f) 3-hydroxybutyrate system.

The observations above may be generalized: α-hydroxycarboxylate ions are bidentate ligands coordinating to alkaline earth metal ions using both the carboxylate and hydroxyl groups to form a five member ring, while β (and probably γ, ...)-hydroxycarboxylate ions act as monodentate ligands using only carboxylate groups. This speculation is reached by virtue of the unique upfield shift of the carboxylate carbon signal with increasing magnesium ion concentration in the magnesium systems. The difference in the coordination manner between the α- and $\beta(\gamma, \ldots)$-hydroxycarboxylate ions may be attributable to the difference in the $-COO^- \cdots OH^-$ distance between them; the carboxylate group and the hydroxyl group of $\beta(\gamma, \ldots)$-hydroxycarboxylate ions are not geometrically close enough to each other for a ring to be formed.

3.3 *Coordination of Hydroxyl Group*

In the above, we suggested that the hydroxyl group plays an important role in the coordination of an α-hydroxycarboxylate ion to alkaline earth metal ions. To understand better the function of the hydroxyl group in such coordination, the interaction of 1,3-butanediol with alkaline earth metal ions was investigated. The metal ion concentration dependence of the ^{13}C NMR signal positions of carbons of the diol is depicted in Figs. 5(a) ~ 5(d). As is seen, the signals exhibit no alkaline earth metal ion concentration dependence, showing that there to be little interaction between the diol and metal ions. This may be generalized. The hydroxyl group alone interacts very little with alkaline earth metal ions; it can interact with them only when there exists a negatively charged group nearby.

3.4 *Interactions Observed in Alkaline Earth Metal Polycarboxylate Systems*

Malonic acid is an α-dicarboxylic acid with its two carboxyl groups being equivalent. According to its dissociation constants and complex formation constants [8], its major species is the monovalent anion (free and bound) at pH = 4.2, and the divalent (free and bound) anion is practically only the species of the malonic acid at pH = 7.0 within the present experimental conditions. The magnesium ion concentration dependence of the ^{13}C NMR signal of the carboxylate/carboxyl carbon of malonic acid species at pH = 4.2 and 7.0 is depicted in Figs. 6a and 6b, respectively. The signal shifts downfield with increasing magnesium ion concentration at pH = 4.2, which indicates that the hydrogenmalonate ion acts as a monodentate ligand, like the monocarboxylate anions examined above. At pH = 7.0, the signal shifts upfield. A consideration analogous to that on the magnesium-α-hydroxycarboxylate systems leads to the speculation that the malonate ion acts as a bidentate ligand coordinating to magnesium ions using its two carboxylate groups to form a six member ring as shown in Fig. 2 (Structure V).

Succinic acid is a β-dicarboxylic acid, and its two carboxyl groups are equivalent. A geometrical consideration shows that, unlike a β-hydroxycarboxylic acid, a β-dicarboxylic acid can act as a bidentate ligand coordinating to alkaline earth metal ions using its two carboxylate groups to form a seven member ring. Its structure is easily inferred from that of the six member ring. Dependence of the ^{13}C NMR signal positions of carboxylate/carboxyl carbons of succinic acid species on the magnesium ion

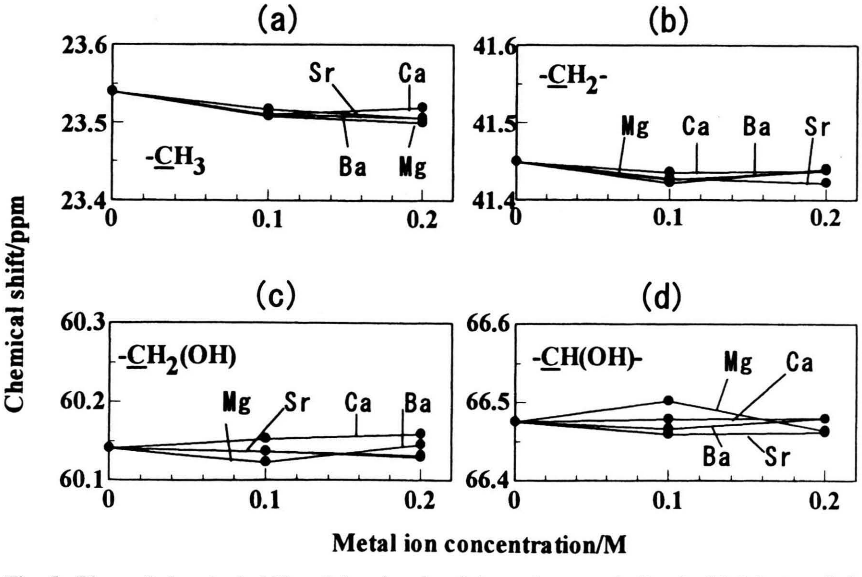

Fig. 5. Plots of chemical shifts of the signals of the carbons underlined of 1,3-butanediol in the 1,3-butanediol systems with pH = 7.0 against alkaline earth metal ion concentrations.

