May, 1975] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48 (5), 1455—1458 (1975) 1455

# The Coprecipitation of Strontium with Hydroxyapatite.

Osamu Fujino
*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611*

(Received December 16, 1974)

The distribution behavior of the strontium ion between hydroxyapatite and the parent solution was investigated. The hydroxyapatite was formed by the extremely slow addition of diammonium hydrogenphosphate to solutions of calcium and strontium nitrate buffered with ethylenediamine at 80 °C. The precipitate yielded a typical X-ray diffraction pattern of hydroxyapatite and had a composition in which the Ca/P molar ratio was 1.67 at pH 6.80. The strontium ion was coprecipitated in the apatite, obeying the Doerner and Hoskins logarithnmic distribution law. The distribution coefficient was scarcely affected by the strontium concentration on the pH value in the parent solution, and had a value of 0.26±0.02 at 80 °C. On the other hand, the apparent distribution coefficient was a little affected by such organic anions as acetate, citrate, lactate, glycinate, and glutamate ions. The lattice constants of the precipitates prepared in the research were measured in order to confirm the formation of the solid solutions.

There is a considerably extensive literature[1)] dealing with the abundance of trace elements in such geochemical samples as phosphate minerals and sediments, and in such biological samples as human bones and teeth. However, there are still very scant experimental data on the coprecipitation of trace elements with the hydroxyapatite, which is the pricipal inorganic constituent of these samples.

In previous papers we have ourselves reported data on the coprecipitation of copper[2)] and zinc[3)] with hydroxyapatite. The coprecipitation of strontium with the apatite is also of great interest because strontium-90 has such a long biological half life as 18000 days in bone and is well known as one of the most dangerous radioisotopes for the human body.

## Experimental

*Apparatus.* The radioactivity countings were made with a Packard Tri-carb Liquid Scintillation Spectrometer, Model 3320. A Hitachi-Horiba pH Meter, Model F-5, was used for the pH measurements. The X-ray patterns of the hydroxyapatite samples were measured by using a Shimadzu X-ray Diffractometer, Model AZ-2A, with nickel-filtered copper radiation.

*Reagents.* The strontium-89 was supplied as chloride in hydrochloric acid by the Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A., and was diluted with 0.1 M nitric acid to make a solution of $\simeq$1 μCi/ml. The strontium and calcium solutions were prepared from their respective reagent-grade nitrates and were standardized by EDTA titration. A standard EDTA solution containing EDTA–magnesium complex was standardized with a calcium solution which had been prepared from dry calcium oxide. A liquid scintillator for the measurement of the strontium activity was prepared by dissolving 100 mg of 1,4-bis(5-phenyloxazolyl)-benzene (POPOP), 4 g of 2,5-diphenyloxazole (DPO), and 75 g of naphthalene with dioxane and by then diluting them to 1 l with the solvent.

The other chemicals were reagent-grade materials.

*Preparation of Hydroxyapatite.* The following procedure was used to prepare the hydroxyapatite precipitates. Twenty milliliters of a 0.5 M calcium nitrate solution and 25 ml of 5 M ethylenediamine were placed in a one-liter Erlenmeyer flask, and the mixture was diluted to around 400 ml with water. The solution, after pH adjustment, was diluted to 500 ml. The flask equipped with a reflux condenser was placed in a thermostat, and nitrogen gas was passed through to prevent the formation of calcium carbonate. The diammonium hydrogenphosphate solution (0.1 M) was then pumped, drop by drop into the calcium solution, which was being stirred by a magnetic stirrer. The supernatent solution was pulled off by an aspirator working through a sintered glass filter, and the precipitate was washed with water repeatedly. The precipitate was then filtered and dried at 110 °C.

*Chemical Analysis of Hydroxyapatite.* The calcium and phosphate ions in the hydroxyapatite precipitate were determined by the EDTA titration method as follows. *Determination of calcium*: EDTA and buffer (pH 10) solutions were added to a sample solution which had been prepared by dissolving the dry sample in hydrochloric acid, and the calcium ions were determined by titrating an excess of EDTA with a magnesium standard solution, using Eriochrome Black T as the indicator. *Determination of phosphate ions*: The phosphate ions in the sample solution above were precipitated as magnesium ammonium phosphate. The precipitate was filtered and dissolved with hydrochloric acid. The phosphate ions could then be determined by titrating the magnesium with the EDTA solution.

