

# The Novel Synthesis and Characterisation of Hydroxyapatite and its Application in Leather Making

WANG, Y. Z.<sup>1</sup>, WANG SHUAI<sup>2</sup> and SHAN, Z. H.<sup>1,2</sup>

<sup>1</sup>The Key Laboratory of Leather and Engineering (Sichuan University), Ministry of Education, Chengdu 610065

<sup>2</sup>National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065

## Abstract

Hydroxyapatite(HAP) has been extensively used in clinics for such as bone tissue repair because of its outstanding performance. But, whether HAP can be utilized in other fields still remains unknown, for example leather making. In the present work HAP was synthesized using a hydrothermal method with diammonium hydrogen phosphate and calcium nitrate as phosphorus and calcium precursors respectively. The Fourier transform infrared spectroscopy analysis of products shows that HAP can be produced using the wet-chemical method. The condition optimization test results indicated that high purity HAP can be obtained when the reaction was carried out at 80°C and pH11 with surfactant added and ultrasonic treatment for 8 hours. To study the effect of HAP on cow hide and whether it can be used as tanning agent, *vitro* and *vivo* tests of adding HAP into cow hide were conducted. The cow hide was first treated in the wet end process of leather making and then cut into several parts. The parts were treated using different HAP addition methods and the experimental results were compared under different conditions. Fourier transform infrared spectroscopy analysis indicated that HAP can react with the cow hide using both methods. Cow hide physicochemical properties were improved after reaction with HAP, the thermal stability was especially enhanced. The thermal shrinkage temperature of the treated hide was 78°C which was higher than the sample in the control group of 54°C. The hide tensile strength was greatly enhanced because HAP penetrated into the hide and reacted on hide collagen. So, HAP has the potential to be used as chrome-free tannage. This meant that the modification of cow hide by HAP was feasible and HAP tannage or HAP combination tannage was of practical significance.

## 1 INTRODUCTION

Hydroxyapatite(HAP) crystals are mostly in a hexagonal system with the molecular structure of apatite<sup>1</sup> although there are some exceptions in a monoclinic system. HAP structures are formed by the tetrahedral arrangement of phosphate( $\text{PO}_4^{3-}$ ).<sup>2</sup> The monoclinic form of HAP formed at high temperature is more ordered and thermodynamically stable,<sup>3</sup> but there is no evidence of its presence in calcified tissues. It is worth noting that the closer the value of Ca/P to 1.67, the higher stability of HAP inside the human body.<sup>4</sup>

Bone is a biocompatible and non-toxic composite organic/inorganic material primarily comprising HAP and collagen and was introduced as a biomaterial in bone repair<sup>5</sup> and allied systems. However, with prepared collagen/hydroxyapatite composites, there are still low mechanical strength, rapid degradation and other defects limiting its application.<sup>6</sup> Improving the HAP effect on collagen is still a problem faced by many researchers. The problem can not only be solved by the synthesis of HAP, but also by the materials it combined with. Collagen is a major structural protein composing the raw hide and the 80-90% collagen belongs to type I collagen with triple helical structure.<sup>7</sup> The conformational stability and helical structure of collagen is a function of variables like temperature, pH and treatment condition. The low hydrothermal stability of collagen limits type I collagen's application in the

clinic and other fields. Tanning can improve the physicochemical properties especially thermal stability of hide collagen in leather making process. Conclusions can be drawn from tanning researches that the tannage produces some special materials.<sup>8</sup> Those materials react with collagen *in-situ* producing a network structure (matrix structure).<sup>9</sup> This matrix structure can effectively prevent the thermal deformation of collagen from heating, in other words, the thermal shrinkage temperature of hide was improved. So, leather's high thermal shrinkage temperature is the result of stable appropriate size module produced by tannage reaction in collagen. It is well known that HAP can react with collagen. Whether HAP can penetrate and react in hide is unknown. HAP has the potential to be a novel modified hide producing industrial material.

