Materials Science Forum Vols. 530-531 (2006) pp. 587-592
online at http://www.scientific.net

Obtention of TCP porous ceramic using albumin

Ribeiro,C., Bressiani, J.C., Bressiani, A. H. A.

IPEN - Instituto de Pesquisas Energéticas e Nucleares, CCTM – Av. Prof. Lineu Prestes, 2242, 05508-000, São Paulo, SP, Brasil.
cribeiro@ipen.br

Keywords: tricalcium phosphate, macroporous, albumin

Abstract: In the last years, the porosity in ceramic materials for implants production has motivated the development of various technologies. Calcium phosphate ceramics, in special the tricalcium phosphate - TCP, are very promising as bone substitutes and scaffolds for tissue engineering. The macroporosity incorporation in TCP ceramics by porogenic, foaming and consolidator agent, as globular protein (ovalbumin) was the focus of this work. Preliminary studies of zeta potential were made to have a suitable suspension. Ovalbumin amounts (5-7 wt%) were added to the ceramic slurries and suspensions with a solid percentage higher than 60 wt% were obtained. The interaction albumin/surfactant with detergency properties was evaluated by pH and viscosity measurements. The foam was produced by mechanical stirring. The results suggested that the presence of the surfactant increase the volume and stability of foam. After drying, burnout and sintering (1200^{o}C/30 min.) the phase composition of the foams was determined by X-ray diffraction. The microstructure and porosity were evaluated by scanning electron microscopy. SEM micrographs of the foam show that the structure consists of a permeable porous network, being observed spherical and interconnected pores.

Introduction

Tricalcium phosphate (TCP) ceramics are promised materials used in tissue engineering and as temporary bone implants because of the similarities with the mineral fraction of bone, its osteoconductive potential and resorbable nature [1]. The later allows the incorporation and release of different drugs or biologically active substances to stimulate cell function [2, 3]. Scaffolds utilized for simultaneous tissue support and delivery of specific factors must exhibit an appropriate pore structure, release kinetics, and suitable resorption rates [3].

Recent advances in colloidal processing method for near net shape ceramic have been incorporated into the biomedical field, to obtain more reliable grafts and implants. Consolidation by proteic action has been recently developed in order to serve this particular requirement. In this method, globular proteins, such as albumin of egg white (ovalbumin) are used as a promising alternative method, due its ability for pore formation and gelation in water, that occurs under specific conditions (thermal and chemical) [4,5].

The phenomenon of gelation involves the formation of a three-dimensional matrix mainly through inter-protein hydrogen bonding that allows the immobilization of water within the gel structure. Prior to the gel formation a denaturation process occurs. This process can be accomplished by UV or X-rays irradiation, heating, pH adjustment, interfacial area increasing, or addition of an organic solvents, salts, urea, detergents, others [4,6].

Egg white has been successful used in the food industry as the major foaming protein. This is possible due to the ability of the ovalbumin to have homogeneous foams of great volume, while improving the foam stability in the presence of other compounds or heating. Low interfacial tension, high viscosity of liquid phase and strong elastic film of the absorbed protein stabilize the foams. The interest in surfactants mixture systems (albumin/ foaming) is related with better performance and stability of the foam. Considering that different synergy can affect the foam volume, the use of additives, such as albumin, in the presence of a surfactant with detergency property, improves the foam action due to the albumin adsorption in the films surface which stabilizes the foam [6, 7].

Therefore, the use of ovalbumin in ceramics shape forming has many advantages in the porous ceramic processing; ovalbumin is nontoxic, biodegradable, cheap, and widely available. Ovalbumin based slurries do not need any chemical additives to initiate and to complete the gelation, the net binder amount in the dried green body is lower or comparable to that in gelcasting (0.6-4.0%) [4,5].

The aim of this work was to study the production parameters of suspensions ceramics based ovalbumin in the processing of porous ceramics.

Experimental Procedure

Tricalcium phosphate, TCP (Fluka) was characterized by N_2 adsorption carried out on a Micromeritics ASAP 2010 instrument. The specific surface area was evaluated by BET method and the particle size distribution was determined by laser diffraction (CILAS 1064). Phase composition was analyzed by X-ray diffraction (XRD) in a Bruker-AXS, D8 Advance.

