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Comparison of the defluoridation efficiency of calcium phosphate and chitin in the exoskeleton of Antarctic krill

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Abstract Calcium (Ca), phosphorus (P), and chitin are the main components of the exoskeleton of krill. Defluoridation of a solution of sodium fluoride (NaF) using calcium phosphate ($Ca_3(PO_4)_2$) and chitin as defluoridation agents was studied. Orthogonal experiments were designed to find the optimum reaction conditions for defluoridation, to obtain the maximum defluoridation efficiency and fluoride removal capacity of calcium phosphate and chitin. At the same time, a comparison of the capacity of the two defluoridation agents was made. The results suggest that calcium phosphate has a far greater capability than chitin for the removal of fluoride (F) from water under similar reaction conditions. It is also suggested that Antarctic krill is likely to adsorb fluoride via compounds such as calcium phosphate, hydroxyapatite, and other compounds of Ca and P with the general form $(Ca, X)_x(PO_4, HPO_4, Y)_y(OH, Z)_z$, in addition to chitin.

Keywords **calcium phosphate, chitin, defluoridation, krill, orthogonal design**

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0 Introduction

Fluorine (F) is an essential trace element for human beings. As F is a dual-threshold element, a deficiency or an excessive can have adverse effects on human health. Fluorine is normally present in bones and teeth, although excessive amounts can be toxic and lead to debilitating fluorosis in humans and animals[1-3]. Antarctic krill (*Euphausia superba*) is rich in F and they contain greater than 1 000 $mg \cdot kg^{-1}$[4], with the exoskeleton containing as much as 5 477 $mg \cdot kg^{-1}$[5].

Calcium (Ca), phosphorus (P), and chitin are the main components of the exoskeleton of krill. It has been reported that the chitin structures in the exoskeleton play an important role in F concentration[6-7]. Chitin accounts for 20%—30% of the dry weight of the shrimp's exoskeleton, while Ca, P and other inorganic mineral elements make up 30%—40%[8]. According to some reports, the F content of chitin in the krill exoskeleton is only about 200 $mg \cdot kg^{-1}$[4]. The comparatively low F content of chitin compared with the overall F content of the krill exoskeleton suggests that chitin may not be the main reason that krill adsorb F from sea water. However, the Ca and P content have been reported to be proportional to the F content in different parts of the krill's body[4]. Thus, it is possible that Ca and P also contribute to the high F content in the krill's exoskeleton. To determine how krill adsorb F, by either chitin or calcium phosphate, we designed various orthogonal experiments. In this study, we optimized the reaction conditions, analyzed how much F was removed from a sodium fluoride (NaF) solution by calcium phosphate and chitin, and compared the capabilities of the two defluoridation agents. Finally, we present a tentative explanation for the high F content of krill.

1 Materials and methods

NaF standard solution (F^-, 100 $mg \cdot L^{-1}$) was used to draw the standard curve for the F ion-selective electrode (ISE)[9]. Chitin, calcium phosphate, and NaF (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 100 mL PTFE beakers and deionized water

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(18 MΩ·cm water obtained from a Milli-Q water purification system) were used to minimize loss or gain of F, which could cause experimental error. A 10-channel analog magnetic stirrer with several PTFE magnetons was used for mixing the F solutions. The pHs of the solutions were measured using a DELTA-320 pH meter (Mettler-Toledo CO., Ltd, Shanghai, China). A PXSJ-226 ion-activity meter (Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, China) was used with the ISE.

To optimize the defluoridation efficiency of chitin and calcium phosphate, orthogonal tests were designed with 3 factors at 3 different levels and the analysis of the F concentration was conducted using the ISE. Many factors were taken into consideration, such as particle size, defluoridation time, pH, reaction temperature, and mass of defluoridation agents. Initial experiments were conducted to determine the three main factors, which were determined to be the defluoridation time (*t*), pH, and mass of defluoridation agents (*m*). Each factor had three levels, which were listed in Table 1. The parameters of the $L_9(3^3)$ orthogonal tests are shown in Tables 2—4.

