Removal of Phosphate from Dilute Phosphate Solution by an Iron Chitosan Complex to Be Used as an Oral Sorbent

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The long-period administration of aluminum-containing phosphate binders for the treatment of hyperphosphatemia has been shown to carry the risk of aluminum accumulation associated with many bone diseases. An iron chitosan complex prepared by chitosan and iron(II) sulfate was examined as an alternative intestinal phosphate binder. In-vitro experiments have shown that the phosphate-binding capacity of iron chitosan increases with increasing the iron content of iron chitosan, the concentration of phosphate, and the pH of the solution. The phosphate-binding capacity of iron chitosan (iron content 32 mg g^{-1}) was over 30 mg g^{-1} when 10 mg of iron chitosan was admitted to 10 ml of a phosphate solution of 10 mg dl^{-1} (pH 7.5) at 37 °C for 6 h. This value is much higher than those of aluminum hydroxide, calcium carbonate, etc., which were practically tried before. No elution of the iron from iron chitosan could be detected under these conditions.

It is still pointed out that the treatment of hyperphosphatemia is necessary in order to prevent secondary hyperparathyroidism and renal osteodystrophy in patients with chronic renal failure undergoing dialysis.[1] Usually, aluminum-containing phosphate binders are prescribed in order to control the serum phosphate levels. However, this therapy carries the well-known risk of aluminum intoxication.[2,3] Some investigators have demonstrated that although calcium carbonate, as well as magnesium carbonate or magnesium hydroxide, could partly substitute for aluminum-containing phosphate binders, they might lead in 10—40% of the cases to hypercalcemia, hypermagnesemia and other severe gastrointestinal problems.[4,5]

Chitosan is a nontoxic natural polysaccharide with good physiological adaptability, used as food additives and medical materials. Because of having an extra electron pair of nitrogen, chitosan can form polymer–metal complexes with many metal cations, and is thus used as a metal-ion sorbent.[6,7] For the purpose of investigating alternate intestinal phosphate binders, an iron(II) chitosan complex prepared from chitosan and iron(II) sulfate has been examined. The phosphate-binding capacity of iron chitosan was evaluated in vitro.

Experimental

Materials. Chitosan was obtained from Nacalai Tesque Inc., and used without further purification. Iron(II) sulfate, aluminum hydroxide, calcium carbonate, magnesium carbonate, and other chemicals were commercially available and of guaranteed grade.

Iron Chitosan Complex. The prescribed amount of chitosan was dissolved in a 1—2% acetic acid aqueous solution (pH 2.5) by heating. After cooling, a prescribed amount of iron(II) sulfate was added to the solution. A yellow precipitate was obtained after stirring for a long time at 30—40 °C, washed with water three times, then ethanol, and finally dried in vacuo.

Phosphate Adsorption. A definite quantity (usually 10 mg) of sorbents was contacted with a definite volume (usually 10 ml) of phosphate solution in a T-tube. After shaking for 6 h at 37 °C, the sorbent and solution were separated by centrifugation. The phosphate concentration of the solution was measured by using a molybdenum blue spectrophotometric method. The phosphate-binding capacity was caculated using

$$Q=\frac{C_0-C}{W}\times V.$$

Here, Q is the phosphate binding capacity/mg g^{-1}, C_0 the initial concentration of phosphate/mg dl^{-1}, C the phosphate concentration of adsorbed solution/mg dl^{-1}, V the volume of adsorbed solution/dl, and W the amount of sorbent added/g.

Iron Content. Strictly weighed iron chitosan (0.100 g) was dissolved in concentrated hydrochloric acid (5 ml) and decomposed by strong heating. The mixture was dissolved in 0.1 M HCl (10 ml, 1M=1 mol dm^{-3}); the resulting solution was diluted to 1000 ml with distilled water. The iron concentration of the solution was measured by using a 1,10-phenanthroline spectrophotometric method, and the iron content of iron chitosan was then calculated.