*Coprecipitation Procedure.* The procedure of the coprecipitation was essentially the same as those of the hydroxyapatite preparation. The carrier and the radiotracer of strontium were added to the calcium solution in the thermostat described above. At appropriate intervals of time, an aliquot of the supernatent solution was pipetted out through a sintered glass filter. The calcium ions in the solution phase were determined by EDTA titration. The strontium ions were counted with a liquid scintillation spectrometer after the supernatent had been ashed with a mixture of perchloric, nitric and hydrochloric acids and after 4 ml of 0.1 M hydrochloric acid and 13 ml of liquid scintillator had been added to it.

The homogeneous[4,5)] and logarithmic[6)] distribution coefficients, $K_\mathrm{D}$ and $K_\lambda$, were calculated by means of the following equations:

$$\left(\frac{\text{micro-component}}{\text{macro-component}}\right)_{\text{cryst.}} = K_\mathrm{D}\left(\frac{\text{micro-component}}{\text{macro-component}}\right)_{\text{soln.}} \tag{1}$$

$$\log\left(\frac{\text{initial quantity}}{\text{quantity left in soln.}}\right)_{\text{micro-component}} = K_\lambda \log\left(\frac{\text{initial quantity}}{\text{quantity left in soln.}}\right)_{\text{macro-component}} \tag{2}$$

*Lattice Constant.* Hydroxyapatite is hexagonal, with two lattice constants, $A_0$ and $C_0$. These were determined by measuring the diffraction angles, $2\theta$, of the three planes—(312), (213), and (321).

## Results and Discussion

*Composition of Apatite Precipitate.* On the apatite precipitates formed from an aqueous solution, it is well known that solid phases may be precipitated which yield X-ray diffraction patterns typical of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, while deviating considerably from it in composition.[7,8] Figure 1 shows the influence of the precipitation rate on the Ca/P ratio of the apatites, which were prepared by precipitating 50 per cent of calcium with a phosphate solution at pH 6.80 and 8.60. The Ca/P ratio became constant in the range of addition rates slower than 10 ml/hr. The apatite prepared at pH 6.80 gave X-ray diffraction patterns typical of hydroxyapatite, although that produced by the rapid addition of phosphate had diffraction patterns with poorly crystallized materials.

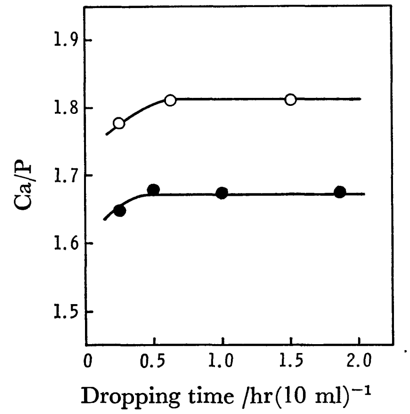

Fig. 1. The Ca/P ratio in the precipitates formed at various precipitation rates.
$Ca(NO_3)_2$, 0.02 M; ethylenediamine, 0.25 M; $(NH_4)_2HPO_4$, 0.1 M; ●, pH 6.80; ○, pH 8.60; initial volume, 500 ml; temperature, 80 °C.

In order to examine the effect of the percentage of calcium precipitation on the Ca/P ratio, the composition of the precipitates was determined; they had been prepared by changing the addition volume of phosphate. As is shown in Fig. 2, the Ca/P ratio was constant throughout the precipitation process of calcium. These precipitates showed X-ray patterns typical of hydroxyapatite.

The pH value affected the Ca/P ratio in the synthesized apatite (50% precipitation), as may be seen in Fig. 3; the Ca/P ratio decreased as the pH decreased. The Ca/P ratio in mineral bone when organic matter has been removed is between 1.57 and 1.61,[9] which implies that the apatite is a little calcium-deficient. As the value of pH 6.80 at 80 °C corresponds to about 8.0 at room temperature, the results in Fig. 3 may not be inconsistent with the fact that the accretion and resorption in bone occur around the neutral pH range.

At pH 6.80, the Ca/P ratio was 1.67; this corresponded to the composition of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. This pH value was, therefore adopted

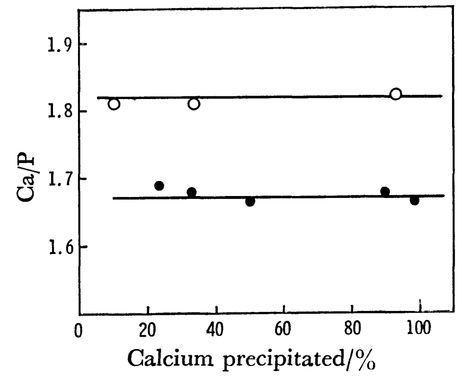

Fig. 2. The Ca/P ratio in the precipitation process.
●, pH 6.80; ○, pH 8.60; dropping velocity, 10 ml/hr. Other are the same as in Fig. 1.

