ELSEVIER

Ceramics International 30 (2004) 139–143

CERAMICS INTERNATIONAL

www.elsevier.com/locate/ceramint

Adsorption/desorption of acidic and basic proteins on needle-like hydroxyapatite filter prepared by slip casting

O. Takagi, N. Kuramoto, M. Ozawa, S. Suzuki*

Ceramics Research Laboratory, Nagoya Institute of Technology, Asahigaoka, Tajimi 507-0071, Japan

Received 2 September 2002; received in revised form 20 November 2002; accepted 20 January 2003

Abstract

The adsorption/desorption of proteins, such as the bovine serum albumin (BSA) as an acidic protein and the lysozyme chloride (LYZ) as a basic protein, was investigated with the needle-like hydroxyapatite(N-HAp) filters after fired at several fixed temperatures. The filters were prepared by a slip casting with the aqueous N-HAp(commercial powder:Ca/P = 1.54) slips supplemented with calcium hydroxide [$Ca(OH)_2$] for the lack of calcium. The cast filters were fired in H_2O vapor at the range of 600–1200 °C. The adsorbed amounts were measured with the N-HAp fired filters. For both the BSA and LYZ, the adsorbed amounts increased with increasing the fired temperatures. Furthermore, the proteins desorption was also investigated by a column chromatograph using the linear gradient method with the sodium phosphate buffer solution (PBS). The PBS concentration at which the BSA was desorbed from the filters was independent to the firing temperature of the filters. However, for the LYZ, the PBS concentration increased with increasing the fired temperatures. From the results, it was found on the filters fired more than 1000 °C that the LYZ adsorbed stronger to the HAp filters than the BSA.

Keywords: A. Firing; A. Slip casting; C. Chemical properties; E. Biomedical applications

1. Introduction

Hydroxyapatite (HAp) has a useful property for a separation of proteins with activity and can be expected as packing filler for the separation in the column chromatography. The HAp synthesized by Tiselius et al. [1] with a wet method in 1956 was applied as the packing filler, but the HAp was plate-like particles, and clogged the flow in the column chromatography due to the orientation of particles. Next, the large aggregated particles of needle-like HAp (N-HAp) obtained by Iwamura et al. [2] in 1986 held the tissue for the smooth flow in the column. However, the N-HAp was insufficient for the mass separation in the long period as the separation agents in the columns since the mechanical strength of the N-HAp aggregated particles was insufficient. So, we examined the preparation of the fired filter of N-HAp with enough compressive strength. In the present work, the filters prepared by a slip casting after refilling the lack amounts of Ca with $Ca(OH)_2$ were fired at several fixed temperatures, and investigated on the adsorption/desorption of proteins.

2. Experiment procedure

2.1. Preparation of slurry and filter

The commercial needle-like hydroxyapatite (N-HAp; Toagosei Chemical Industry Co. Inc. Japan, av. Ca/P = 1.54) was adopted as the starting powder, and refilled with $Ca(OH)_2$ for the lack amounts of Ca [3]. The aqueous N-HAp slurries were prepared with 25 mass%HAp and 1.0 mass% cation type dispersant (SN7347-C, the Sunnopko Co Ltd, Japan, molecular weight; ca.1000, pH12.0, n.v.20). The aqueous slurries were mixed by a ball mill with resin coated ball for 2 h and cast into the pluster mold at 0.2 MPa by the pressure casting apparatus as shown in Figs. 1 and 2. The cast filter was burnt out at 500 °C for 1 h and then fired at 400–1200 °C for 2 h in vapor.

* Corresponding author. Tel.: +81-572276811; fax: +81-572276812.
E-mail address: suzuki@crl.nitech.ac.jp (S. Suzuki).