TCP powder surface was investigated by electrophoretic mobility measurements to determine the zeta potential and establish the better dispersion conditions and effectiveness of anionic dispersant (dispex A40-Ciba, an ammonium polyacrylate). The analysis were performed under several amounts of dispersant and pH values. In this analysis it was used Zeta Plus – Brookhaven Instruments Corporation. The TCP powder was dispersed in electrolyte solution KCl of 10^{-3} M; with a solid loading of 0.04 vol.%. Potassium hydroxide or cloridric acid were used to adjust the pH of the solutions.

The albumin (Schettert – Nutraceutic) was investigated by thermogravimetric analysis (ATG/DTG, Schimadzu 500), in air flow, heating rate of 5^oC/min up to 1000^oC in platinum crucible.

Stability and foaming properties were investigated by expansion and syneresis measurements according to eq.1 and eq.2 [8], using 1 wt.% of foaming in deionized water and 3 minutes stirring:

$$\% \text{ expansion} = [v_2 - v_1/v_1] \times 100 \quad \text{(eq.1)}$$

$$\% \text{ syneresis} = [v_3/v_2 - v_1] \times 100 \quad \text{(eq.2)}$$

v_1= vol initial liq. before agitation; v_2=vol. after agitation; v_3= vol. of set free liquid of the foam after certain time.

The procedure of foam production involves a dispersion of an aqueous suspension of TCP powder using Dispex A40. The albumin (5-7 wt %) was incorporated into the suspensions to promote the gelation. The foaming action was improved by a mixture of albumin and Genaminox (dimethyl alkyl amine oxide), a nonionic foaming agent. The interaction albumin/Genaminox was analyzed by rheological studies in rheometer Brookfield

and pH was monitored to assure the protein activity in the presence of this nonionic surfactant.

The foams were kept in oven at 60°C/12 h and subsequently annealed at 700 °C/2h using a heating rate of 3 °C/min. in order to degrade of the organic material. The samples were sintered in oxidative atmosphere using heating rate of 5°C/min up to 1200°C/30 minutes.

Scanning electron microscopy (Philips-XL 30) of gold-coated specimens was carried out in order to observe the morphology of the porous structure.

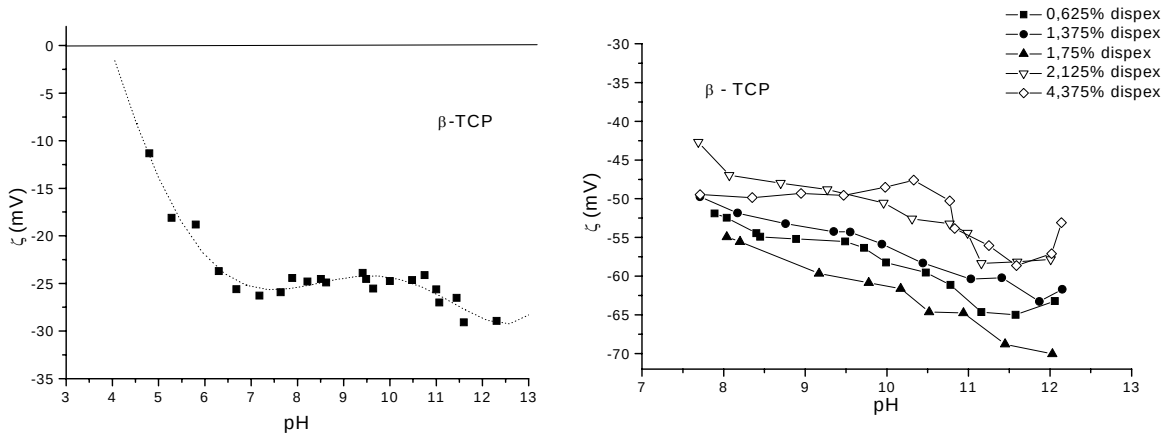
Results and Discussion

The specific surface area (BET) and particles size distribution data of the TCP powder are shown in Table 1. Fine powders hinders the dispersion process due its tendency of adsorb more water volume for to decrease its free energy and supply deficient bond [9]. Normally, small particles tend to agglomerate due high intensity attractive forces compared to repulsive forces. This phenomenon has a substantial influence in the rheological behavior