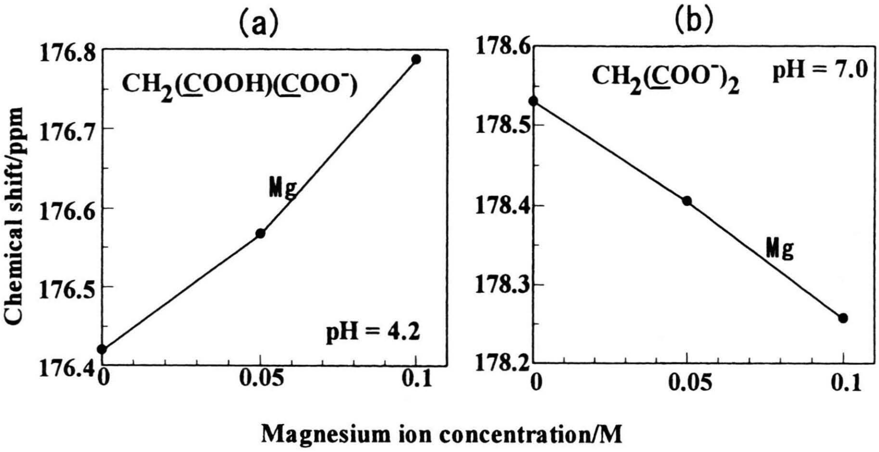

Fig. 6. Plots of chemical shifts of the signals of the carboxyl/carboxylate carbons underlined in the magnesium malonate system against the magnesium ion concentration. (a) pH = 4.2; (b) pH = 7.0.

concentration at pH = 4.6 and at pH = 7.0 is drawn in Figs. 7a and 7b, respectively. The major species of succinic acid is the monovalent anion at pH = 4.6, the divalent anion being practically the only species of the acid at pH = 7.0. Unlike the case of malonate system above, the signal shows downfield shifts both at pH = 4.6 and 7.0. The trend in the signal shift at pH = 4.6 is as expected, since the major species is monovalent at this pH value. The trend at pH = 7.0 may seemingly indicate that the divalent succinate ion acts as a monodentate ligand coordinating to magnesium ions using only one of its two carboxylate groups. However, this cannot explain a notable feature of the magnesium succinate system that the magnesium ion concentration dependence of the carboxylate carbon signal is larger at pH = 4.6 than at pH = 7.0. This observation is best understood by assuming that the succinate ion acts both as monodentate and bidentate ligand and ^{13}C NMR signals of their carbons shift downfield and upfield, accordingly, with increasing magnesium ion concentration. The proportion of the species acting as bidentate ligand is higher at pH = 7.0 than at pH = 4.6, and as a result, concentration dependence of the signal position larger at pH = 4.6 than at pH = 7.0.

A similar feature is also observed for the magnesium methylsuccinate system shown in Fig. 8 [11], where the two carboxyl groups are no equivalent, and for the magnesium tricarballylate system in Figure 9. In addition, the β-carboxylate (β against both the hydroxyl group and the other carboxylate group) carbon signal of the malate ion in the magnesium malate system shifts upfield with increasing magnesium ion concentration at pH = 7.0 (vide infra). These observations support the formation of a ring

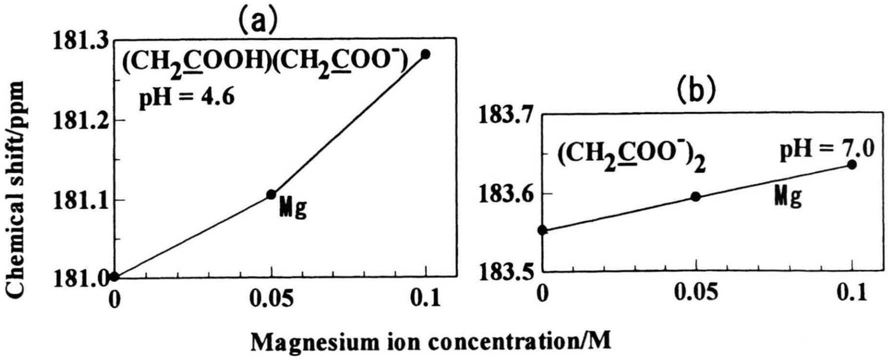

Fig. 7. Plots of chemical shifts of the signals of the carboxyl/carboxylate carbons underlined in the magnesium succinate system against the magnesium ion concentration. (a) pH = 4.6; (b) pH = 7.0.