0272-8842/03/$30.00
doi:10.1016/S0272-8842(03)00061-0

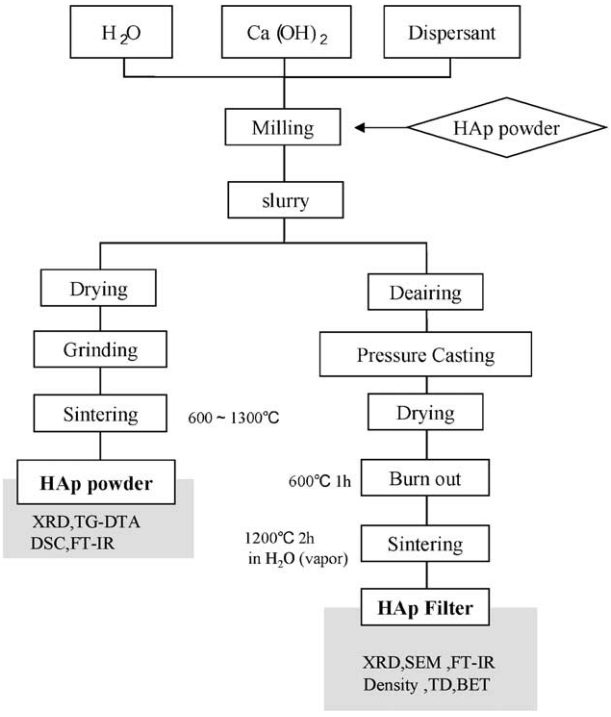

Fig. 1. Experimental flow chart.

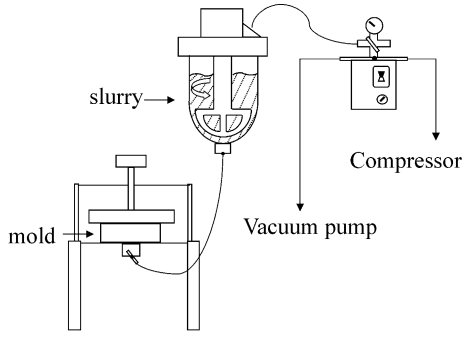

Fig. 2. Schematic diagram of apparatus for slip casting.

2.2. Adsorption/desorption of proteins

The adsorbed amounts of proteins were measured on the filters fired at several fixed temperatures. The protein chromatograms were also investigated with the liquid chromatography apparatus (CCPM: Tosoh Co. Inc., Japan) as shown in Fig. 3. Each fired filter of N-HAp was fixed into the apparatus as shown in Fig. 4. The bovine serum albumin (BSA, Fraction V, Wako pure Chemical Industry Ltd., Japan, molecular weight; 65,000, p.I.4.8) as an acidic protein and the lysozyme chloride (LYZ; the egg white, Wako pure Chemical Industry Ltd., Japan, molecular weight; 14000, p.I.11) as a basic protein were used in the present experiment. First, the proteins were dissolved in the 10mmol/dm^3 buffer solutions of sodium phosphate (PBS, pH6.8), and the concentration of the solution was diluted to 0.001 mg/dm^3. The adsorbed amounts were measured by a batch method. Next, the chromatogram was obtained by a linear gradient method [4]. Two PBS solutions, A (10 mmol/dm^3) and B (500 mmol/dm^3), were applied to the elution. The flow rate of the solutions was kept at 2×10^3 mm^3/min invariably. The chromatograms were measured with the absorbance at 280 nm wavelength. First, the 100%A (0%B) solution was flowed for 10 min after applied with the proteins into the column, and then the concentration of B with using the linear-gradient method arrived to 100% for 20 min.

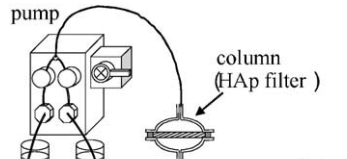

Fig. 3. Schematic apparatus of liquid chromatography.

