Conversion mechanism of a bioactive Si-Ca-P system wet gel in aqueous K_2HPO_4 solution

ZHIQIANG FENG, JIAZHE GUO, YADONG LI*, YANLIN HUANG

College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China

A bioactive Si-Ca-P system (designated 58S) wet gel was prepared via a sol-gel method. The wet gel was reacted continuously in 0.25*M* K_2HPO_4 solution with a starting pH value of 9.0 at 37 °C for different time. The structural and compositional changes resulting from the conversion reaction were characterized using X-ray diffraction, scanning electron microscopy, Fourier transform infrared spectroscopy and inductive coupled plasma spectroscopy. The results indicated that the induction period for the CDHA nucleation on the surface of the 58S bioactive wet gel was about 3 days. The conversion mechanism of the products was also discussed.

(Received January 6, 2011; accepted January 26, 2011)

Keywords: Bioactive wet gel, Conversion mechanism, Ca-P precipitations, Characterization

1. Introduction

Research in the past two decades has shown that a group of bioactive glasses and glass-ceramics with specific compositions can adhere to bone through chemical bonding [1, 2]. These bioactive materials have been already used clinically for the replacement and reconstruction of damaged bone [3-5]. It has been found that all bioactive glasses and glass ceramics hitherto known bond to bone through a hydroxyapatite (HA) layer that is formed on their surfaces in the body [6, 7], such as the well-recognized composition designated 45S5. The mechanism by which the 45S5 bioactive glass converts to HA in an aqueous phosphate solution was discussed in detail by Hench [3, 4]. It is well established that an initial step in the reaction is the formation of a SiO_2-rich gel layer on the 45S5 glass surface by ion-exchange reactions. The SiO_2-rich gel that is formed on the surfaces of bioactive glasses in the body plays an important role in forming the apatite layer [8]. The reaction of the 45S5 glass with an aqueous K_2HPO_4 solution to form HA on the SiO_2-rich gel has been investigated, but the conversion mechanism of a bioactive silicate-based wet gel in the aqueous K_2HPO_4 solution has not been studied in previous studies.

The objectives of the present work were to determine the conversion mechanism of a bioactive Si-Ca-P system wet gel in the aqueous K_2HPO_4 solution and to characterize the crystal structure and composition of the reaction products. The bioactive Si-Ca-P system wet gel can provide alternative materials for use as noninvasive bone replacement. As such, a better understanding of the conversion process for the Si-Ca-P system wet gel in the aqueous K_2HPO_4 solution is important.

2. Experimental

A bioactive Si-Ca-P system wet gel was prepared via sol-gel method in the present work. The wet gel was designated 58S, with a composition (in mol %) of 60 SiO_2, 36 CaO and 4 P_2O_5. The wet gel was synthesized using commercially obtained tetraethyl orthosilicate (TEOS, Lingfeng, Shanghai), $Ca(NO_3)_2 \cdot 4H_2O$ (Lingfeng, Shanghai), Tri-Butyl-Phosphate (TBP, Lingfeng, Shanghai) and HCl (Lingfeng, Shanghai). To prepare the 58S bioactive wet gel, the TEOS alcoholic solution was stirred vigorously at 50 °C, and then $Ca(NO_3)_2 \cdot 4H_2O$ and TBP alcoholic solutions were slowly added drop by drop. The pH value of the mixed solution was adjusted to 3 using HCl and the mixture was stirred continuously at 50 °C for 2h. The obtained sol (5 ml) was aged at 37 °C, and changed into wet gel.

The aqueous K_2HPO_4 solution used in the conversion reaction was prepared by dissolving K_2HPO_4 in deionized water to give a solution with a concentration of 0.25*M* and a starting pH value of 9.0. The much higher phosphate ion concentration was used to enhance the conversion rate of the transformation. This solution was able satisfactorily to reproduce apatite formation on the surfaces of various kinds of bioactive glasses and glass-ceramics in vitro [9, 10].

The 58S wet gels were soaked in 50 ml aqueous K_2HPO_4 solution, and the system was placed in an oven at 37 °C for different time. After soaked for various periods, the reacted products were removed from the solution, filtrated and washed with deionized water, then rinsed with acetone to terminate any ongoing reactions. Finally, the products were dried in an oven at 90 °C for 1 day.