Table1- Specific surface area and particles size distribution data of TCP powder

$D_{90\%}$ (μm)	$D_{50\%}$ (μm)	$D_{10\%}$ (μm)	Medium diameter (μm)	Specific Superficial Area (m^2/g)
9.77	2.67	0.60	4.0	2.3 ± 0.1

Studies of zeta potential of TCP powder were realized in order to obtain a good dispersion condition. Modification of surface charge in function of the H^+ and OH^- ions interaction was monitored through electrophoretic mobility measurements, obtaining the zeta potential by Smoluchovski equation [9]. Zeta potential (Fig.1) is dependent on surface charge density and is defined as the electrostatic potential at the hydrodynamic shear plane which is located nearly the stern surface.



(a) (b)

Fig.1- Zeta potential curves versus pH (a) TCP starting (b) TCP with several amount of dispersant.

The negative sign charge of TCP measured in different pH's is in agreement with the literature data [10]. The solubility of this ceramic, mainly in pH values below 6 hinders the

detect ion of the isoelectric point. The β-TCP particles showed high mobility in pH above 7 (interval of maximum potential). However, for pH values higher than 7, zeta potential were almost constant.

Dispex A-40 seems to be adsorbed in particles surface and modify the double electric layer, by an increase in the negative superficial charge (Fig.1). A better stabilization of the suspension can achieved 10-11 pH range. Higher values than literature [11] (up to – 65 mV) were observed data, which leads to suitable stability when concentrated suspensions were desirable. Zeta potential measured in pH 10.5, was more effective for dispersant amount of 1.75 vol.%, which corresponds to 0.3 wt. % of final suspension.

The thermogravimetric results of the albumin are present in Fig 2. The data shows a two-step pyrolysis. The first weight loss (~ 9.2%) was attributed to the protein dehydration process and the second step, characterized by the higher weight loss range, (~ 70%), is due to degradation process. After annealing the albumin present a residual amount lower than 9%, probably due to substances that would be eliminated. According to this respect, heated rates of 3^{o}C/min was used during annealing in order to eliminate completely the residue.

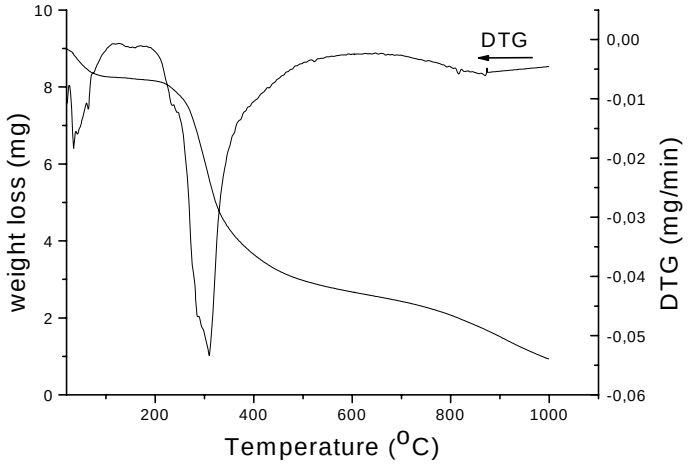


Fig.2 – TGA/DTG curves of albumin, heated rate 5^{o}C/min in synthetic air.

Genaminox was selected as surfactant to study the interaction albumin/foaming agent due to its nonionic characteristic and, consequently higher resistance in the presence of several ions, such as Ca^{+2}. Stability of the foaming agent was evaluated by syneresis measurements (eq.2), Table 2. These results suggest that was occurred a slow drainage, sufficient to solidify the foam, being observed by syneresis values in the Table 2.