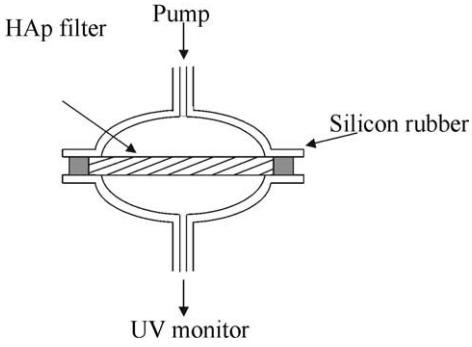

Fig. 4. HAp column.

3. Results and discussion

3.1. Property of filters

The filters fired at several fixed temperatures were checked by the X-ray diffraction (XRD; Rigaku, RINT-2200, Japan), FT-IR (Nicolet, Impact 400) and the scanning electron microscope (SEM; Jeol, JSM-6100). The obtained XRD patterns and FT-IR spectra were shown in Figs. 5 and 6, respectively. From Fig. 5, the β-$Ca_3(PO_4)_2$ (β-TCP) phase appeared slightly in the filters fired at 600 and 800 °C, but the filters fired more than 1000 °C were returned again to the HAp phase only. The crystallization of HAp progressed even more at the higher temperature. The sintering of the HAp filters proceeded also with increasing firing temperatures from the SEM photographs as shown in Fig. 7. The peak of OH group on the filters fired at 1200 °C appeared at 3570 cm^{-1} in FT-IR spectra (Fig. 6).

Therefore, the starting Ca-deficient HAp refilled with $Ca(OH)_2$ seemed to be restrained to convert to oxyapatite (OAp) and β-TCP after fired in vapor although no difference between the OAp and the HAp was clearly shown from the XRD patterns [5,6].

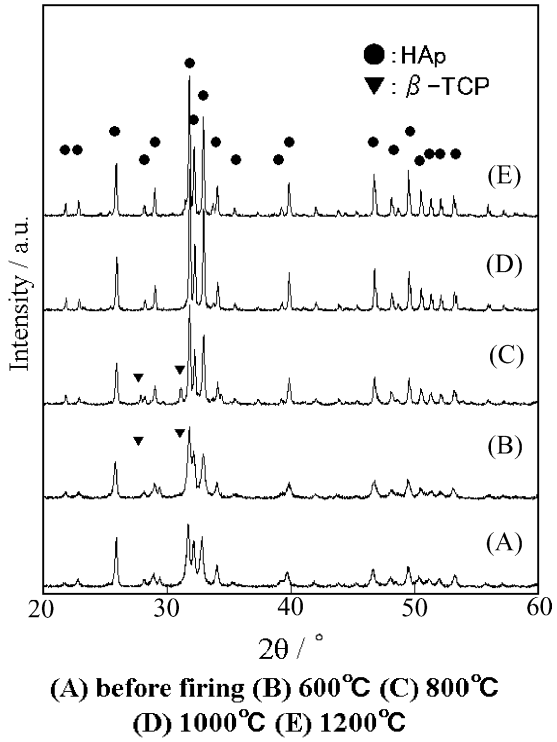

Fig. 5. XRD patterns of HAp added with $Ca(OH)_2$ after fired at several fixed temperatures.

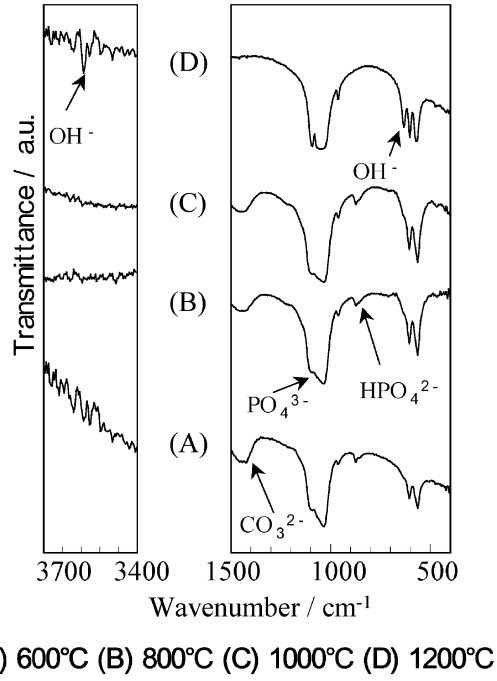

Fig. 6. FT-IR spectra of HAp added with $Ca(OH)_2$ after fired at several fixed temperatures.