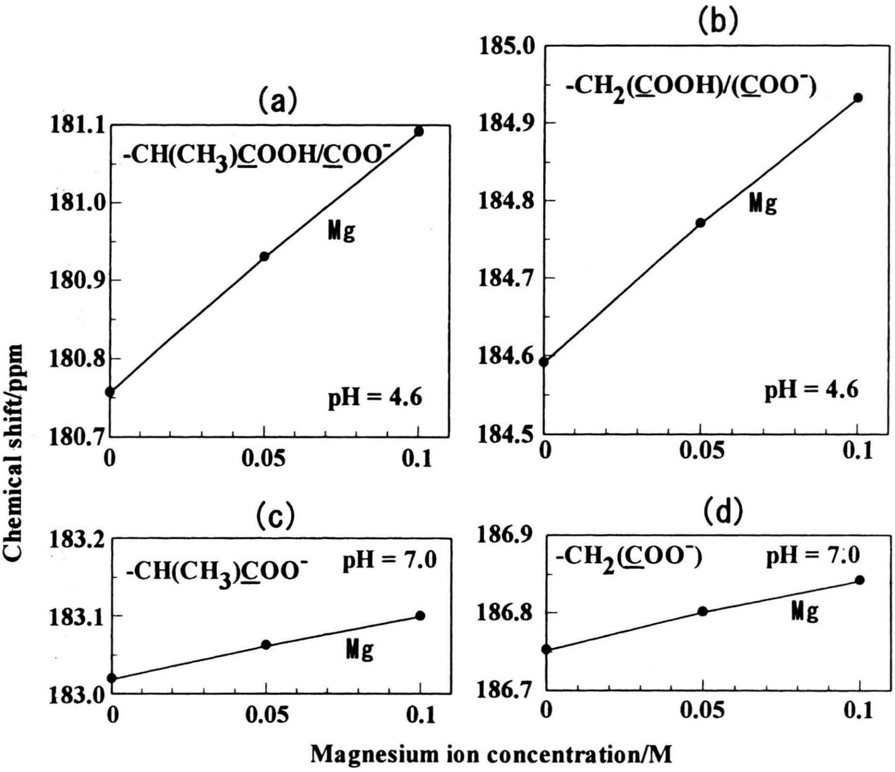

Fig. 8. Plots of chemical shifts of the signals of the carboxyl/carboxylate carbons underlined in the magnesium methylsuccinate system against the magnesium ion concentration. (a) and (b) pH = 4.6; (c) and (d) pH = 7.0.

structure consisting of two carboxylate groups located at the β positions to each other.

3.5 The Sequence of Dissociation of Different Kinds of Carboxyl Groups in a Hydroxypolycarboxylic Acid

It is a unique feature of the magnesium hydroxycarboxylate and polycarboxylate systems that ^{13}C NMR signals of carboxylate carbons shift upfield with increasing magnesium ion concentration only when carboxylate anions act as bidentate ligand forming ring structures. This feature provides information on the sequence of the dissociation of carboxyl groups of different kinds in a hydroxypolycarboxylic acid.

In Figs. 10a and 10b, we show the magnesium ion concentration dependences of the ^{13}C NMR signals of carboxylate/carboxyl carbons of two different kinds in malic acid in the magnesium malate system at various pH values. In this system, the major species of malic acid at pH = 3.2 are the neutral malic acid molecule and the monovalent anion. At pH = 4.0, the monovalent anion is the major species. At pH = 4.7, the monovalent anion and the divalent anion are the major species. And at pH = 5.5 and 7.0, the major species is the divalent anion. In addition, at pH = 7.0, the divalent anion is practically the only species of malic acid. Figs. 10a and 10b show that the two NMR signals shift downfield or shift little with increasing magnesium ion concentration when the pH value is 4.7 or lower, and that they shift upfield only when the divalent malate ion becomes the major species. Similar features are found for the magnesium citrate system shown in Fig. 11 and the magnesium isocitrate system shown in Fig. 12. In any case, upfield shifts of carboxylate carbon signals are observed only when all the carboxylic groups in a polycarboxylic acid dissociate. These facts suggest a correlation of the sequence in dissociation of different kinds of carboxylic groups with their positions relative to the hydroxyl group in a molecule. That is, the closer to the hydroxyl group a carboxyl group is located, the less apt it is to dissociate. This is because, if the α-carboxyl group (α against the hydroxyl group) dissociates at a lower pH than the β-carboxyl group, the upfield shift of the α-carboxylate carbon signal with in-