Table 2– Syneresis evaluation in different time range after foam production.

foaming agent	*syneresis after 4 min*	*syneresis after 9 min*	*syneresis after 25 min*
Genaminox KC180	10%	26%	45%

Suspension with 6wt% of albumin and 0.5wt% of foaming agent was prepared with a constant monitoring of pH values. It is well know that pH variation causes a denaturation of the protein and change in the viscosity (η) of the suspension. In this study several parameters were controlled, such as pH, viscosity and expansion (eq.1), so that the effectiveness of the foaming agent can be evaluated (Table 3).

Table 3 – Parameters during albumin/foaming interaction in the suspension

Foaming agent	*pH*	*η (mPas) without albumin*	*η(mPas) with albumin*	*% expansion*
Genaminox KC 180	9.65	1.74	2.51	236

Albumin increases the viscosity, leading to non-Newtonian properties of the suspensions and inhibits films drainage hydrodynamics mechanisms during the maturation stage of the foam. The pH values in the presence of the surfactant do not alters the albumin action, which is active in this alkaline range. The albumin adsorption produces also an additional elasticity that fits the Laplace pressure gradient, delays the effect Ostwald maturation and improves the foam stability [7]. It can be observed that the syneresis of this suspension was slower than those observed in the absence of the albumin.

The X-ray diffraction patterns of TCP samples and the foam are shown in Fig 3. β-TCP phase was identified in all samples, including the albumin foam without any other crystalline phase.

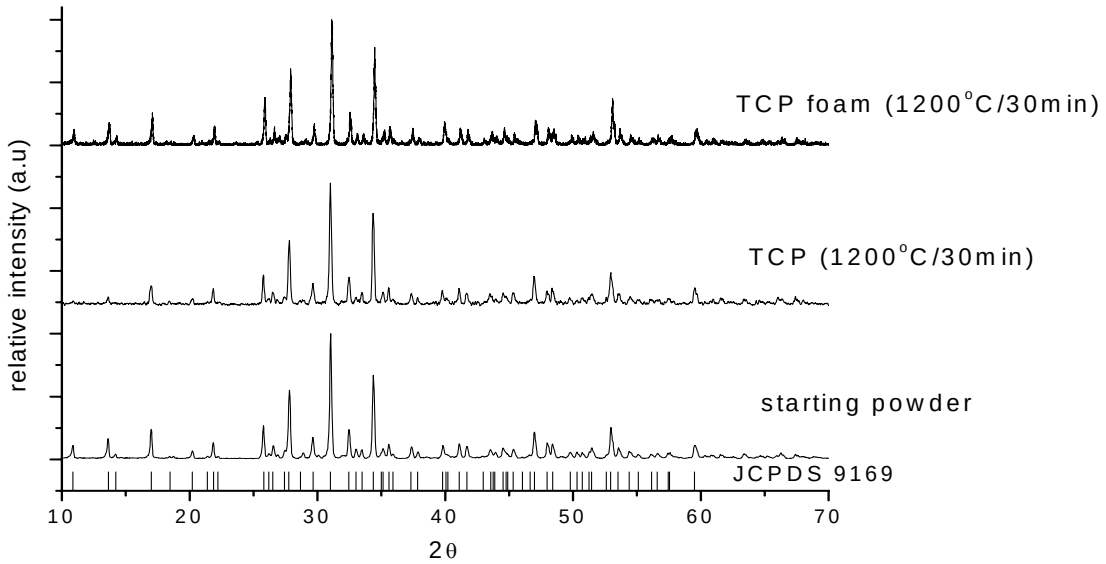
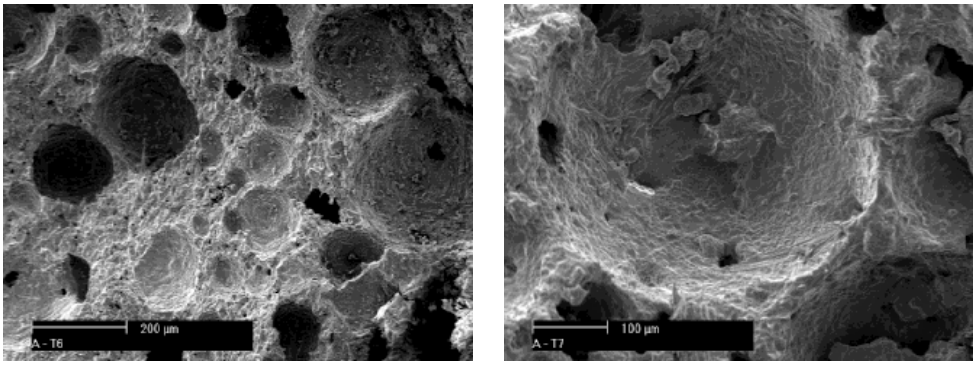