Table 1 The factors and levels for the orthogonal tests

Level	*t*/min	pH	*m*/g
1	80	4	0.2
2	120	5	0.4
3	160	6	0.6

Table 2 F removal rate and the adsorption capacity for *5.553 mg·L^{-1} NaF solution with chitin as the defluoridation agent

Orthogonal test design items		Factors			Tested results	Computed results	
		t/min	pH	*m*/g	*CF/(mg·L^{-1})	F removal rate/%	F adsorption capacity /(mg·kg^{-1})
Levels	T1	80	4	0.2	4.011	27.77	385.5
	T2	80	5	0.4	4.724	14.93	103.6
	T3	80	6	0.6	5.432	2.18	10.1
	T4	120	4	0.4	3.933	29.27	202.5
	T5	120	5	0.6	4.743	14.59	67.5
	T6	120	6	0.2	5.251	5.44	75.5
	T7	160	4	0.6	3.904	29.70	137.4
	T8	160	5	0.2	4.655	16.17	224.5
	T9	160	6	0.4	5.193	6.48	45.0
Range trend analysis of F removal rate	K1 (%)	14.96	28.88	16.46			
	K2 (%)	16.40	15.23	16.86			
	K3 (%)	17.45	4.70	15.49			
	R	2.49	24.18	1.37			
Range trend analysis of adsorption capacity	K-1 (mg·kg^{-1})	166.4	241.8	228.5			
	K-2 (mg·kg^{-1})	115.2	131.9	176.9			
	K-3 (mg·kg^{-1})	135.6	43.5	71.7			
	R	51.2	198.3	156.8			

*Already deducted value of blank F concentration (CF)

A total ionic strength adjustment buffer (TISAB) buffer was prepared by dissolving 14.2 g of $C_6H_{12}N_4$ (AR), 8.5 g of KNO_3 (AR), and 1 g of $C_6H_4Na_2O_8S_2{\cdot}H_2O$ (AR) in 500 mL of deionized water. The pH of the TISAB buffer solutions were then adjusted to the required pH values (pH = 4, 5 or 6) using HCl (aq, 0.01 mol·L^{-1} and 0.001 mol·L^{-1}). The TISAB buffer was prepared for later use and to avoid interference of the F analysis by Fe and Al compounds. A blank TISAB buffer was also prepared without adjusting pH. The 250 mg·L^{-1} F^- solution was prepared by dissolving 0.055 3 g NaF powder in 100 mL of deionized water in a 100 mL PTFE volumetric flask, and then the mixture was shaken well. A 49 mL portion of the blank TISAB buffer was transferred to a 100 mL PTFE beaker, and 1 mL of 250 mg·L^{-1} F^- was added. The original F concentration of the solution was then determined using the ISE.

A 49 mL portion of the TISAB buffer with the required pH (pH = 4, 5 or 6) was then transferred to another 100 mL

PTFE beaker and 1 mL of 250 mg·L^{-1} F^- was added. The required amount (m = 0.2 g, 0.4 g or 0.6 g) of the defluoridation agent (chitin or calcium phosphate) was added. Then, a PTFE magneton was placed in the beaker and the solution was mixed in a 10-channel analog magnetic stirrer for the required time (t = 80, 120 or 160 min). The final F concentration was determined using the ISE. Duplicates were prepared for each treatment. The orthogonal test design is shown in Tables 2—4.

Table 3 F removal rate and the adsorption capacity for *5.881 mg·L^{-1} NaF solution with calcium phosphate as the defluoridation agent

Orthogonal experimental design items		Factors			Tested results	Computed result	
		t/min	pH	m/g	*CF /(mg·L^{-1})	F removal rate/%	F adsorption capacity /(mg·kg^{-1})
	T1	80	4	0.2	0.002	99.966	1 469.8
	T2	80	5	0.4	0.005	99.915	734.5
	T3	80	6	0.6			
	T4	120	4	0.4	0.000	100.000	735.1
Levels	T5	120	5	0.6	0.001	99.983	490.0
	T6	120	6	0.2	0.509	91.345	1 343.0
	T7	160	4	0.6	0.000	100.000	490.1
	T8	160	5	0.2	0.000	100.000	1 470.3
	T9	160	6	0.4	0.907	84.577	621.8

*Already deducted value of blank CF

Table 4 Comparison of the efficiency of the two defluoridation agents chitin and calcium phosphate