Several studies have described diverse methods to synthesize HAP such as microemulsion,<sup>10</sup> precipitation,<sup>11</sup> solid-state reaction and microwave.<sup>12</sup> In the present study, HAP was produced in a novel hydrothermal method. Material analyses such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and inductively coupled plasma-mass spectroscopy (ICP-MS) were used to characterise HAP. In order to improve the physicochemical properties of the hide especially the thermal denaturation temperature, HAP was used to

treat the cow hide both *in vivo* and *vitro*. HAP effects were analysed by FTIR, leather tensile strength test, leather shrinkage temperature meter and derivative thermogravimetric analysis (DTG). HAP may be of use as chrome-free tannage or possibly an exceptional performance material.

## 2 EXPERIMENTAL PROCEDURES

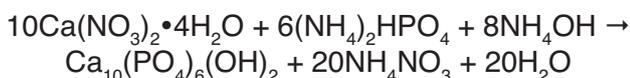
### 2.1 Materials and equipment

$\text{NH}_4 \cdot \text{H}_2\text{O}$ ,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $(\text{NH}_4)_2\text{HPO}_4$ , etc., are all analytical reagent grade. Raw cow hide (purchased from Hoffen Chemical Company in Xinji City, Hebei Province).

Equipment: Sartorius Extend Series electronic balance (Sartorius, Germany); Nicolet 6700 Fourier transform infrared spectrograph (Nicolet, U.S.A.); VG PQ ExCell Inductively coupled plasma-mass spectroscopy (TJA, U.S.A.); TG 209F1 Thermal Gravimetric Analyzer and DSC 200PC Differential Scanning Calorimeter (Netzsch, Germany); D/Max2500 X-ray diffraction (Rigaku, Japan); H-600 Transmission electron microscope (Hitachi, Japan); DL07-PS-83 Leather shrinkage temperature metre (Beijing North Branch of Analytic Instrument Limited Company, China); QX-W400 Leather tensile strength tester (Shanghai Qixiang Testing Instrument Co. Ltd., China).

### 2.2 HAP preparation

In this paper HAP was synthesized by a hydrothermal method using  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $(\text{NH}_4)_2\text{HPO}_4$  as starting materials and ammonia solution as agent for pH adjustment. The solution of 0.30mol/L  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  was prepared by dissolving 70.8g  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in 1000mL absolute ethyl alcohol. It was then gently stirred at the set temperature and pH. The solution of 0.18mol/L  $(\text{NH}_4)_2\text{HPO}_4$  was prepared by dissolving 23.76g  $(\text{NH}_4)_2\text{HPO}_4$  in 1000mL distilled water and then slowly added dropwise into the  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  solution. The solutions were slowly stirred at set temperatures for the given times. That can be explained by the following reaction:



In the next step, the precipitates were removed from solutions by centrifugation at 1000g for 10 minutes. The products were washed by distilled water and alcohol and then dried at 80°C. The crystallinity of the product was further increased by calcination at different

temperatures. The resulting powders were ground and evaluated by FTIR, XRD, ICP-MS and TEM.

### 2.3 Tests of effects of HAP on hide collagen

The raw cow hide purchased from Hoffen Chemical Company was treated by the usual wet-end process of leather making. The salted cow hides were pre-soaked at room temperature with 0.5%  $\text{Na}_2\text{CO}_3$ , 0.2% degreasing agent, 0.2% penetrating agent and 5.0% NaCl. Followed by soaking with 0.5%  $\text{Na}_2\text{CO}_3$ , 0.2% degreasing agent, 0.2% penetrating agent and 1.0% NaCl in the second day. The soaked hides were limed with 1.8%  $\text{Na}_2\text{S}$  and 2.0%  $\text{Ca}(\text{OH})_2$  for 120 minutes and then delimed with 2.5%  $(\text{NH}_4)_2\text{SO}_4$  and 0.2% degreasing agent on the third day. The hide was bated with 0.3% bating enzyme. The cow hide samples were taken after the raw cow hides had gone through washing, liming, deliming, degreasing and pickling processes. 200g cow hide sample and 1.0L distilled water were placed into a reactor. The pH was adjusted to about 11.0 with ammonia solution, stirred and mixed with a magnetic stirrer for 20 minutes. After that, 1.2mol/L  $\text{Ca}(\text{NO}_3)_2$  and 0.72mol/L  $(\text{NH}_4)_2\text{HPO}_4$  solution, respectively, were dropped into the mixture, and stirred fully for 8 hours and aged at 55°C for at least 20 hours. After washing, vacuum drying, and being air-dried, the hide sample marked as HC-1 -treated with HAP was prepared. Another cow hide sample was treated in the same process as a contrast without HAP addition.