Fig. 3 - X-ray diffraction patterns for samples in different conditions (starting powder, TCP, sintered at 1200°C/30min and TCP foam based albumin, sintered at 1200°C/30min).



(a) (b)

Fig.4- SEM micrographs of TCP foams based albumin. (a) 6 wt. % of albumin and (b) 7 wt. % of albumin.

TCP foams micrographs (SEM) are showed in Fig.4. Albumin amount, lower than 6 wt% leads to TCP foams with undesirable handling mechanical resistance. The foams were solidified in maturation stage, due to maintenance of cells with a spherical shape and absence of poliedric geometry in the foam. This characteristic indicates that the rheology and dispersion condition of the suspension were well adjusted to have suitable stable foam, which is able to persist during solidification stage. Spherical pores increase the bone metabolism [2]. The presence of additional micropores which helps the liquids drainage and mainly interconnected porosity are present in all the samples. This kind of porosity and surface roughness improves vascularization and tissue growth, which are promising characteristics for osteointegration of the implant material [2, 3].

Conclusion

- β-TCP suspensions prepared in pH near to 10,5 are efficient for attainment of an excellent condition dispersion, assuring lower values of viscosity and a suitable zeta potential.
- Dispex A-40 modified the powders surface, resulting in higher repulsive forces between particles that were indicated by the increase (in module) of the zeta potential values. The use of 0.3 wt.% dispersant optimized the suspensions with 68 wt. % solids percentage, resulting in adjusted properties in the foam production.
- Foam based albumin and surfactant present a suitable syneresis to the foam solidification. The foams showed slow draining and high superficial viscosity, leading to a higher elasticity of the cells and consequently, improving their stability.
- Pores characteristics obtained such as: size, geometry and interconnectivity are desired for application as implanted TCP.

Acknowledgements

The authors would like to thank the laboratories IPEN by development research and the financial support provided by CNPq, FAPESP and Probral (CAPES/DAAD).

References

[1] A. Ravaglioli, A. Krajewski. Bioceramics: from concept to clinic, Chapman & Half, 1992.
[2] C. Tuck, J.R.G. Evans. Journal of Materials Science Letters 18, (1999), p. 1003-1005.
[3] R. F. S. Lenza, J. R. Jones, W. L. Vasconcelos, L. L. Hench. Journal of Biomedical Materials Research Part A, v. 67A, (2003) p. 121 – 129.
[4] O. Lyckfeldt, J. Brandt, S. Lesca. J.Eur. Ceram. Soc., 20, (2000), p.2551-2559.
[5] I. Garrn, C. Reetz, N. Brandes, L. W. Kroh, H. Schubert. Journal European Ceramics Society . v.24, (2004), p. 579-587.
[6] P.W. Gossett, S.S.H. Rizvi, R.C. Baker. Food Technology. v.38, 5, (1984), p 67-96.
[7] D. Beneventi, B. Carre, A. Gandini. Colloids and surfaces. A: Physicochemical and Engineering Aspects. 189, (2001), p.65-73
[8] S.H. Wang, S. M. Fernandes, L.C. Cabral, F.B.Araújo. Ciência e Tecnologia de Alimentos. v. 20, 2, (2000), p.83-89.
[9] I. R. Oliveira, A. R. Studart, R. G. Pileggi, V. C. Pandolfelli. Dispersão e empacotamento de partículas. Fazendo Arte Editorial, 2000.
[10] Padilla, S., García-Carrodegas, R., Vallet-Regí, M. J. Eur. Ceram. Soc. v.24, A, (2004), p.2223-2232.
[11] D. Gouvêa, B.B.S. Murad. Cerâmica, v.47, 301, (2001), p.51-56.