Analysis. The FT-IR spectra were recorded with a JASCO DP/F-3t spectrometer. The UV-vis spectra were measured with a Shimadzu UV-200 spectrophotometer. The serum analysis were carried out in Laboratories for Biomedical Analysis of Japan (Tokyo).

Results and Discussion

Iron Content. The iron content of iron(II) chitosan can be controlled by the concentration of chitosan and iron(II) sulfate. Some results of the iron content with the preparation conditions are summarized in Table 1. When the iron(II) sulfate concentration and the pH value of the solution were held constant at 3% and 2.5, respectively, the iron content of iron chitosan increased with decreasing the chitosan concentration. Keeping the concentration of chitosan at less than 1% was suitable for obtaining iron chitosan with a high iron content. Under the same conditions, a pure iron(III) chitosan complex with a high iron content could not be obtained with iron(III) sulfate; thus, details concerning the iron(II)

Table 1. Content of Iron in Iron Chitosan Prepared under Various Conditions

Chitosan concentration[a]/%	0.2	0.4	0.6	0.8	1.0	1.0	1.0	1.0
Ferrous sulfate/g dl^{-1}	3.0	3.0	3.0	3.0	3.0	2.0	1.0	0.5
Reaction time[b]/h	>100	>100	100	72	48	48	48	68
Iron content/mg g^{-1}	160	127	93	86	73	55	32	15

a) 1—2% Acetic acid aqueous solution (pH 2.5). b) Temperature: 40°C.

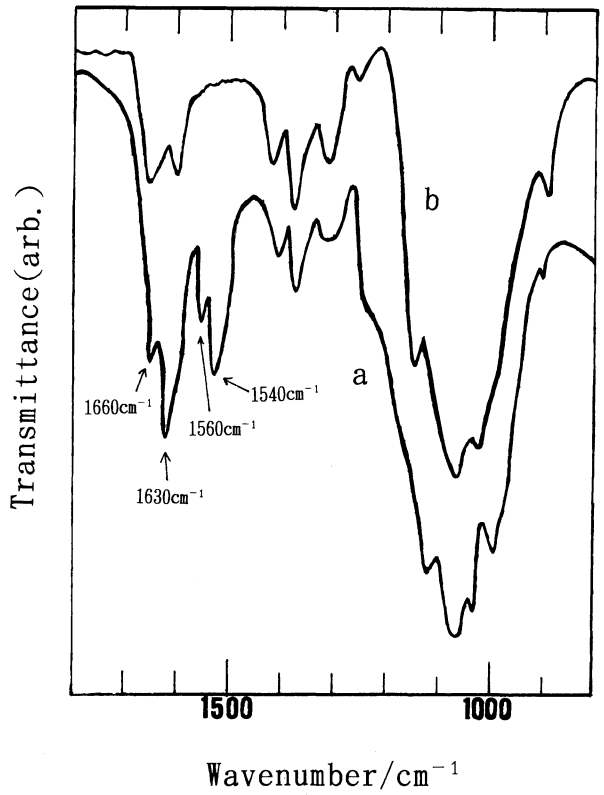

Fig. 1. FT-IR spectra of iron chitosan (a) and chitosan (b).