The crystal structure of the obtained products were detected by X-ray diffraction (XRD, MERCURY CCD,

Japan) using $Cu_{k\alpha}$ (λ = 0.15406 nm) radiation in a step-scan mode (0.03° 2θ per step) in the 2θ range 4°-80°. Each Fourier transform infrared spectroscopy (FTIR, ProStar LC240, USA) spectrum of the products was obtained from 32 scans at a resolution of 2 cm^{-1}, in the range of 400-1500 cm^{-1}. The morphologies of the products were observed using scanning electron microscopy (SEM, S-4700, Hitachi, Japan). The concentration changes of Ca^{2+} and PO_4^{3-} in reacted K_2HPO_4 solution were analyzed using inductive coupled plasma spectroscopy (ICP, Vista MPX, USA).

3. Results and discussion

Fig. 1 shows the XRD patterns of the 58S wet gels soaked in the K_2HPO_4 solution for different periods. In the pattern of the 58S xerogel (Fig. 1a), only an amorphous peak locates at 2θ around 20-30°. The Ca-P precipitations started to form on the surface of the 58S wet gel after 15 minutes, and grew with increasing soaked time. All peaks in Fig.1b and c could be identified as dicalcium phosphate dihydrate ($CaHPO_4{\cdot}2H_2O$, DCPD) and dicalcium phosphate ($CaHPO_4$, DCP) corresponding to the standard JCPDs Card 72-1240 and 75-1250, respectively. When observed closely, the XRD patterns of the early stage of bioactive borosilicate glass in an aqueous phosphate solution were also indicated the presence of the DCPD precipitation in the literature [9]. After soaked for 1 h, the mixture of DCPD and DCP almost changed into the DCPD. As the soaked time increases, the diffraction patterns of Ca-P precipitations (Fig.1d-f) became markedly different. With only a few minor peaks corresponding to DCPD, moreover, a diffraction peak at 2θ = 9.4° (marked with rectangle in Fig. 1d and e) presents in the patterns. This peaks appear to be in the position of the Octacalcium Phosphate (OCP, JCPDs Card 79-0423). The OCP has been generally considered as the precursor of hydroxyapatite (HA) crystal formation [11]. Further reaction for 3 days gave soaked 58S wet gel with diffraction peaks almost identical to those of stoichiometric microcrystalline HA. This suggests that the synthetic products are apatite-like crystals and considered to be CDHA according to JCPDs Card 09-0432 [12].

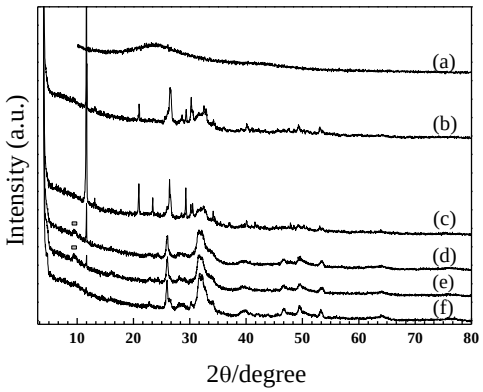

Fig. 1. XRD patterns of the 58S xerogel (a), and wet gels soaked in 0.25 *M* K_2HPO_4 *solution for various periods: 0.25 h (b), 1 h (c), 12 h (d), 1 day (e) and 3 days (f).*

The FTIR spectra of the 58S wet gels soaked in K_2HPO_4 solution for various periods are shown in Fig. 2. The peak at 1385 cm^{-1} in the FTIR spectra is assigned as a Si-O-Si stretching vibration [13]. The P-O bands at 1101, 1075, and 1038 cm^{-1} were attributed to P-O stretching in phosphate (PO_4^{3-}) and hydrogeno-phosphate (HPO_4^{2-}). The formation of Ca-P precipitations for reaction time of 12h-3days shown a typical structure of OCP. After soaked for various periods, the vibration bands located at 1130-1075 cm^{-1} became less sharp and distinct, transmittance maximum degraded into a shoulder, the band (1110-1030 cm^{-1}) separation narrowed. The peaks at 1101 and 1033 cm^{-1} coincided closely with the CDHA (1033 cm^{-1}) [14]. A very weak band near 875 cm^{-1} was the P-O stretching in HPO_4^{2-} groups [15].The peaks at 563 cm^{-1} and 602 cm^{-1} were assigned as P-O banding of PO_4^{3-} group [16]. These two peaks were characteristic of an apatite crystalline phase, and became more pronounced in the spectra for a reaction time of 3 days. As the previous XRD data shown, the conversion of 58S wet gel in 0.25*M* K_2HPO_4 solution initially produced a DCPD layer, which subsequently converted to OCP and CDHA.