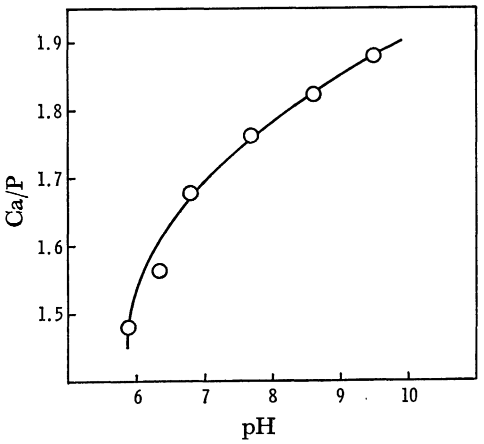

Fig. 3. The pH effect on Ca/P ratio.
Dropping velocity of phosphate, 10 ml/hr. Others are the same as in Fig. 1.

throughout the experiments.

*Coprecipitation of Strontium.* In order to examine the influence of the precipitation rate on the coprecipitation behavior of strontium, the precipitation reaction was performed at the pumping velocities of 7, 12.2, and 65.8 ml of phosphate solutions per hour; the

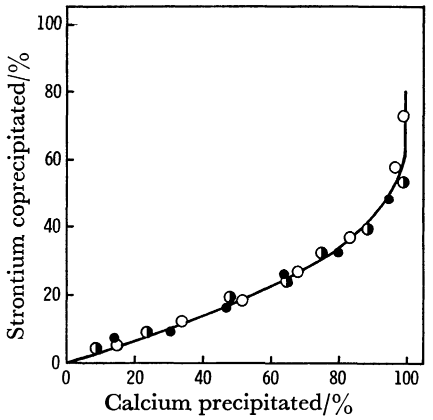

Fig. 4. The coprecipitation of strontium with hydroxyapatite formed at various precipitation rates.
$Ca^{2+}$, 0.02 M; $Sr^{2+}$, $10^{-6}$ M; ethylenediamine, 0.25 M; $HPO_4^{2-}$, 0.1 M; dropping velocity, ●, 7.0 ml/hr; ○, 12.2 ml/hr; ◑, 65.8 ml/hr; pH 6.80; initial volume, 500 ml; temperature, 80 °C.

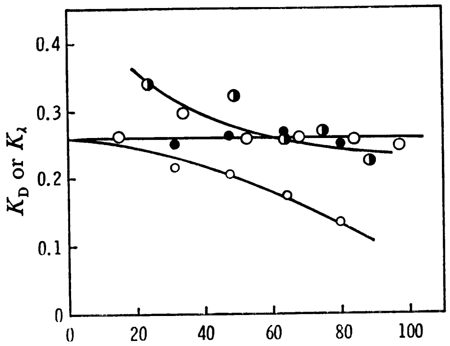

Fig. 5. The distribution manner of strontium.
◑, $K_\lambda$ at 65.8 ml/hr of the dropping velocity;
○, $K_\lambda$ at 12.2 ml/hr; ●, $K_\lambda$ at 7.0 ml/hr;
○, $K_D$ at 7.0 ml/hr, respectively.
Others are the same as indicated in Fig. 4.

concentrations of calcium, strontium and ethylenediamine were $2\times10^{-2}$ M, $1\times10^{-6}$ M, and 0.25 M respectively; the thermostat was regulated at 80 °C. The results are shown in Fig. 4, in which the percentages of the coprecipitated strontium are plotted against the fractions of precipitated calcium. The strontium ion was enriched in the solution phase, as is shown in Fig. 4. The homogeneous and logarithmic distribution coefficients are plotted in Fig. 5 against the percentage of calcium precipitated. In the case of the slower addition of phosphate ions, the strontium ions were coprecipitated obeying the logarithmic law (the coefficient, $K_\lambda=0.26\pm0.02$), while in the more rapid addition the distribution coefficient decreased as the amount of precipitated calcium increased. The X-ray diffraction pattern of the latter solid phase denoted that the material was a poorly crystallized one. The coprecipitation behavior of strontium at pH 6.80, 7.70, and 8.60 is shown in Figs. 6 and 7. The Ca/P ratio of the apatite was affected by the pH, as has been mentioned above, while the logarithmic distribution coefficient did not change with the pH ($K_\lambda$=0.26).