3.2. Adsorption/desorption of proteins

The adsorbed amounts of BSA and LYZ proteins on the filters increased with increasing fired temperatures as shown in Fig. 8. The tendency of adsorption with both proteins related to the growth of HAp crystalline phase as shown in Fig. 5, that is, the most adsorbed amounts of both the acidic and basic proteins were obtained with the filters fired at 1200 °C. For the desorption, the chromatograms of BSA and LYZ resulted in Figs. 9 and

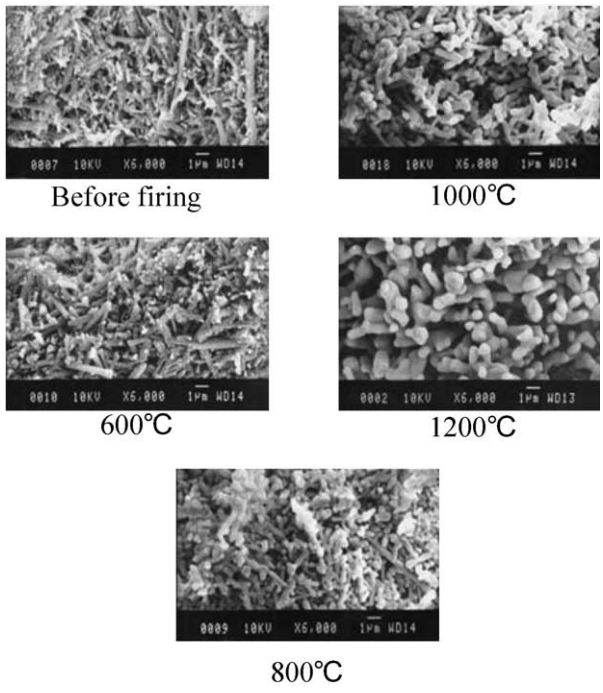

Fig. 7. SEM photographs of surface of HAp filters after fired at several fixed temperatures.

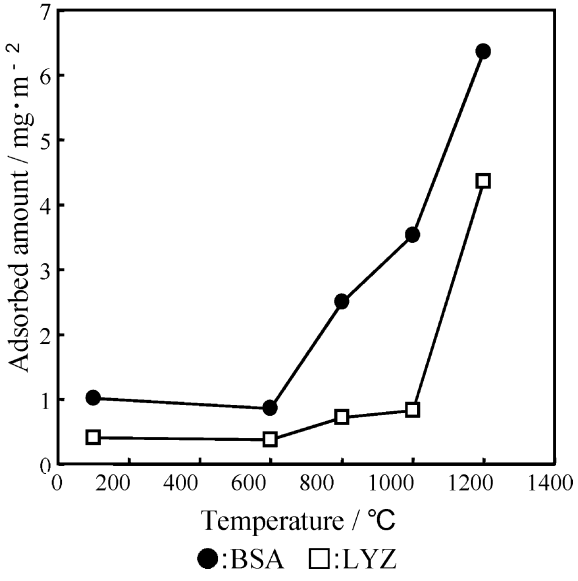

Fig. 8. Adsorption amount of BSA and LYZ at unit area of HAp filters.

10, respectively, and the converted concentrations to the PBS amounts were summarized in Fig. 11. The concentrations of PBS at which BSA were desorbed from the filters were almost independent from all fired temperatures, while the PBS concentrations of the LYZ increased with the fired temperature. It is considered on the acidic protein such as BSA that the adsorption/desorption to the filters is practiced by the competition reaction around the charged site of Ca, that is, the Ca sites charged positively at the surface of the filters were not almost varied at all the fired temperatures [7]. On the other hand, for the LYZ protein, the PBS concentrations of desorption increased with increasing the fired temperatures of filters (Fig. 11).