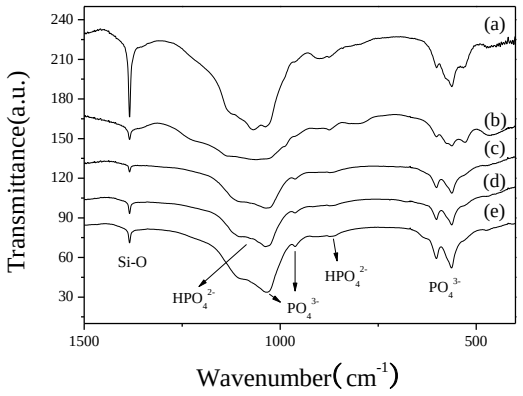

Fig. 2. FTIR spectra of the 58S xerogel (a) and the wet gels soaked in 0.25 *M* K_2HPO_4 *solution for various periods: 1 h (b), 12 h (c) ,1 day (d) and 3 days (e).*

Fig. 3 shows the SEM images of the 58S wet gel soaked in the K_2HPO_4 solution for 1h, 12h, and 3days. After soaked 1h (Fig. 3a), the surface of reacted products had a few plate-like crystals, which had been identified as DCPD by XRD technique. This agrees well with the reports in the literature [9]. A distinct change in the surface of the reacted products was observed after 12h (Fig. 3b). According the XRD patterns (Fig. 1d and e), the SEM revealed the band-like crystals that are identified as OCP. With increasing reaction time, the CDHA with small spherical crystal clusters was found after 3 days. It can be seen from Fig. 3c that each spherical crystal cluster consists of a large number of flakes. The EDS spectra of the 58S wet gel soaked in K_2HPO_4 solution for 1 h, 12h and 3 days are shown in Fig. 4. The atomic ratios of the Ca/P are 1.07, 1.39, and 1.58, respectively. These further confirm that the structure of the Ca-P nano-crystals is chemically and structurally identified to be DCPD, OCP and CDHA [17].

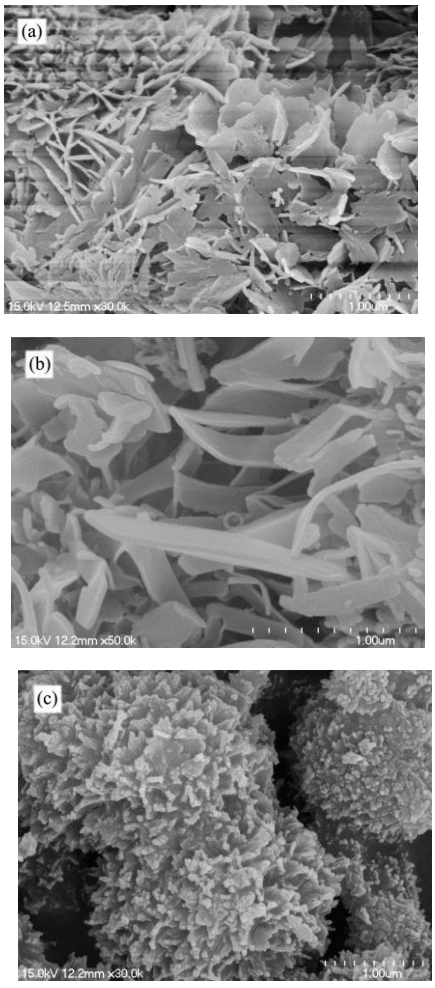

Fig. 3. SEM micrographs of the 58S wet gels soaked in 0.25M K_2HPO_4 solution for 1 h (a),12 h (b) and 3 days (c).