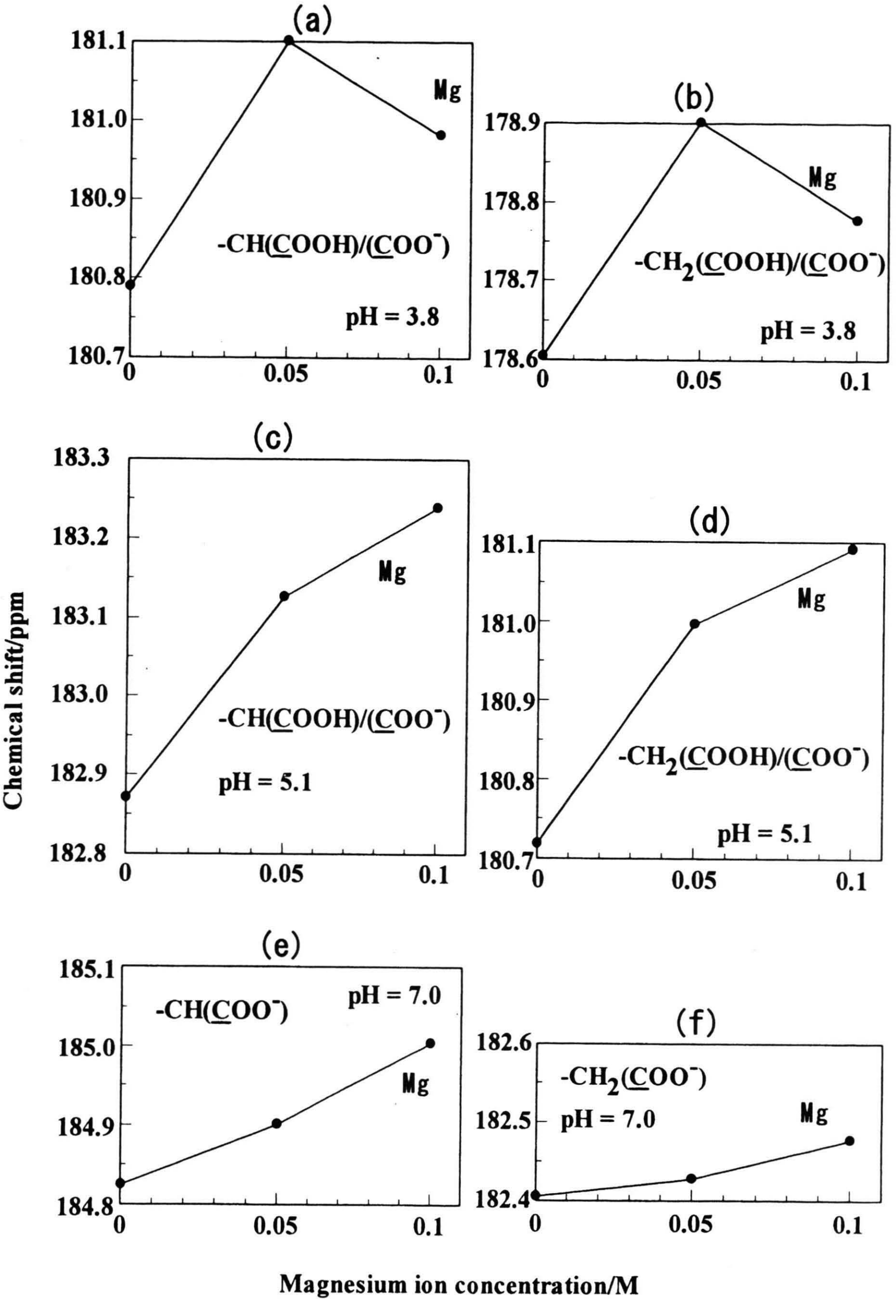

Fig. 9. Plots of chemical shifts of the signals of the carboxyl/carboxylate carbons underlined in the magnesium tricarballylate system against the magnesium ion concentration. (a) and (b) pH = 3.8; (c) and (d) pH = 5.1, (e) and (f) pH = 7.0.

creasing magnesium ion concentration should occur at a lower pH than that of the β-carboxylate carbon, due to the formation of a ring using the α-carboxylate and the hydroxyl groups. The expected sequence of dissociation is that the α-carboxyl group is slightly less apt to dissociate than the β-carboxyl group (which is slightly less apt to dissociate than γ-carboxyl group ...). This trend may be attributable to the fact that the electron-rich hydroxyl group makes the carboxylic group closer to itself less apt to dissociate.