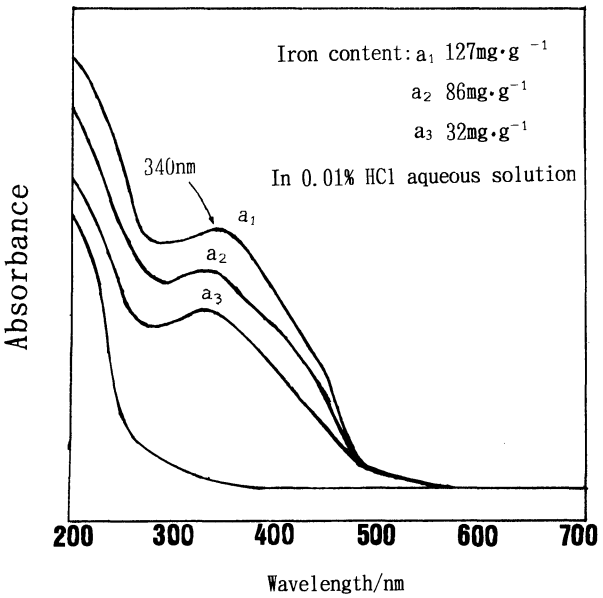

Fig. 2. UV-vis spectra of iron chitosan (a_1, a_2, a_3) and chitosan (b).

chitosan complex (represented iron chitosan) are mainly presented in the following description.

FT-IR and UV-vis Spectra. Figure 1 shows the FT-IR spectra of iron chitosan and chitosan. Compared with the spectra of chitosan, the additional amine-type absorption peaks of iron chitosan at 1540 and 1560 cm^{-1} were observed in addition to the δ-NH (1630 cm^{-1}, 1660 cm^{-1}) absorption peaks of amine. Furthermore, the absorption band at around 340 nm was observed in the UV-vis spectra (Fig. 2). It could be illustrated from those characteristic spectra that the structure of iron chitosan is a kind of metal–polymer complex.[6,7]

Adsorption Isotherm. The phosphate adsorption results of two kinds of iron chitosan (iron content 32 mg dl^{-1}, 20 mg dl^{-1}) at 37°C in dilute potassium dihydrogenphosphate aqueous solutions with a phosphate concentration below 10 mg dl^{-1} could be plotted in accord with the following Langmuir adsorption isotherm equation:

$$\frac{C}{Q}=\frac{1}{a\times b}+\frac{C}{b}.$$

Here, C is the concentration, Q the binding capacity, a the adsorption constant, and b the equilibrium binding capacity. The linear adsorption isotherms (Fig. 3) indicate that the adsorption of phosphate on iron chitosan is a type of chemisorption. The ligand exchange can be assumed as being the adsorption mechanism of phosphate on iron chitosan.

Comparison with Other Sorbents. The serum phosphate concentration of hyperphosphatemia in chronic uremia has been reported to be less than 10 mg dl^{-1}. As an oral sorbent of phosphate, it is required to bind with intestinal phosphate as much as possible, even in a further diluted phosphate solution. The phosphate-binding capacities of iron chitosan, chitosan, chitosan bead,[8] aluminum hydroxide, calcium carbonate, magnesium carbonate, and iron hydroxide in a dilute phosphate aqueous solution (10 mg dl^{-1}) at 37°C are listed in Table 2. The phosphate-binding capacity of iron chitosan is shown to be much greater than those of aluminum hydroxide and others for a dilute phosphate solution. The effectiveness of iron chitosan might be attributable to its insolubility due to complex formation.

Adsorption Rate. The increase in phosphate binding

Table 2. Comparison of Phosphate Binding Capacities of Various Sorbents

	Iron chitosan[a)]	$Fe(OH)_3$	$Al(OH)_3$	$CaCO_3$	$MgCO_3$	Chitosan	Chitosan bead[b)]
Capacity/mg g^{-1}	42	23	<2	<2	<2	<1	<4

Initial phosphate concentration: 10 mg dl^{-1}, Temperature: 37°C, Shaking for 6 h, Sorbent/solution: 10 mg/10 ml. a) Iron content: 32 mg g^{-1}. b) Ref. 8.