The hide samples treated by HAP *in vitro* addition (marked HC-2) were prepared as follows using the synthesised HAP microspheres. A 200g cow hide sample and 1.0L distilled water entered into a reactor. The pH was adjusted to about 11.0 with ammonia water as before. Then 5wt% of synthesised HAP microspheres were added with respect to hide weight. The intermixture was stirred for 8 hours and transferred to a vacuum dryer. The materials obtained above were analysed by leather shrinkage temperature meter, Leather tensile strength tester, FTIR and TG/DTG spectrometer.

## 3 RESULTS AND DISCUSSION

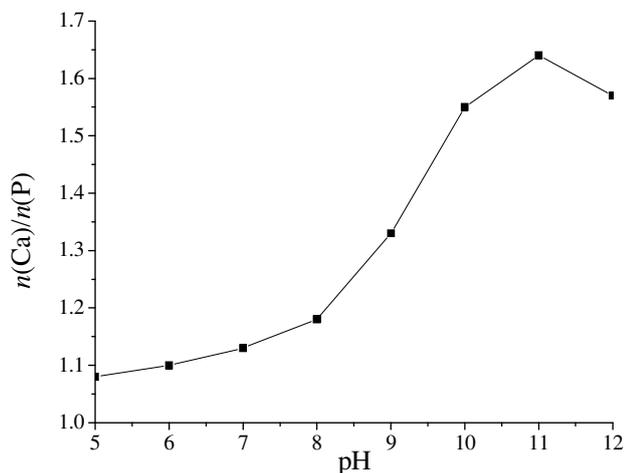
### 3.1 Characterization of HAP

The tests were conducted at different pH values which varied from 5.0 to 12.0 adjusted by ammonia. The product Ca/P mole ratio ( $n(\text{Ca})/n(\text{P})$ ) was increased with the pH value rise (Fig. 1). The reaction pH may affect the solubility properties of reactants and

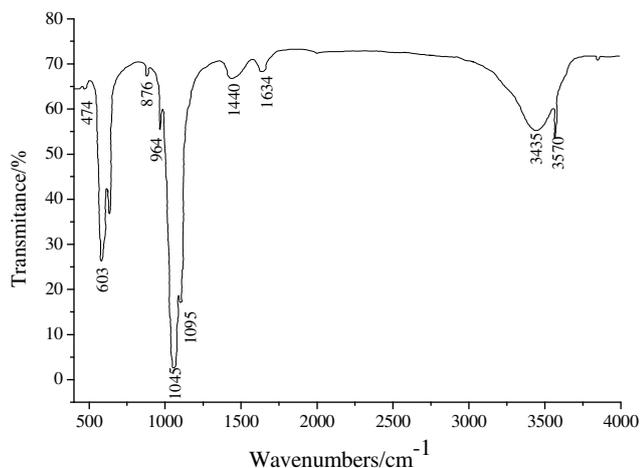
TABLE I  
The n(Ca/P) value range of different products