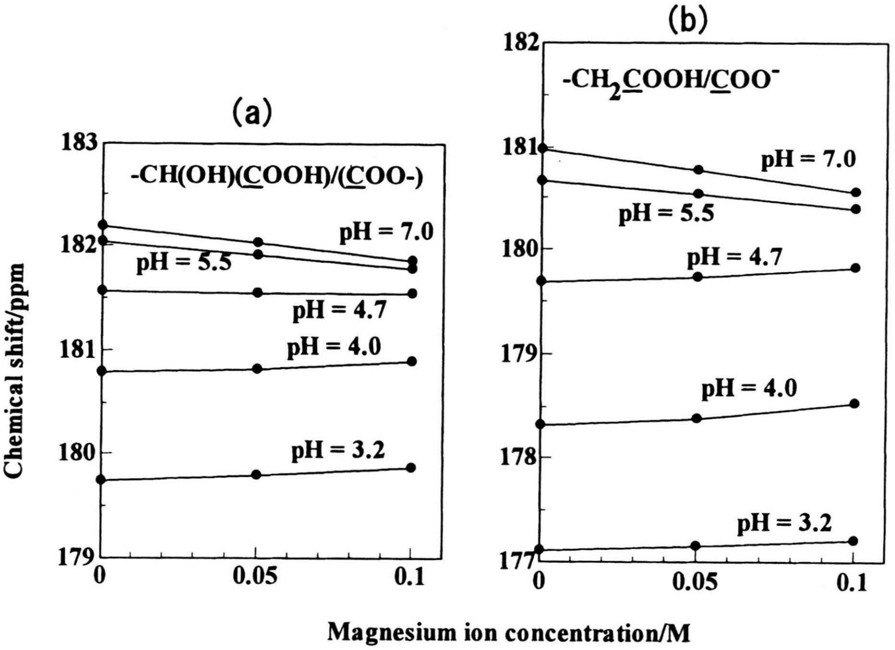

Fig. 10. Plots of chemical shifts of the carboxyl/carboxylate carbon signals in the magnesium malate system against the magnesium ion concentration at various pH values. (a) α-carboxyl/carboxylate carbon (α against the hydroxyl group); (b) β-carboxyl/carboxylate carbon.

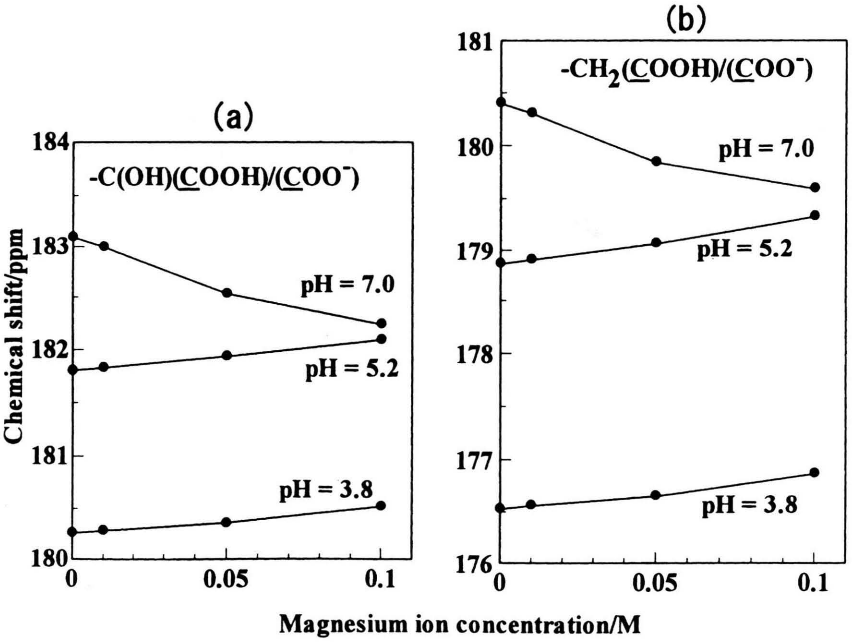

Fig. 11. Plots of chemical shifts of the carboxyl/carboxylate carbon signals in the magnesium citrate system against the magnesium ion concentration at different pH values. (a) α-carboxyl/carboxylate carbon (α against the hydroxyl group); (b) β-carboxyl/carboxylate carbon.