The hydroxyapatite was precipitated at pH 6.80 from solutions containing strontium ranging from $10^{-8}$ M to $2.5\times10^{-2}$ M, with the other circumstances kept identical. The results are shown in Fig. 8; no significant change in the coprecipitation behavior of strontium was observed between $10^{-8}$ M and $5\times10^{-4}$ M. However, the distribution coefficient in the calcium-strontium apatite system tended to decrease at higher concentrations of strontium. It was observed from the X-ray diffraction pattern that this precipitate crystallized somewhat poorly; this tendency became clearer as the strontium content increased. Calcium-strontium apatite may crystallize more slowly than the pure calcium apatite.

The effect of the concentration of ethylenediamine used as a buffering agent was examined at pH 6.80 with regard to solutions of 0.10, 0.25, 0.50, and 1.0 M ethylenediamine; the effect was found not to be

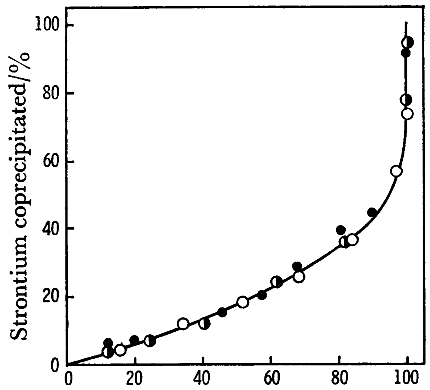

Fig. 6. The coprecipitation of strontium at different pH values.
○, pH 6.80; ◑, pH 7.70; ●, pH 8.60.

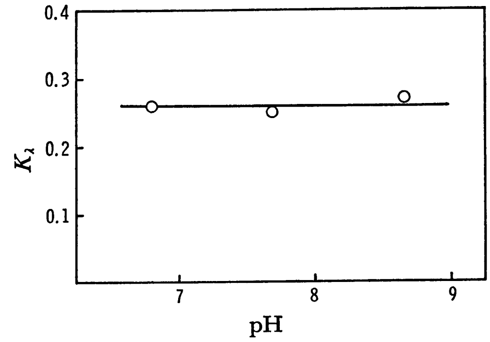

Fig. 7. The effect of pH on the logarithmic distribution coefficient.

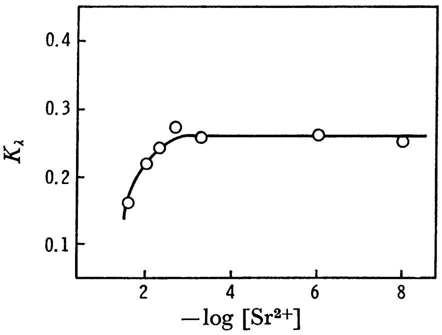

Fig. 8. The effect of strontium concentration.

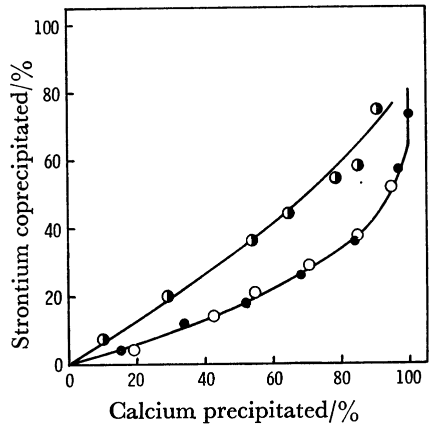

Fig. 9. The temperature effect on the coprecipitation of strontium.
●, 80 °C $K_\lambda$=0.27; ○, 55 °C $K_\lambda$=0.26;
◑, 25 °C $K_\lambda$=0.56; dropping velocity, 10 ml/hr.

noticeable in the range investigated. This may be caused by the fact that calcium and strontium ions do not form complexes with ethylenediamine so stable as to influence the distribution coefficient.