w (HAP)/%	w ( $\text{Ca}_3(\text{PO}_4)_2$ )/%	w ( $\text{CaHPO}_4$ )/%	n (Ca/P)(theoretical value)	n (Ca/P) ± 0.02 (measured value)
80	15	5	1.6	$1.568 \leq n(\text{Ca/P}) \leq 1.632$
85	10	5	1.609	$1.577 \leq n(\text{Ca/P}) \leq 1.641$
90	5	5	1.617	$1.585 \leq n(\text{Ca/P}) \leq 1.650$
95	5	0	1.658	$1.625 \leq n(\text{Ca/P}) \leq 1.691$
100	0	0	1.667	$1.637 \leq n(\text{Ca/P}) \leq 1.703$

products that will change the ion balance in solution. So the growth of HAP crystals is also changed by the change of pH values. These factors may lead to the different  $n(\text{Ca})/n(\text{P})$  of products at different reaction pHs. The products would mainly be composed of  $\text{CaHPO}_4$  in acidic conditions and  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  in alkaline conditions. When the product was obtained at pH11.0, the  $n(\text{Ca})/n(\text{P})$  ratio reached 1.66 very close to the theoretical value. The HAP obtained at pH11.0 has the highest purity amongst all the products.



**Figure 1.** The relationship between  $n(\text{Ca})/n(\text{P})$  and the reaction pH.

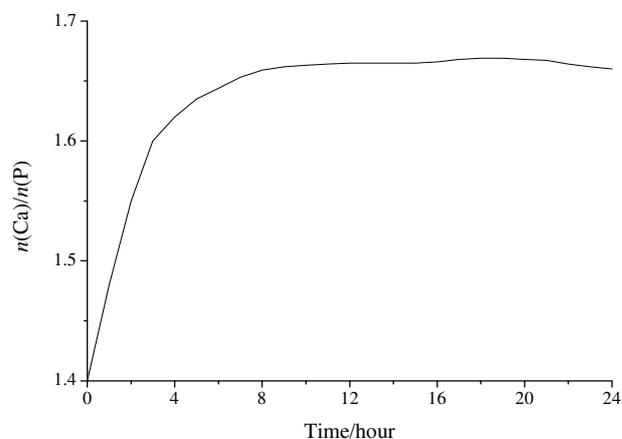


**Figure 2.** The FTIR spectra of synthesized HAP.

There were two anion groups in the HAP particle including  $\text{PO}_4^{3-}$  and  $\text{OH}^-$ . The FTIR pattern of HAP particle synthesised at pH11.0 indicated that the characteristic absorption peaks  $571\text{cm}^{-1}$ ,  $602\text{cm}^{-1}$ ,  $963\text{cm}^{-1}$ ,  $1050\text{cm}^{-1}$  and  $1089\text{cm}^{-1}$  of  $\text{PO}_4^{3-}$  can be detected in the synthesized product (Fig. 2). The characteristic  $\text{OH}^-$  absorption peaks appeared at  $631\text{cm}^{-1}$ ,  $3570\text{cm}^{-1}$ . The conclusion can be drawn that high purity HAP can be produced by hydrothermal method described in this paper.

In the HAP preparation system, the phosphate ion will almost only contain  $\text{PO}_4^{3-}$  with very small concentrations of  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  ions at a reaction pH of 11. The  $n(\text{Ca})/n(\text{P})$  ratio of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ,  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  and  $\text{CaHPO}_4$  precipitates are 1.67, 1.50

and 1.00 respectively. Different precipitate proportions in the product results in different  $n(\text{Ca})/n(\text{P})$  ratios (Table I). It can be concluded that  $n(\text{Ca})/n(\text{P})$  will range at 1.625~1.703 when the HAP content exceeds 95%. So the  $n(\text{Ca})/n(\text{P})$  can be an index evaluation of HAP purity.