Orthogonal experimental design items		Factors			F removal rate/%		F adsorption capacity/(mg·kg^{-1})	
		t/min	pH	m/g	Chitin	Calcium Phosphate	Chitin	Calcium phosphate
	T1	80	4	0.2	27.77	99.966	385.5	1 469.8
	T2	80	5	0.4	14.93	99.915	103.6	734.5
	T3	80	6	0.6	2.18		10.1	
	T4	120	4	0.4	29.27	100.000	202.5	735.1
Levels	T5	120	5	0.6	14.59	99.983	67.5	490.0
	T6	120	6	0.2	5.44	91.345	75.5	1 343.0
	T7	160	4	0.6	29.70	100.000	137.4	490.1
	T8	160	5	0.2	16.17	100.000	224.5	1 470.3
	T9	160	6	0.4	6.48	84.577	45.0	621.8
Maximum					29.79[1]	100.000	385.5	1 470.3

[1]The maximum F removal rate for chitin was obtained using the optimum reaction conditions determined from the orthogonal experiments (defluoridation time: 160 min, pH = 4, and amount of chitin: 0.4 g).

2 Results and discussion

2.1 Defluoridation rate of chitin

The F removal rate with chitin as the defluoridation agents was calculated from the final F concentration (Table 2). As shown in Table 2, the F removal rate of chitin in T7, T4, and T1 are the highest, and are much higher than the other experiments. All the F removal rates are less than 30%. The highest F removal rate (29.70%) was observed with T7 treatment (defluoride time: 160 min, pH: 4, and chitin: 0.6 g).

The range trend analysis of F removal rate for the different levels and factors was calculated and the results are listed in Table 2. The values of K1, K2, and K3 represent the individual F removal rate for each selected level and factor. For example, the K1 value of column pH means that

the F removal rate of level 1 (pH = 4) is 28.88%, which is the average value of the data of level 1 (27.77%, 29.27%, and 29.70%), and the K3 value of column t means that the F removal rate of level 3 (t = 160 min) is 17.45%, which is the average value of the data of level 3 (29.70%, 16.17%, and 6.48%). The value of R represents the range of K1, K2, and K3 in the same column: R_{pH} (24.18) > R_t (2.49) > R_m (1.37), indicating that these three factors affect the F removal rate in the order: pH value of the solution > defluoridation time > amount of chitin.

The range trend of F removal rate for the different levels and factors is shown in Figure 1. The optimal levels of the three factors are level 3 for defluoridation time (160 min), level 1 for pH (pH = 4), and level 2 for amount of chitin (0.4 g). Therefore, the optimal reaction conditions for the orthogonal experiment are t3-pH1-m2, which was not included in the orthogonal test design. In a supplementary experiment under the optimal reaction conditions, the F removal rate was 29.79% (Table 4), which is slightly higher than the value of T7 (29.70%).

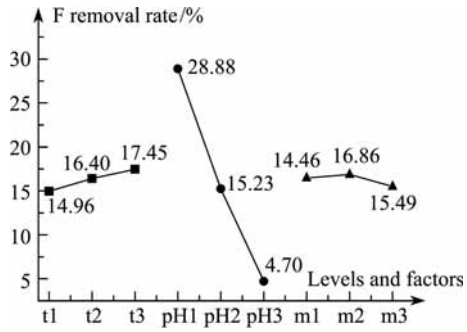

Figure 1 The range trend of the F removal rate for different levels and factors.

2.2 Fluoride adsorption capacity of chitin

The adsorption capacities with chitin as the defluoridation agent are listed in Table 2. The adsorption capacity was calculated using the formula:

$$\text{Adsorption capacity (mg·kg}^{-1}) = (C_o - C_f) \times V / m, \quad (1)$$

where C_o and C_f denote the F concentration (mg·L^{-1}) of the original and final solution, V and m denote the volume (mL) of the F solution and the mass (g) of chitin added as the defluoridation agent. The F adsorption capacities of chitin show significant differences (Table 2), with the maximum adsorption capacity (385.5 mg·kg^{-1}) observed for T1 treatment (defluoride time: 80 min, pH: 4, and chitin: 0.2 g).