Calcium-strontium apatite was precipitated at 20, 55, and 80 °C. As is shown in Fig. 9, the coprecipitation behavior of strontium was identical at both 55 and 80 °C, while at 20 °C the behavior was different. The precipitate prepared at 20 °C had broad peaks in its X-ray diffraction pattern. The Ca/P mole ratio of the precipitate was 1.36 at the beginning of precipitation and decreased to 1.07 at 90 per cent precipitation. The abnormal distribution of strontium was probably caused by an incompleteness of apatite crystallization such as was observed above.

Collin prepared homogeneous solid solutions of strontium and calcium hydroxyapatite by heating precipitates formed from an aqueous solution to 950 °C,10) and the lattice constants of these solid solutions were measured accurately. In the present apatites, the lattice constants were also measured in order to confirm the formation of the solid solutions (Table 1); the values were found to be close enough to those in Collin's paper, though there was a little error caused by the imcompleteness of crystallization.

Table 1. Lattice constants of calcium and strontium apatites

| Sr/(Sr+Ca), % | $A_0$ | $C_0$ |
|---|---|---|
| 0 | 9.417 Å | 6.870 Å |
| 14.5 | 9.479 | 6.924 |
| 20.0 | 9.494 | 6.966 |
| 42.3 | 9.559 | 7.039 |

It is also interesting to investigate the influence of chelating agents on the present coprecipitation system, because considerably strong chelate-forming substances should exist in the calcium-metabolism system. Acetate, citrate, lactate, glycinate, and L-glutamate ions were used as the organic anions. The results are summarized in Table 2. As is shown in the table, the apparent distribution coefficient, $K_\lambda$, increases as the concentration of these organic anions increases.

Solid solutions were formed between calcium and strontium apatite by slow precipitation from a neutral or weakly basic solution at 80 °C, and the distribution coefficient of the strontium ions was about 0.26. A direct extension of these results to the processes occurring in biological systems may be difficult. However, it is possible that the very long biological half-life of strontium is caused by the formation of solid solutions.

Table 2. The effect of organic acids on the distribution coefficient

| Organic acid | Concn of acid | |
|---|---|---|
| | M | $K_\lambda$ |
| Acetic acid | 0 | $0.24_5$ |
| | 0.1 | 0.26 |
| | 0.3 | $0.27_3$ |
| | 0.4 | $0.29_3$ |
| | 0.5 | $0.33_7$ |
| | 0.7 | $0.35_7$ |
| Citric acid | $2\times10^{-4}$ | $0.28_4$ |
| | $1\times10^{-3}$ | $0.41_8$ |
| Lactic acid | $3\times10^{-2}$ | $0.27_9$ |
| | $5\times10^{-2}$ | $0.31_7$ |
| | 0.1 | $0.35_2$ |
| Glycine | 0.1 | $0.24_2$ |
| | 0.4 | $0.31_5$ |
| Glutamic acid | 0.04 | $0.27_8$ |
| | 0.1 | $0.31_6$ |

pH 6.80, Ca 0.02 M, ethylenediamine 0.25 M, Sr $10^{-6}$ M, temperature 80 °C

The author would like to express his thanks to Professor Eiji Suito for his helpful discussions and to Mr. Tsunoru Yoshida for his X-ray mesurements. The author would also like to thank Professor Tsunenobu Shigematsu, Professor Masayuki Tabushi, and Dr. Masakazu Matsui for their helpful discussions and comments during the preparation of this paper.

## References

1) F. W. Spiers, "Radioisotopes in the Human Body," Academic Press, New York (1968).

2) T. Shigematsu, O. Fujino, and M. Matsui, *Bull. Inst. Chem. Res., Kyoto Univ.*, **50**, 645 (1972).

3) O. Fujino, M. Matsui, M. Tabushi, and T. Shigematsu, *Radioisotopes*, **24**, 7 (1975).

4) W. Chlopin, *Z. Anorg. Allg. Chem.*, **143**, 97 (1925).

5) L. M. Henderson and F. C. Kracek, *J. Amer. Chem. Soc.*, **49**, 738 (1927).

6) H. A. Doerner and W. M. Hoskins, *ibid.*, **47**, 662 (1925).

7) A. S. Posner, J. M. Stutman, and E. R. Lippincott, *Nature*, **188**, 486 (1960).

8) E. E. Berry, *J. Inorg. Nucl. Chem.*, **29**, 317 (1967).

9) F. C. McLean and M. R. Urist, "Bone," the University of Chicago Press, Chicago (1961), p. 53.

10) R. L. Collin, *J. Amer. Chem. Soc.*, **81**, 5275 (1959).