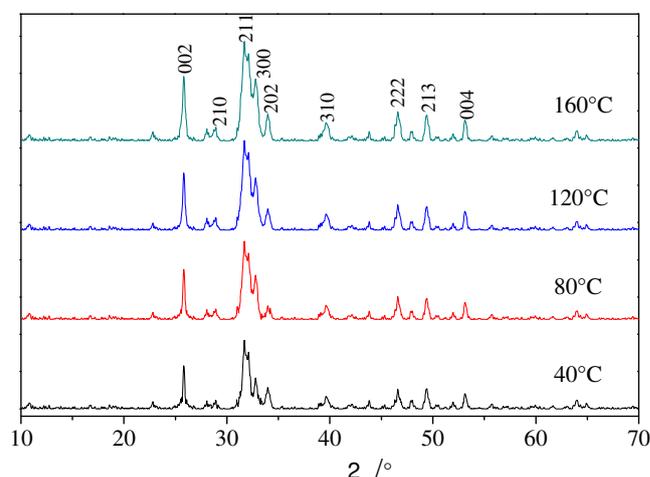


**Figure 3.** The corresponding relationship between products  $n(\text{Ca})/n(\text{P})$  with time.

In the synthesis process of HAP, the reaction liquid was taken out and cooled down in ice water after reaction times from 1 to 24 hours. The products  $n(\text{Ca})/n(\text{P})$  ratios were tested by ICP-MS (Fig.3). HAP particles were synthesised soon after the beginning of the synthetic reaction. The product's  $n(\text{Ca})/n(\text{P})$  ratio reached 1.66 close to the theoretical value when the reaction time was 8 hours.

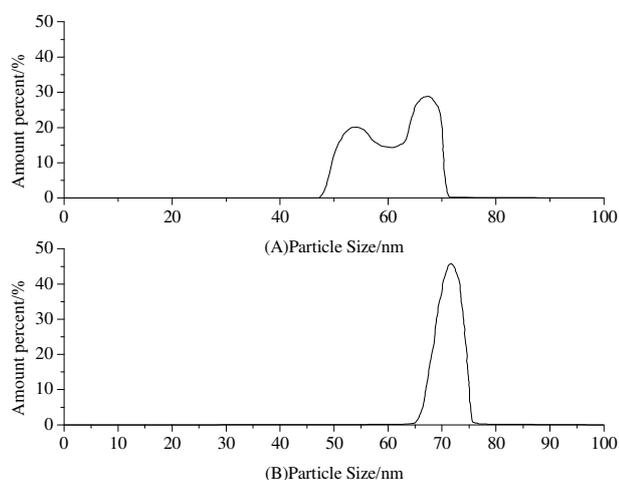
The production particles' XRD patterns for products synthesised at different temperatures  $40^\circ\text{C}$ ,  $80^\circ\text{C}$ ,  $120^\circ\text{C}$  and  $160^\circ\text{C}$  were all consistent with the standard HAP XRD pattern (Fig. 4). The developments of HAP crystals are different under different temperatures. With the increase of temperature, the products' XRD pattern peak line becomes a little sharper and higher. This indicates that the crystallinity of HAP increases a little with the increase of temperature.

In order to obtain finer particles, dimethylformamide was added in the production of HAP in experiment A. It can be concluded that the particle size of the HAP



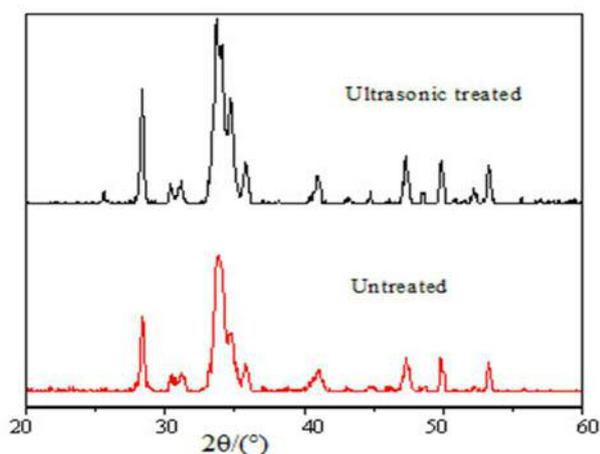
**Figure 4.** XRD patterns of HAP particles synthesised at different temperature.

particles obtained decreases with the addition of surfactant, but the particle size distribution becomes wider (Fig. 5). The surfactant can be absorbed on the particle surface which will cause repulsion between the particles. That means surfactants can reduce the particle size by reducing particle aggregation.