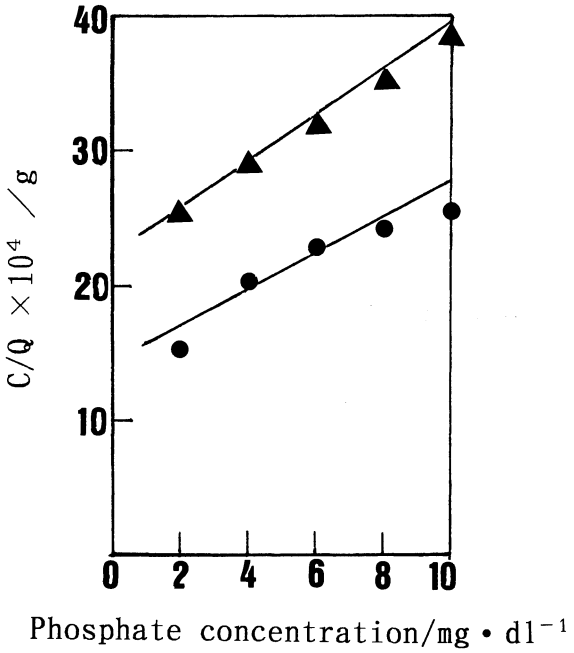

Fig. 3. Phosphate adsorption isotherm for iron chitosan. Iron content: (●) 32 mg g^{-1}, (▲) 20 mg g^{-1}, Iron chitosan/solution: 10 mg/10 ml, Temperature: 37°C, Shaking for 16 h.

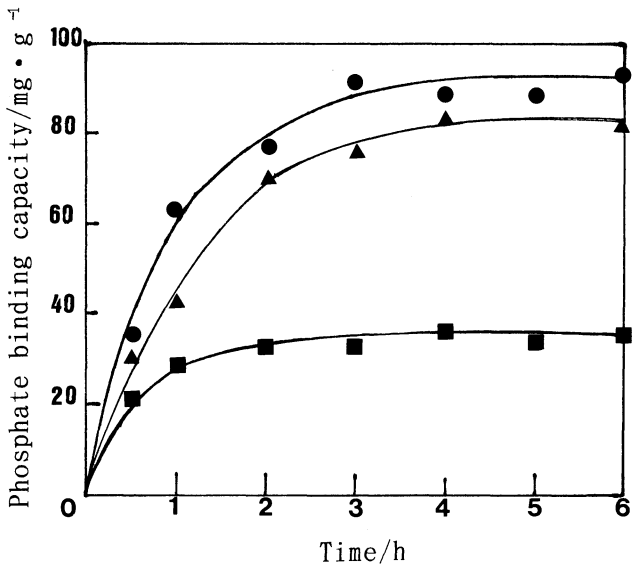

Fig. 4. Phosphate binding rate of iron chitosan. Iron content: (●) 127 mg g^{-1}, (▲) 86 mg g^{-1}, (■) 32 mg g^{-1}, Iron chitosan/solution: 10 mg/10 ml, Initial phosphate concentration: (●) 30 mg dl^{-1}, (■) 10 mg dl^{-1}, Temperature: 37°C, Tris buffer (pH 7.5).

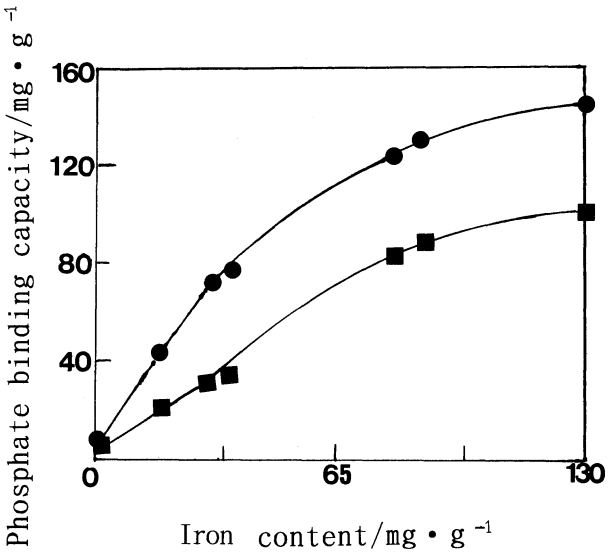

Fig. 5. Phosphate binding capacity of iron chitosan with iron content variation. Initial phosphate concentration: (●) 60 mg dl^{-1}, (■) 10 mg dl^{-1}, Iron chitosan/solution: 10 mg/10 ml, Temperature: 37°C, Shaking for 16 h.

by iron chitosan with the duration time is shown in Fig. 4. The phosphate-binding capacity of iron chitosan becomes constant after 3 h. This adsorption rate is a satisfactory value for the removal of intestinal phosphate.