A range trend analysis of F adsorption capacity rate for the different levels and factors was conducted and the calculated results are listed in Table 2. The F removal rate is in the order R_{pH} (198.3) > R_m (156.8) > R_t (51.2), indicating that the three factors affect the F adsorption capacity of chitin in the order: pH value of the solution > amount of chitin > defluoridation time.

The plots of the adsorption capacity for the different levels and factors (Figure 2) shows the optimal levels of the three factors are level 1 for defluoridation time (80 min), level 1 for pH (pH = 4), and level 1 for mass of chitin (0.2 g), so the optimal reaction conditions for the orthogonal experiment are t1-pH1-m1. In conclusion, we get a maximum F adsorption capacity of 385.5 mg·kg^{-1} using chitin as the fluoride removal agent.

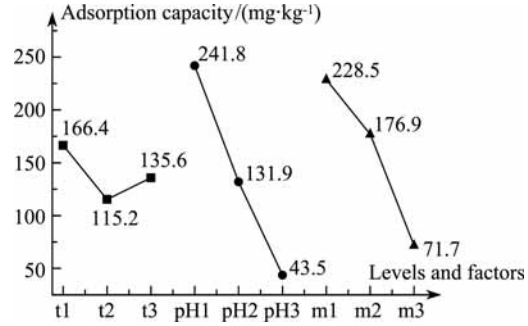

Figure 2 The range trend of the F adsorption capacity for different levels and factors.

2.3 Defluoridation capability of calcium phosphate

The F removal rate and F adsorption capacity of calcium phosphate are given in Table 3. The orthogonal experimental design using calcium phosphate as the defluoridation agent was the same as for chitin. The same reaction conditions are used to enable direct comparison of the efficiency of chitin and calcium phosphate. After determining the final fluoride concentration, the F removal rate and F adsorption capacity of calcium phosphate were calculated. The fluoride concentration for T3 treatment could not be obtained because of an error in the ISE. Hence, the range trend analysis for calcium phosphate could not be carried out.

It was observed that the final fluoride contents are all very low, with most of them close to zero (Table 3). Thus, the F removal rates are all close to 100%. In contrast, the F adsorption capacities are different. The maximum F adsorption capacity is 1 470.3 mg·kg^{-1} with T8 conditions. The F adsorption capacities with T1 and T6 conditions are close to that of the T8 treatment, and significantly higher than the others. The values of the fluoride content close to zero indicate that there is an excess of calcium phosphate for the remove of F. In other words, the actually maximum adsorption capacity of F using calcium phosphate as the defluoridation agent will be greater than 1 470.3 mg·kg^{-1}.

2.4 Comparison of the efficiency of the two defluoridation agents

The efficiency of the two defluoridation agents (chitin and calcium phosphate) was compared using the same reaction conditions and the results are shown in Table 4. Compared with chitin, both the F removal rate and the F adsorption capacity of calcium phosphate are higher. The maximum F removal rate of chitin is 29.79%, while that of calcium phosphate is 100%. Similarly, the maximum F adsorption capacity of chitin is 385.5 mg·kg^{-1}, while it is at least 1 470.3 mg·kg^{-1} for calcium phosphate. These results indi-

cate that calcium phosphate is more effective than chitin for removing F from water, and thus calcium phosphate is a more effective defluoridation agent.

2.5 Possible reasons for high F content of krill

In general, Antarctic creatures have high F content, and a strong ability for fluorine accumulation and a high F tolerance[10]. Krill is an important species in Southern Ocean ecosystems, because it is an important food source for seals and other Antarctic animals. To investigate whether Ca and P, or their compounds, can increase the F content in the exoskeleton of krill, we made a rough calculation of the F content of krill exoskeleton. 1 kg of krill exoskeleton contains 4 028 mg F[4], and the chitin component is about 250 g, because it contains 20%—30% chitin[8]. It has been reported that the F content in chitin is not high, only about 200 $mg \cdot kg^{-1}$[4]. In our orthogonal experiments, the maximum F adsorption capacity of chitin was estimated to be 385.5 $mg \cdot kg^{-1}$. Therefore, the F content of chitin in the krill exoskeleton constitutes only 2.4% of the total exoskeleton F, indicating that chitin isn't the main reason for the high F content of krill. The Ca and P content in krill exoskeleton are reported to be 3.55% and 5.59%[4]. Assuming that the Ca and P in the krill exoskeleton only exist in the form of calcium phosphate, it can adsorb 1 470.3 $mg \cdot kg^{-1}$ F based our orthogonal experiments, although this is an underestimation of the actual maximum F adsorption capacity. The estimated F content contributed by calcium phosphate in the krill exoskeleton is about 3.4% of the total exoskeleton F using the percentage of Ca, and 10.2% using the percentage of P. Although it also only contributes a small portion of the total F in the krill exoskeleton, it is higher than that of chitin. Moreover, the actual F adsorption capability of calcium phosphate is expected to be considerably greater than that estimated in the present study. Thus, calcium phosphate adsorbs more F from solution than chitin, and this partly explains the high F content of krill. In the next section we will attempt to explain the source of the remainder of F in krill.