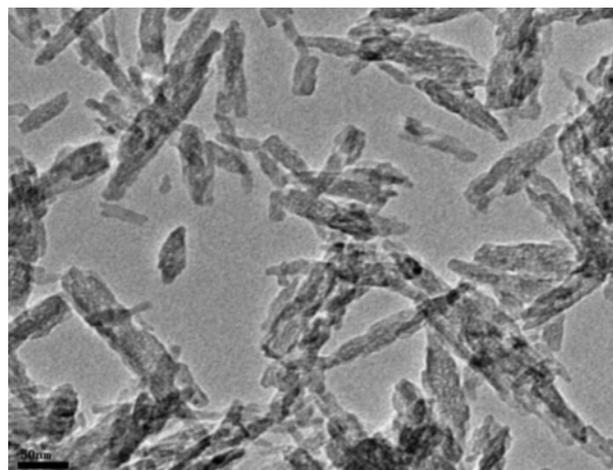
**Figure 5.** Surfactant effect on the HAP particle size. (A) Add surfactant (B) No surfactant.

The ultrasonic wave treatment can produce a different sound intensity that causes fluid to move at high speed. Then ultrasonic cavitation in water results in the formation, growth and break-up of micro bubbles in solution. The result indicates that ultrasonic treatment can affect both crystal growth and crystallinity of HAP (Fig. 6). The HAP particle synthesised with ultrasonic treatment has higher crystallinity than the untreated group.



**Figure 6.** Ultrasonic effect on HAP synthesis.

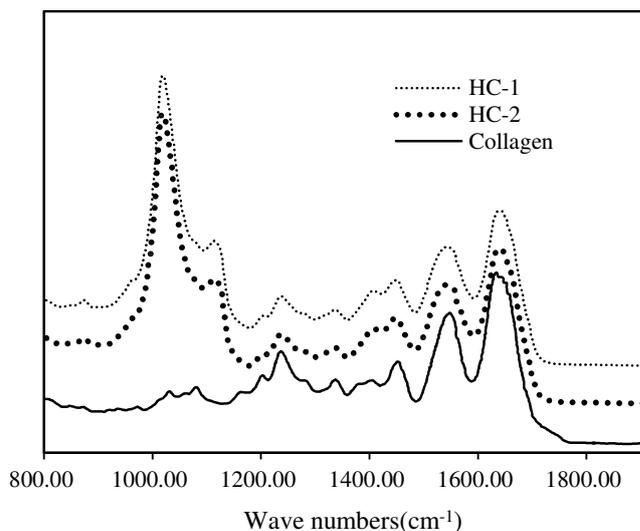
So, high crystallinity HAP can be synthesised by the hydrothermal method using  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $(\text{NH}_4)_2\text{HPO}_4$  as starting materials at pH11 and  $80^\circ\text{C}$  with surfactant addition and ultrasonic treatment for 8 hours as described in this paper. The synthesized HAP particle is 100nm length and 45nm width (Fig. 7). So HAP particles can penetrate into the depths of hide as the basis of the reaction between HAP and hide collagen.



**Figure 7.** The TEM photograph of synthesized HAP.

### 3.2 Effect of HAP on collagen

The bands of hide collagen can be observed with C=O stretching at  $\sim 1645\text{cm}^{-1}$  for the amide I, and N-H deformation at  $\sim 1545\text{cm}^{-1}$  for the amide II. Compared to pure hide collagen, these products show phosphoric groups with a typical stretching vibration band located at  $1050\text{cm}^{-1}$  in IR spectra of HC-1 and HC-2 (Fig. 8). So HAP can bind to hide collagen both in the *vitro* and *vivo* tests.