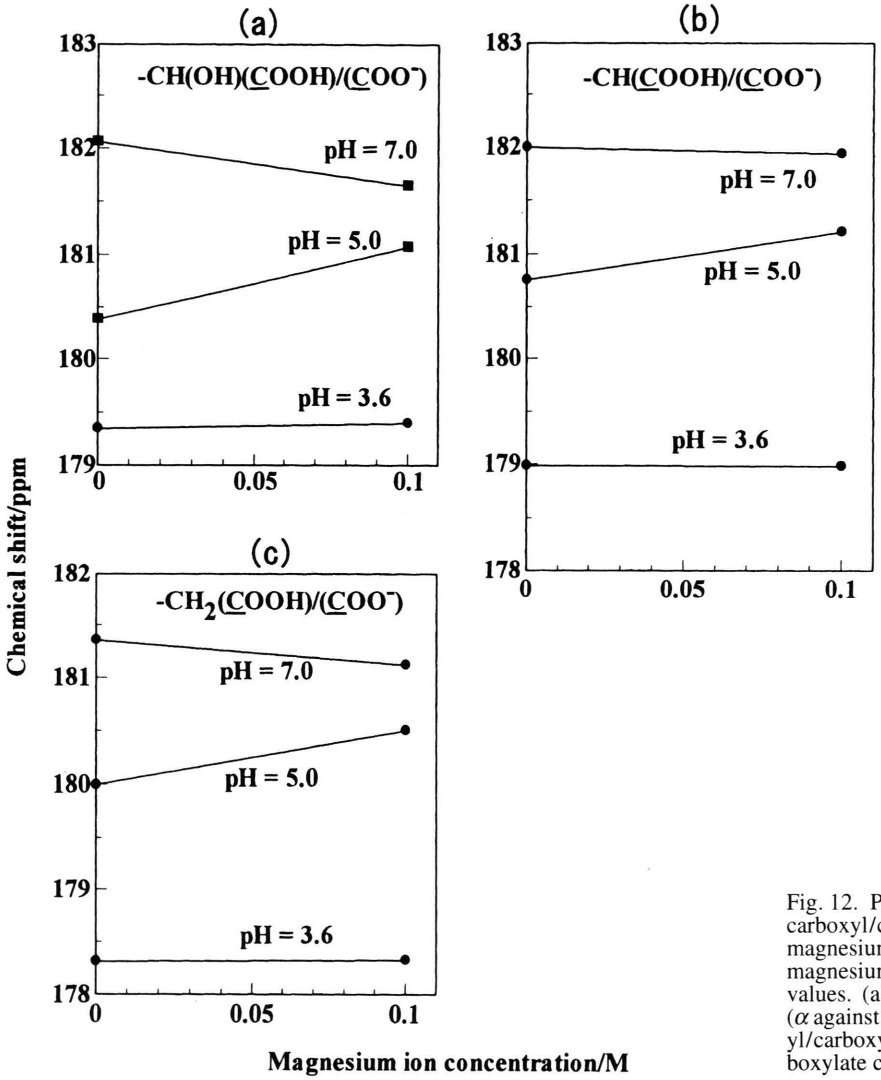

Fig. 12. Plots of chemical shifts of the carboxyl/carboxylate carbon signals in the magnesium isocitrate system against the magnesium ion concentration at different pH values. (a) α-carboxyl/carboxylate carbon (α against the hydroxyl group); (b) β-carboxyl/carboxylate carbon; (c) γ-carboxyl/carboxylate carbon.

3.6 Interaction of Citric Acid with Alkaline Earth Metal Ions

As mentioned above, the metal ion concentration dependence of the change in ^{13}C NMR signals in alkaline earth metal citrate systems is as a whole similar to that of the corresponding signals in the metal malate systems. That is, at lower pH values, the signals of the carboxyl/carboxylate carbons in every alkaline earth metal system show downfield shifts with increasing metal ion concentration. At pH = 7.0, at which the citrate ion is practically the only species of citric acid, the signals of the carboxylate carbons in the magnesium system shift upfield with increasing magnesium ion concentration.

The striking feature of the citrate systems, which the other carboxylate systems examined lack, is that new signals are observed at pH = 7.0 (and very slightly even at pH = 5.2). The relative intensities of the new signals increase with increasing alkaline earth metal ion concentrations but their positions are nearly independent of the kind of metal ions. As an example of the ^{13}C NMR spectra obtained, the spectrum of the solution with pH = 7.0 containing 0.10 M magnesium chloride and 0.050 M citric acid is shown in Fig. 13 (the upper spectrum). It should be compared with the spectrum of the citric acid solution at pH = 7.0 containing no alkaline earth metal ion (the lower spectrum). In addition to the signals at around $\delta = 77$, 180, and 182.5 ppm (the signal of the $-CH_2-$