2.6 What is the main source of fluorine in krill?

Ca and P are the principal components of the bones of animals, with Ca and P making up 39.9% and 18.5% of the weight of bone. The ratio of Ca to P is 2.16, and the major form of inorganic calcium is $Ca_{10}(PO_4)_{6-x}(CO_3)_x(OH)_{2+x}$, which is deposited in the collagen molecule clearance[11]. Similarly, in the exoskeleton of krill, P often exists in Ca compounds[12], and Ca usually exists as calcium carbonate and calcium phosphate[13]. It has been reported that Ca and P are very rich in Antarctic krill[4, 14-15]. We suggest that F would be physically or chemically adsorbed by chitin in the krill exoskeleton during the Antarctic krill growth process, since we found that chitin has a F adsorption capacity of about 385.5 $mg \cdot kg^{-1}$. This may be caused by the structure and strong ion exchange ability of chitin. We propose that Ca, P, and chitin may have a synergistic effect in the F enrichment of Antarctic krill. During the growth process of Antarctic krill, Ca and P would be transported to the clearance of chitin in the krill exoskeleton by the krill body, generating stable compounds in the form of various calcium phosphates. These compounds would fill in the clearance of chitin structure and tightly integrate with chitin. The concentration of F is high in sea water, and it will slowly seep into the krill exoskeleton via the chitin structure, and then react with the stable compounds made of Ca and P, forming a stiff crust that can protect the soft body from physical damage. Deposition of F in the Antarctic krill exoskeleton can also prevent excess F from entering the krill body.

Fluoride uptake by various calcium phosphates, such as hydroxyapatite[$Ca_{10}(PO_4)_3(OH)_2$, HAP], octacalcium phosphate[$Ca_8H_2(PO_4)_6 \cdot 5H_2O$, OCP], and dicalcium phosphate dihydrate[$CaHPO_4 \cdot 2H_2O$, DCPD]) has been studied by Yang et al.[16]. They found that the calcium phosphates absorb fluoride through fluorapatite formation via dissolution and recrystallization. Chen et al. has also studied the reaction of DCPD, HAP with F[17]. The reaction products were anhydrous dicalcium phosphate[$CaHPO_4$, DCPA], fluor-hydroxyapatite[$Ca_{10}(PO_4)_3Fx(OH)_{2-x}$, FHAP], fluorapatite[$Ca_{10}(PO_4)_6F_2$, FAP], and calcium fluoride [CaF_2], depending on the F ion concentration. These results combined with our experiment data suggest that F may deposit with calcium phosphates in the chitin structure, forming substances like $Ca_x(PO_4, HPO_4)_y(OH, F)_z$. Moreover, cations like Mg^{2+}, Sr^{2+}, Ba^{2+}, and Zn^{2+} have similar properties to Ca^{2+}, and anions like CO_3^{2-}, HCO_3^-, SO_4^{2-}, Cl^-, and NO_3^- have similar properties to PO_4^{2-} and HPO_4^-, and they are all abundant in the ocean. Thus, we suggest that they may also play a role in the enrichment of F in krill, by forming compounds like $(Ca, Mg)_x(PO_4, HPO_4, CO_3)_y(OH, Cl, F)_z$, and $(Ca, Sr)_x(PO_4, HPO_4, SO_4)_y(OH, NO_3, F)_z$.