**Figure 8.** The FTIR spectra of collagen, HAP/collagen composite.

Only 20% of the pure hide will remain when the processing temperature reaches  $660^\circ\text{C}$ . But the HC-1 and HC-2 products both show very little mass loss (Fig. 9). The HAP's binding to hide collagen reduces the collagen's mass loss in the thermal degradation process. That indicates the HAP in hide supports the thermal stability of the hide.<sup>13</sup>

Hide collagen's three strand spiral structure will be gradually degraded in the process of heating. The hide collagen was gradually decomposed with the release of the energy of the three strands of the spiral structure. The hide will shrink when the hide collagen

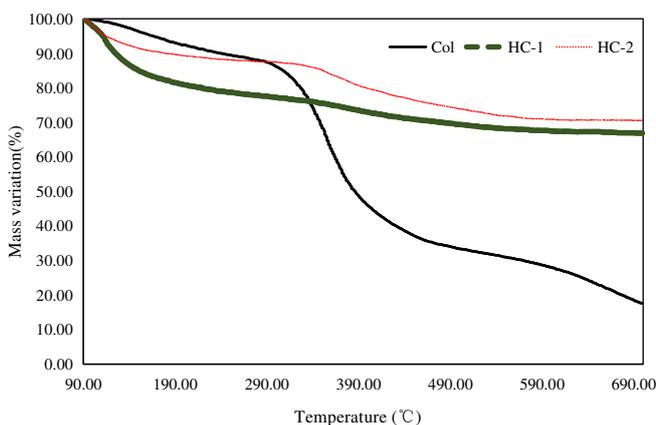


Figure 9. The TG test of pure collagen and HAP/collagen.

degradation reaches a certain level. The thermal shrinkage temperature ( $T_s$ ) of HAP treated hide reached, at 78°C, a level higher than pure hide collagen whose  $T_s$  was 54°C. So the conclusion can be drawn that HAP combined with hide and the thermal stability was improved.

The reaction processes of HAP and hide can be explained in Figure 10. The HAP particle infiltrated into the hide and attached on the hide collagen. The stability of hide can be improved, whether it is by chemical combination or physical support. The tensile strength of HAP treated hide greatly improved since HAP penetrates into hide and 'grows' on hide collagen. Hide's other physicochemical properties may also be modified by reaction with HAP which will be discussed in the following article. HAP may be suitable to use as a chrome-free tanning agent and may yield novel medical or industrial materials with practical value.

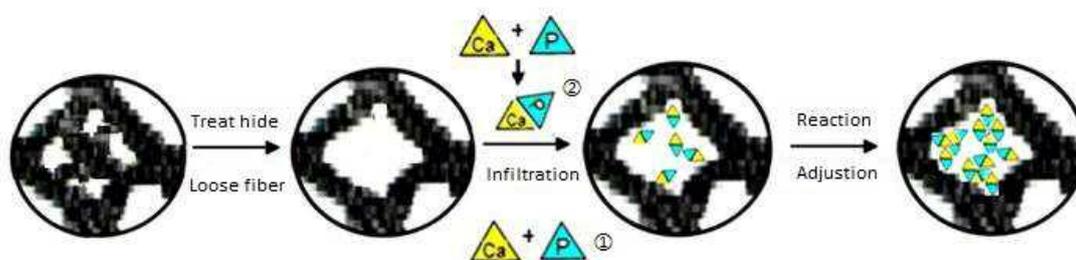


Figure 10. The reaction mechanism of HAP and hide collagen.

#### 4. CONCLUSION

HAP has been widely used as a biomaterial in bone repair, bone implants and bone drug delivery systems due to its outstanding performance. Researches on HAP are of great significance and have become the most active field of researches in recent years. The FT-IR spectra analysis results revealed that HAP was produced by the hydrothermal method in this paper. High purity HAP can be obtained when the reaction is performed at 80°C and pH11 for 8 hours with surfactant addition and ultrasonic treatment. In order to improve the thermal stability of hide collagen and study whether HAP can be used as chrome-free tanning agent, tests of HAP's effect on cow hide was performed. Both *in vitro* and *in vivo*, tests were conducted adding HAP into the cow hide. FTIR spectra analysis indicated that HAP can react with the hide in both methods. Hide's thermal stability was improved after reaction with HAP and the  $T_s$  of treated hide reached 78°C. The hide tensile strength was greatly enhanced since HAP penetrated into hide and reacted on hide collagen. Other physicochemical properties may also be improved which will be tested and more related experiments will be performed. Conclusions can be drawn that HAP can be used to treat the hide to modify the cow hide. This research is of significance to the application of hide and HAP and to get innovative medical or industrial materials with practical value. HAP has the potential to be used as chrome-free tanning agent. In order to