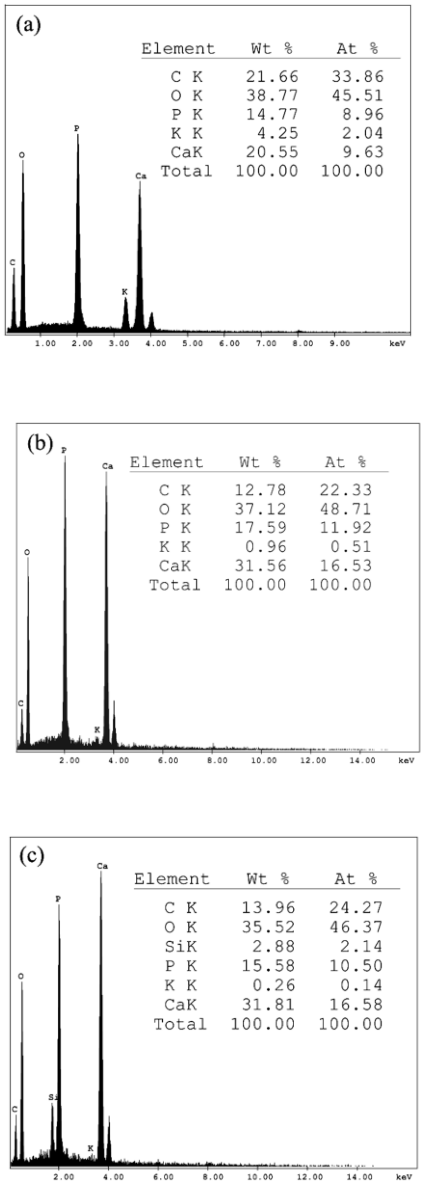

Fig. 4. EDS spectra of the 58S wet gels soaked in 0.25M K_2HPO_4 solution for 1 h (a), 12 h (b) and 3 d (c).

Fig. 5 shows the concentration changes of the Ca^{2+} and PO_4^{3-} in reacted K_2HPO_4 solution with the different soaked time. Reaction process began to respond by adding the K_2HPO_4 solution, the Ca^{2+} concentration increased at the first 12 hours and reached a maximum value of about 32.8 ppm. This is because the Ca^{2+} gradual dissolved into the solution from the wet gel during the initial stage. The DCPD precipitation becomes possible when the concentrations of calcium and phosphate ions increase to a higher than normal level in SBF [18]. Since Ca^{2+} and PO_4^{3-} combine rapidly produced DCPD, the Ca^{2+} concentration increased slowly. After soaked for 12h, the concentration of Ca^{2+} reached maximum and gradually decreased, this is because the DCPD gradual changed into the OCP and CDHA. The decreases of the PO_4^{3-} concentration in the

reacted K_2HPO_4 solution is attributed to the growth of the Ca-P precipitations nuclei formed on the surface of the 58S wet gel. This is consistent with the results from the XRD, FTIR and SEM data described above.

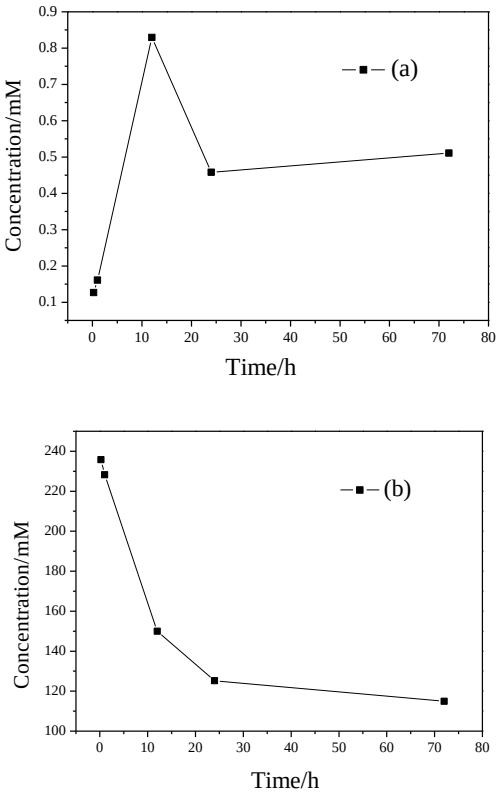

Fig. 5. Ca^{2+} (a) and PO_4^{3-} (b) concentration changes of the reacted 0.25 M K_2HPO_4 solution during the mineralization reaction.

In the present work, the concentration of PO_4^{3-} in K_2HPO_4 solution was relatively high (0.25*M*). Although thermodynamically very favorable to the formation of HA, the reactions were limited by diffusion kinetics of the Ca^{2+} ions in the 58S wet gel, so in the initial reaction there were many types of Ca-P precipitations. In fact, DCPD, OCP and CDHA have been considered as a precursor phase for HA formation [19]. Theoretical analysis based on nucleation kinetics indicates that the OCP and CDHA nucleation rates could be much faster than that of HA in the physiological environment [18]. In accordance with the balance principle, the Ca-P precipitations with relatively high solubility will be transformed into the Ca-P precipitations with low solubility. Solubility of CDHA is much smaller than DCPD and OCP, therefore, the DCPD and OCP under certain conditions are gradually converted to CDHA [20, 21]. On the other hand, the Ca-P precipitation nucleation rates are significantly affected by the pH value. High pH environment is favorable for HA nucleation [22]. However, the precipitations of DCPD, OCP and CDHA are more kinetically favorable than that of stoichiometric HA [23]. In the present experiments performed at a starting pH 9.0, the formation of CDHA at longer reaction time (3days) is consistent with stability data for Ca-P precipitations. The conversion order of 58S wet gel in 0.25*M* K_2HPO_4 solution can be summarized as: DCPD → OCP → CDHA.