Iron Content and Binding Capacity. Figure 5 illustrates the results of a phosphate adsorption experiment carried out with 6 kinds of iron chitosan with various iron contents in aqueous phosphate solutions (phosphate concentration: 60 mg dl^{-1}, 10 mg dl^{-1}). The phosphate-binding capacity of iron chitosan increases with an increase in the iron content. This affords another support for the above-mentioned adsorption mechanism of ligand exchange.

Influence of pH. Figure 6 shows the influence of the pH on the phosphate-binding capacities of iron chitosan in phosphate Tris buffer solutions with phosphate concentrations of 10 mg dl^{-1} and 60 mg dl^{-1}. With a decrease from pH 1.5 to pH 7.5, especialy between pH 4 and pH 3, the phosphate-binding capacity decreases drastically. As can be seen from Fig. 7, the main reason for the decrease is attributable to the elution of iron, as well as the dissolving of iron chitosan. No iron elution from iron chitosan could be detected when the pH was greater than 5.

When an oral sorbent is practically fed from the

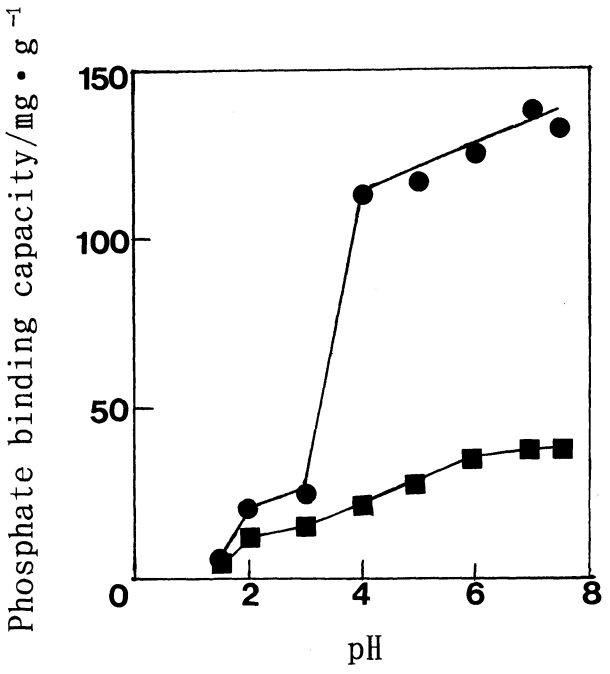

Fig. 6. Phosphate binding capacity with pH variation. Iron content: (●) 127 mg g^{-1}, (■) 32 mg g^{-1}, Initial phosphate concentration: (●) 60 mg dl^{-1}, (■) 10 mg dl^{-1}, Iron chitosan/solution: 10 mg/10 ml, Temperature: 37°C, Shaking for 16 h.