explore the mechanism of the reaction, test conditions and the application, further researches will be conducted.

#### ACKNOWLEDGMENTS

The authors thank the Science Fund of National Bureau of Quality Inspection and also express our gratitude to the National Engineering Laboratory for Clean Technology of Leather Manufacture for providing instruments. Thanks to teacher Shan's guidance and the support of our country.

(Received March 2018)



in association with  
China Leather

#### References

- Guerra-López, J. R., Echeverría, G. A., Güida, J. A. *et al.*, Synthetic hydroxyapatites doped with Zn(II) studied by X-ray diffraction, infrared, Raman and thermal analysis. *J. Physics & Chemistry of Solids*, 2015, **81**, 57.
- Hawthorne, F. C., Structure and chemistry of phosphate minerals. *Mineralogical Magazine*, 2004, **62**(2), 141.
- Medina, D. A. V., Ferreira, A. P. G., Cavalheiro, E. T. G., Thermal investigation on polymorphism in sodium saccharine. *J. Thermal Analysis and Calorimetry*, 2014, **117**(1), 361.
- Tan, C. Y., Singh, R., The, Y. C. *et al.*, The effects of calcium-to-phosphorus ratio on the densification and mechanical properties of hydroxyapatite ceramic. *Internat. J. Applied Ceramic Technology*, 2014, **12**(1), 223.

5. Yang, W., Both, S. K., Zuo, Y. *et al.*, Biological evaluation of porous aliphatic polyurethane/hydroxyapatite composite scaffolds for bone tissue engineering. *J. Biomedical Materials Research Part A*, 2015, **103**(7), 2251.
6. Gholami, F., Cytocompatibility and mechanical strength of hydroxyapatite reinforced with multi-walled carbon nanotubes. *J. Bioengineering & Biomedical Science*, 2015.
7. Usha, R., Maheshwari, R., Dhathathreyan, A. *et al.*, Structural influence of mono and polyhydric alcohols on the stabilization of collagen., *Colloids & Surfaces B Biointerfaces*, 2006, **48**(2),101.
8. Covington, A. D., Song, L. and Suparno, O., An explanation of the chemical stabilisation of collagen. *J. Soc. Leather Technol. Chem.*, 2007, **92**(1), 1.
9. Covington, A. D., Lampard, G. S., Hancock, R. *et al.*, Studies on the origin of hydrothermal stability: a new theory of tanning. *J. Amer. Leather Chem. Ass.*, 1998, **93**, 107.
10. Yang, Y., Wu, Q., Wang, M. *et al.*, Hydrothermal synthesis of hydroxyapatite with different morphologies: Influence of supersaturation of the reaction system. *Crystal Growth & Design*, 2014, **14**(9), 4864.
11. Nurul Huda, A., Subuki, I., Hussain Ismail, M., Synthesized hydroxyapatite powder from clamshell via chemical precipitation method. *Advanced Materials Research*, 2014, **911**, 72.
12. Li, X., Shang, X., Kong, H. *et al.*, Synthesis of hydroxyapatite from cuttlefish bone via hydrothermal solid-state conversion. *J. Biomaterials Tissue Eng.*, 2016, **6**(10), 775.
13. Covington, A. D., Lampard, G. S. *et al.*, Extended X-ray absorption fine structure studies of the role of chromium in leather tanning. *Polyhedron*, 2001, **20**, 461.