4. Conclusions

The bioactive Si-Ca-P system wet gel was successfully prepared using the sol-gel method. The surface transformation reactions of the wet gel were studied in 0.25*M* K_2HPO_4 solution with a starting pH value of 9.0 at 37 °C. For reaction time of 1h, the results indicated the formation of DCPD with plate-like crystals. In comparison, the formation of OCP with thin, band-like crystals was confirmed during reaction time of 12h-1day and the CDHA with small spherical crystal clusters was found at later time. This indicated that the induction period for the CDHA nucleation on the surface of the 58S bioactive wet gel was about 3 days. This early-stage conversion reaction of the 58S wet gel in 0.25*M* K_2HPO_4 solution can be followed as the order: DCPD → OCP → CDHA.

Acknowledgements

This work was supported by Program for Postgraduates Research Innovation in University of Jiangsu Province (2010).

References

[1] L. Hench, R. Splinter, W. Allen, T. Greenlee, . J. Biomed. Mater. Res. **5**, 117 (1971).
[2] L. Hench, H. Paschall, J. Biomed. Mater. Res. **7**, 25 (1973).
[3] L. Hench, J. Am. Ceram. Soc. **74**, 1487 (1991).
[4] L. Hench, J. Biomed. Mater. Res. A **41**, 511 (1998).
[5] M. N. Rahaman, R. F. Brown, B. S. Bal, D.E. Day, Semin. Arthroplasty, **17** (2006) 102.
[6] K. Ohura, T. Nakamura, T. Yamamuro, T. Kokubo, Y. Ebisawa, Y. Kotoura, M. Oka, J. Biomed. Mater. Res. **25**, 357 (1991).
[7] K. Ohura, M. Ikenaga, T. Nakamura, T. Yamamuro, Y. Ebisawa, T. Kokubo, Y. Kotoura, M. Oka, J. Appl. Biomater. **2**, 153 (1991).
[8] K. Karlsson, K. Fr berg, T. Ringbom, J. Non-Cryst. Solids **112**, 69 (1989).
[9] Y. Li, M. Rahaman, B. Bal, D. Day, Q. Fu, J. Am. Ceram. Soc. **91**, 1528 (2008).
[10] R. Brown, M. Rahaman, A. Dwilewicz, W. Huang, D. Day, Y. Li, B. Bal, J. Biomed. Mater. Res. A **88**, 392 (2009).
[11] Y. Miake, S. Shimoda, M. Fukae, T. Aoba, Calcif. Tissue Int. **53**, 249 (1993).
[12] S.-C. Liou, S.-Y. Chen, H.-Y. Lee, J.-S. Bow,

Biomaterials **25**, 189 (2004).
[13] R. Bell, P. Dean, Discuss. Faraday Soc. **50**, 55 (1970).
[14] G. Sauer, R. Wuthier, J. Biol. Chem. **263**, 13718 (1988).
[15] A. Siddharthan, S. Seshadri, T. Kumar, J. Mater. Sci. Mater. Med. **15**, 1279 (2004).
[16] J. Ning, A. Yao, D. Wang, W. Huang, H. Fu, X. Liu, . X. Jiang, X. Zhang, Mater. Lett. **61**, 5223 (2007).
[17] L. Madsen, E. Hans, J. Cryst. Growth **310**, 2602 (2008).
[18] X. Lu, Y. Leng, Biomaterials **26**, 1097 (2005).
[19] W. Brown, N. Eidelman, B. Tomazic, Adv. Dental Res. **1**, 306 (1987).
[20] I. Izquierdo-Barba, D. Arcos, Y. Sakamoto, O. Terasaki, A. Lo pez-Noriega, M. Vallet-Regi, Chem. Mater. **20**, 3191 (2008).
[21] X. Lu, Y. Leng, Biomaterials **25**, 1779 (2004).
[22] M. Iafisco, M. Marchetti, J. Go mez Morales, M. Herna ndez-Herna ndez, J. Garci a Ruiz, N. Roveri, Cryst. Growth Des. **9**, 4912 (2009).
[23] R. Boistelle, I. Lopez-Valero, J. Cryst. Growth **102**, 609 (1990).

*Corresponding author: liyadong@suda.edu.cn