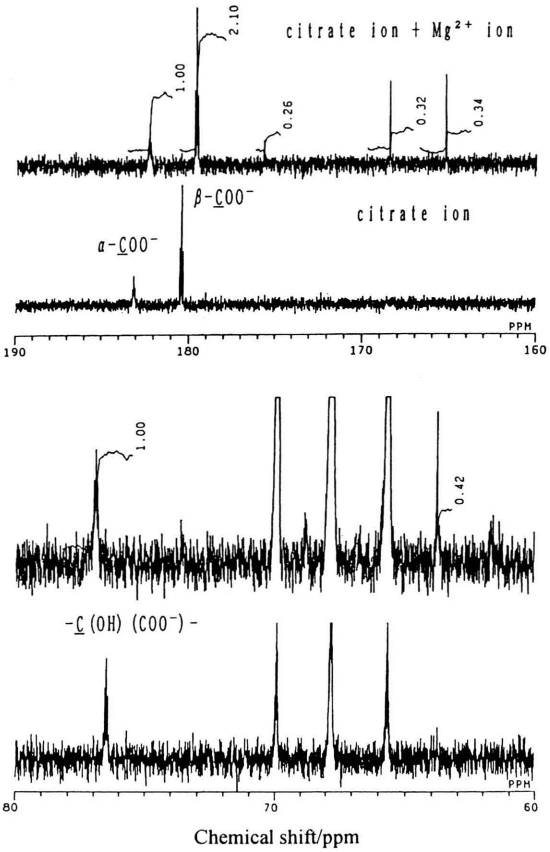

Fig. 13. ^{13}C NMR spectra of the citrate systems. The upper spectrum: magnesium ion concentration, 0.10 M; citric acid concentration, 0.050 M; pH, 7.0. The lower spectrum: magnesium ion concentration, 0.0 M; citric acid concentration, 0.050 M; pH, 7.0. Peak assignments are given in the text.

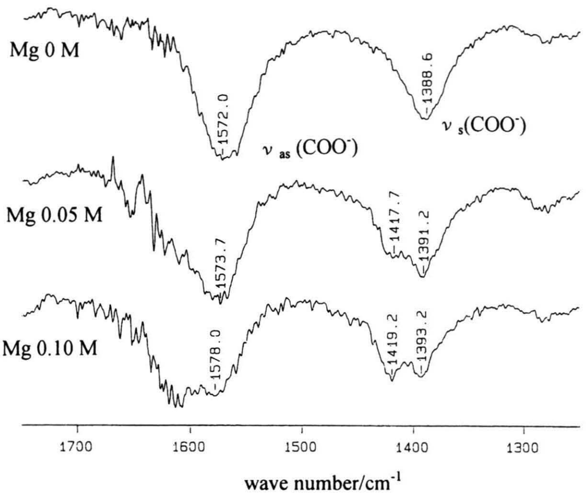

Fig. 14. The IR spectra in the symmetric and asymmetric COO$^-$ frequency region of aqueous solutions with pH = 7.0 of the magnesium citrate system.

carbon observed at around $\delta = 45.5$ ppm is not shown), which are also observed for the solutions with the lower pH values, new signals are observed at around $\delta = 63.8$, 165.2, 168.5, and 175.6 ppm. The observation of those four signals suggests the formation of a relatively stable magnesium-citrate complex whose life time is long on the ^{13}C NMR time scale. The existence of the stable complex is confirmed by the IR spectra of the magnesium citrate systems with the pH value of 7.0, as shown in Figure 14. A new COO$^-$ vibrational mode appears at around 1418 cm^{-1}, and its intensity increases with increasing magnesium ion concentration.

The existence of the three distinct carboxylate carbon signals shows that the three carboxylate groups are in electronically different environments. The hydroxyl group is expected to participate in the formation of the stable complex because no such complex is evidenced in tricarballylate systems where there are three carboxyl groups in a molecule but the hydroxyl group is absent. Based on the different electronic environments of the three carboxylate carbons and participation of the hydroxyl group in the complex formation, we propose Structure VI in Fig. 2 as the structure of the new stable complex between the alkaline earth metal ion and the citrate ion. The citrate ion acts as a tridentate ligand.

The new signal at around $\delta = 63.8$ ppm is attributable to the C̲(OH)(COO$^-$) carbon and the other three signals to the carboxylate carbons. The signal at around $\delta = 175.6$ ppm belongs most probably to the non-coordinating *β*-carboxylate carbon because the deviation from that of the free carboxylate carbon is smallest. The signals at around 165.2 ppm and 168.5 ppm may be assignable to the coordinating *β*-carboxylate carbon and the coordinating *α*-carboxylate carbon, respectively, judging from their positions relative to those of the free carboxylate carbons.