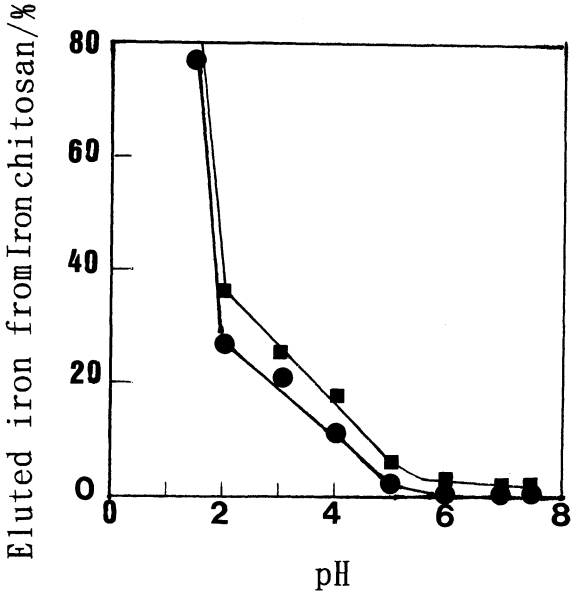

Fig. 7. Iron elution from iron chitosan with pH variation. Iron content: (●) 127 mg g^{-1}, (■) 32 mg g^{-1}, Iron chitosan/solution: 10 mg/10 ml, Initial phosphate concentration: 10 mg dl^{-1}, Temperature: 37°C, Shaking for 16 h.

mouth, it goes through the stomach to the intestine, along with pH changes from 1.5 (pH of gastric juice) to 7.5 (pH of intestinal juice). The sorbent must thus keep a high phosphate binding capacity, in spite of the changing pH. After 2 h of adsorption with 10 mg of iron chitosan in 5.0 ml of a phosphate dilute Tris buffer solution (pH 1.5), the pH of the solution was changed to 7.5 by adding 45 ml of a phosphate Tris buffer solution (pH 7.5) and continued shaking for another 2 h. The total phosphate binding capacity decreased by about 20%, in comparison with the result with 4 h of adsorption in a pH 7.5 buffer solution under the same conditions (Table 3). The results, however, still maintain satisfactorily high levels.

Test in Serum. The results described above are the results of experiments carried out in aqueous solutions. Finally, the adsorption in human serum enriched phosphate by adding potassium dihydrogenphosphate was examined (Table 4). More than 65% of the phosphate in the serum was removed by iron chitosan under the prescribed conditions. The iron-elution from iron chitosan was much less than that of basic iron(II) sulfate, which is ascribed to the formation of the chitosan complex. Besides phosphate, lesser ratios of calcium, sodium and potassium were found to be removed by iron chitosan.

Table 3. Effect of pH Exchange on Phosphate Binding Capacities

Iron content	Treatment/h		Binding capacity
mg g^{-1}	pH 1.5	pH 7.5	mg g^{-1}
127	0	4	88
	2	2	69
86	0	4	81
	2	2	65
32	0	4	33
	2	2	26

Initial phosphate concentration: 10 mg dl^{-1}, Temperature: 37°C, Iron chitosan/solution: 10 mg/50 ml.

Table 4. Results of Adsorption Test in Phosphate-Enriched Serum

Sorbents	PO_4^{3-}/mg dl^{-1}	Ca^{2+}/mg dl^{-1}	K^+/mEq l^{-1}	Na^+/mEq l^{-1}	Fe/μg dl^{-1}
Control[a)]	8.8	12.9	9.4	203	157
Iron chitosan[b)]	1.2	9.2	7.2	158	5700
Iron chitosan[c)]	2.9	—[d)]	—	—	415
$Fe_n(OH)_mSO_4$	1.7	—	—	—	25800

Sorbent/serum: 0.1 g/10 ml, Temperature: 37°C, Shaking for 6 h. a) Serum phosphate enriched by addition of extra potassium dihydrogenphosphate. b) Iron content: 127 mg g^{-1}. c) Iron content: 32 mg g^{-1}. d) Not measured.

Conclusions

The iron chitosan complex is a more effective chemical sorbent for binding phosphate in dilute phosphate solutions than those used before. Because of metal–polymer complex formation, the elution of iron from iron chitosan is efficiently depressd. It is expected to be used for a new intestinal phosphate binder, which is prevented from iron elution and insolublization of iron chitosan into a solution of pH 1.5.

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