For the desorption of the LYZ, the competition reaction [4] seemed to occur also in the site of PO^{3-} or OH^{-} charged negatively at the surface of filters. The negativity of the charged site increased gradually with increasing fired temperatures (Fig. 6). So, the surface state of the filters was investigated by a colloidal vibration potential apparatus (CVP; PenKem SYSTEM7000S) since the CVP is correlated with the zeta-potential of a particle surface. [8] The CVP increased with increasing the fired temperature (Fig. 12). From the FT-IR spectra, the structural OH group appeared at 3570 cm^{-1} and the conversions to PO_4^{3-} from HPO_4^{2-} were admitted (Fig. 7). From the above results, the N-HAp filters supplied with $Ca(OH)_2$ to the lack amount of Ca seemed to be converted as following steps, (1) before firing; Ca-deficient N-HAp coexisted with $Ca(OH)_2$. (2) 600–800 °C (after burnt out); The TCP phase derived in the N-HAp filter slightly although the adsorbed amounts increased gradually. (3) The range more than 1000 °C; the HAp crystalline phase was only grown again after reacted with $Ca(OH)_2$. Furthermore, the compressive strength of the filters after fired at 1200 °C was obtained with 8.2 MPa by using the compression apparatus (AGS-G, Shimadzu, Japan). As mentioned earlier, the N-HAp filters fired at 1200 °C can be applied enough practically as a useful separation filter of a column chromatography for mass separation with the enough mechanical strength held on industrial pressure without damping the activity of proteins.

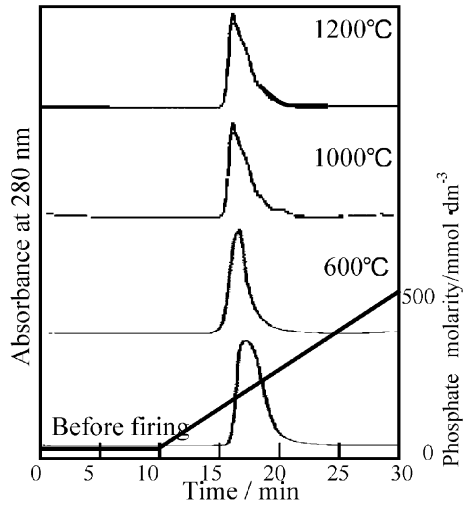

Fig. 9. Chromatogram of BSA with HAp filter after fired at several fixed temperatures.

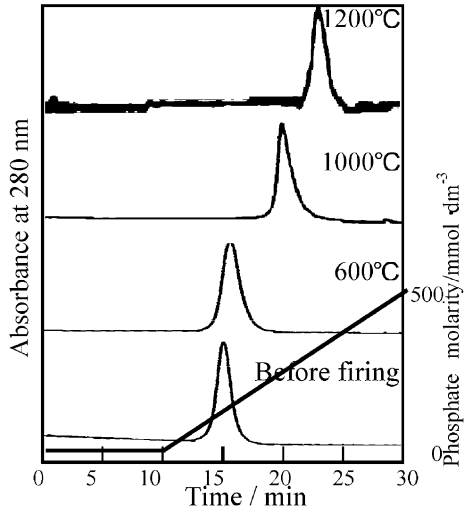

Fig. 10. Chromatogram of LYZ with HAp filter after fired at several fixed temperatures..