3 Conclusions

In this study, the defluoridation of solutions of sodium fluoride (NaF) using calcium phosphate and chitin as defluoridation agents was studied. We designed orthogonal experiments to determine the optimum reaction conditions for defluoridation. The maximum defluoridation efficiency and fluoride removal capacity of calcium phosphate and chitin were determined. Calcium phosphate was found to have a greater F removal capacity than chitin under similar reaction conditions. Based on the results of our experiments, the mechanism of the F enrichment in Antarctic krill can mainly be explained by the existence of substances such as calcium phosphate, hydroxyapatite, and other compounds of Ca and P with the general form $(Ca, X)_x(PO_4, HPO_4, Y)_y(OH, Z)_z$, where $X = Mg^{2+}$, Sr^{2+}, Ba^{2+} or Zn^{2+}, and $Y = CO_3^{2-}$, HCO_3^-, SO_4^{2-}, Cl^-, or NO_3^-. Further research into the mechanism of Antarctic krill F enrichment is required.

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References

1 Boulton I C, Cooke J A, Johnson M S. Fluoride accumulation and toxicity in wild small mammals. Environ Pollut, 1994, 85(2): 161-167.
2 Choubisa S L. Endemic fluorosis in Southern Rajasthan, India. Fluoride, 2001, 34(1): 61-70.
3 Li Y M, Liang C K, Slemenda C W, et al. Effect of long-term exposure to fluoride in drinking water on risks of bone fractures. J Bone Mineral Res, 2001, 16(5): 932-939.
4 Zhang H S, Xia W P, Cheng X H, et al. A study of fluoride anomaly in Antarctic krill. Antarctic Research, 1991, 3(4): 24-30 (in Chinese).
5 Sands M, Nicol S, McMinn A. Fluoride in Antarctic marine crustaceans. Mar Biol, 1998, 132(4): 591-598.
6 Yin X B, Chen L A, Sun L G, et al. Why do penguins not develop skeletal fluorosis? Fluoride, 2010, 43(2): 108-118.
7 Zhu B Y, Wang X Y, Hu Q X. A study of fluoride in Antarctic krill. Antarctic Research, 1988, 1(1): 51-55 (in Chinese).
8 Zhang X G, Zhou A M, Lin X X, et al. Comparative study of chemical compositions of white shrimp head and shell. Modern Food Science and Technology, 2009, 25(3): 224-227 (in Chinese).
9 Xie Z Q, Sun L G. Fluoride content in bones of Adelie penguins and environmental media in Antarctica. Environ Geochem Health, 2003, 25(4): 483-490.
10 Xiang J H. Antarctic krill and fluorine. Marine Science, 1985, 9(3): 57-59 (in Chinese).
11 Xu S Q, Wang J, Cheng B B, et al. Concentrations of Ca, P and Sr and characteristics of Ca/P and Ca/Sr in the bones of typical seabirds in the Antarctic. Journal of University of Science and Technology of China, 2007, 37(8): 995-1002 (in Chinese).
12 Brannon A C, Rao K R. Barium, strontium and calcium levels in the exoskeleton, hepatopancreas and abdominal muscle of the grass shrimp, Palaemonetes pugio: relation to molting and exposure to barite. Comparative Biochemistry and Physiology, 1979, 63(2): 261-274.
13 Deshimaru O, Yone Y. Requirement of prawn for dietary minerals. Bulletin of the Japanese Society of Science Fisheries, 1978, 44(8): 907-910.
14 Sun S, Yan X J. Active substances in the Antarctic krill. Chinese Journal of Polar Research, 2001, 13(3): 213-216 (in Chinese).
15 Zhu Y Y, Yin X B, Zhou S B. A preliminary study of selenium and mineral elements in Antarctic krill. Chinese Journal of Polar Research, 2010, 22(2): 135-140 (in Chinese).
16 Yang T, Kim C, Jho J, et al. Regulating fluoride uptake by calcium phosphate minerals with polymeric additives. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2012, 401: 126-136.
17 Chen F, Feng Z D, Lin C J. Effect of sodium fluoride solution on the hydrolysis of $CaHPO_4 \cdot 2H_2O$ and the solubility of its hydrolysate. Journal of Xiamen University (Natural Science), 2001, 40(1): 52-58 (in Chinese).