When a carboxylate ion coordinates to a metal ion, the chemical shift of the carboxylate carbon in general shows a downfield shift, as exemplified by the uranyl ion systems [1]. Another example is shown in Fig. 15, in which are depicted the free and coordinating carboxylate carbon signals in the cobalt acetate system [12]. Contrary to those examples, the signals of the coordinating carboxylate carbons of the citrate ion in every alkaline earth metal citrate system examined shift upfield. A geometri-

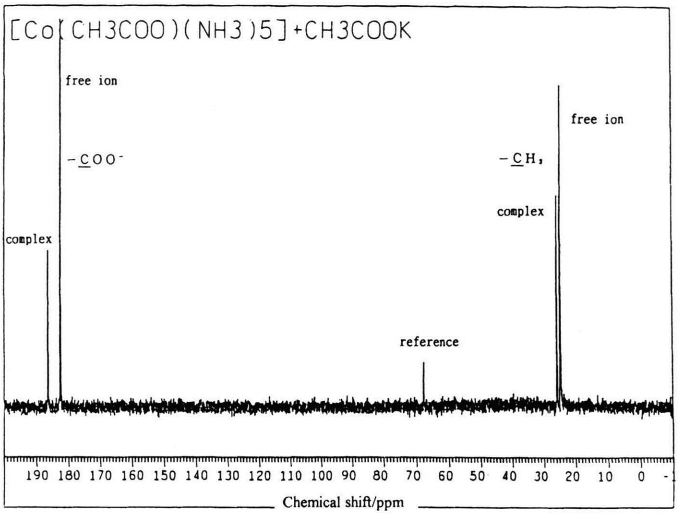

Fig. 15. A ^{13}C NMR spectrum of the cobalt acetate system. The carboxylate carbon signal shows a downfield shift upon coordination.

cal consideration indicates that it is geometrically impossible for a citrate ion to act as a tridentate ligand coordinating to a dehydrated alkaline earth metal ion. Thus, the citrate ion is expected to coordinate to alkaline earth metal ions from outside the first hydration sphere around the metal ions. The existence of the hydrating waters explains the upfield shifts of the carboxylate carbon signals upon complex formation, as has been discussed in [3].

4. Conclusion

The major findings of the present ^{13}C NMR spectroscopic study on various alkaline earth metal carboxylate solutions are as follows:

1) No distinct ^{13}C NMR signals corresponding to free and bound carboxylate ions are observed in any of the carboxylate systems examined but the citrate system.

2) A monocarboxylate ion acts as a monodentate ligand coordinating to alkaline earth metal ions using the single bonded oxygen of the carboxylate group. An α-hydroxycarboxylate ion most likely acts as a bidentate ligand coordinating alkaline earth metal ions using the carboxylate group and the hydroxyl group, while a $\beta(\gamma$-, …)-hydroxycarboxylate acts as a monodentate ligand. Both α- and β-dicarboxylate ions can act as bidentate ligands coordinating to alkaline earth metal ions using the two carboxylate groups.

3) A sequence of dissociation of non-equivalent carboxyl groups in a hydroxypolycarboxylic acid is suggested; the carboxyl group located farther away from the hydroxyl group seeming to dissociate slightly earlier, i.e. at a lower pH.

4) The formation of a relatively stable complex is observed in every alkaline earth metal citrate system with pH = 7.0. The most likely coordination manner of the citrate ion in the complex is that the citrate ion acts as a tridentate ligand coordinating to alkaline earth metal ions using the α-carboxylate group, one of the β-carboxylate groups and hydroxyl group. The metal ion in the complex is expected to maintain the first hydration sphere.

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[9] The concentration of tricarballylic acid is 0.30 M.

[10] The ^{13}C NMR signal at around $\delta = 181$ ppm in Fig. 1, observed as a quartet, is assigned to the carboxylate/carboxylic carbon {$-CH(OH)(\underline{C}OO^-)/(\underline{C}OOH)$, α-carboxy-

late/carboxylic carbon}, the signal at around $\delta = 178$ ppm, observed as a quartet, is assigned to the other carboxylate/carboxylic carbon {$-CH_2\underline{C}OO^-/\underline{C}OOH$, β-carboxylate/carboxylic carbon}, the signal at around $\delta = 71$ ppm, observed as doublet-triplet, is assigned to the methine carbon ($-\underline{C}H(OH)-$), and the signal at around $\delta = 42$ ppm, observed as triplet-doublet, is assigned to the methylene carbon ($-\underline{C}H_2-$).

[11] The two carboxyl groups are non-equivalent. The ^{13}C NMR signals of $-CH(CH_3)\underline{C}OOH$ and $-CH_2\underline{C}OOH$ are doublet-multiplets and triplet-multiplets, respectively, at pH = 4.6.

[12] The complex $[Co(CH_3COO^-)(NH_3)_5]ClO_4$ used for the ^{13}C NMR measurement was obtained by the substitution of an ammonium in Co $(NH_3)_6^{2+}$ with an acetate ion. Acetate ions are considered to coordinate directly to cobalt(II) ions.