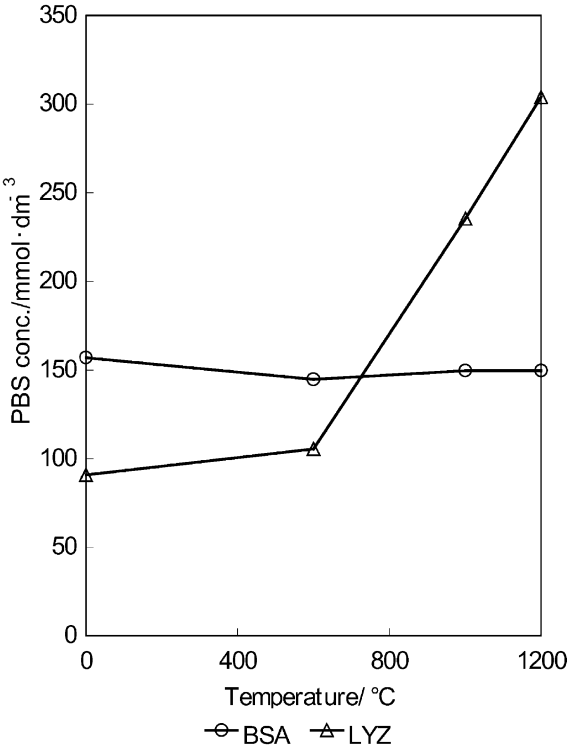

Fig. 11. Concentration of PBS at desorption of protein as a function of fired temperature.

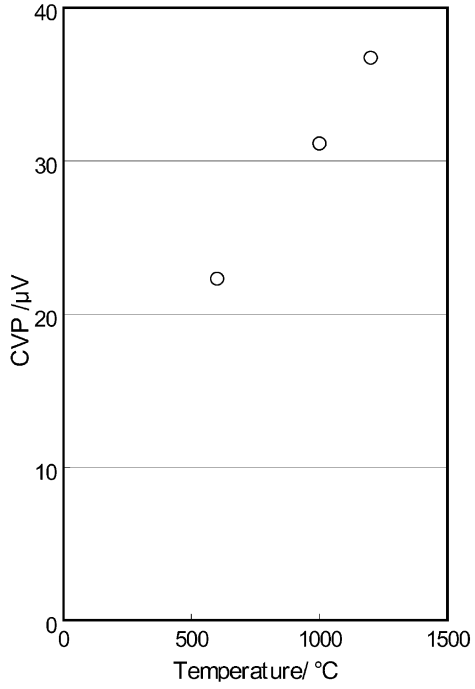

Fig. 12. CVP of HAp.

4. Conclusions

The N-HAp filters for using a column chromatography practically were fired at several fixed temperatures after prepared by a slip casting, and investigated for the adsorption/desorption of acidic (BSA) and basic (LYZ) proteins. The adsorbed amounts of both proteins increased with increasing the fired temperatures. For the desorption, the chromatograms were measured by the linear gradient method, and converted to the concentrations of PBS. The PBS concentrations of the acidic protein (BSA) were almost independent from all the fired temperatures, while the PBS concentrations of the basic protein (LYZ) increased with the fired temperature. As the compressive strength of the filters after fired at 1200 °C was obtained with 8.2 MPa, the N-HAp filters can be applied enough practically as a useful separation filter of a column chromatography for mass separation with the enough mechanical strength held on industrial pressure without damping the activity of proteins.

References

[1] A. Tiselius, S. Hjerten, O. Levin, Protein chromatography on calcium phophate columns, Archives of Biochemistry and Biophysics 65 (1965) 132–155.
[2] H. Hirano, M. Iguchi, T. Nishimura, T. Iwamura, Agricultural Biology Chemistry 50 (1) (1986) 219–221.
[3] S. Suzuki, K. Itou, M. Ohgaki, M. Ohtani, M. Ozawa, Ceramics International 25 (1999) 287–291.
[4] T. Kawasaki, M. Niikura, Y. Kobayashi, Journal of Chromatography 515 (1990) 91–123.
[5] T. Kanazawa, T. Umegaki, J. Yamashita, Ceramics 25 (8) (1990) 724–725.
[6] T. Kijima, M. Tsutsumi, Journal of the American Ceramic Society 62 (1979) 455.
[7] T. Kawasaki, Syokubutusaiboukougaku 3 (2) (1991) 57–61.
[8] J.A. Enderby, Proceedings of the Physical Society 207A (1951) 321.