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Preparation of Hollow Porous HAP Microspheres as Drug Delivery Vehicles

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Abstract: Hollow HAP microspheres in sub-millimeter size were prepared and investigated as a drug delivery vehicle. The LCB (lithium-calcium borate) glass microspheres, which were made through flame spray process, were chosen as precursor for hollow HAP microspheres. The LCB glass microspheres reacted with phosphate buffer (K_2HPO_4) solution for 5 days at 37 ℃. During the reaction the Ca-P-OH compound precipitated on the surface of LCB glass microspheres and formed porous shells. Then the microspheres turned to be hollow ones with the same diameter as the glass microspheres after LCB glass run out in the chemical reaction. After heat-treated at 600 ℃ for 4 h, the Ca-P-OH compound became HAP, thus the hollow HAP microspheres were produced. The mechanism of forming hollow HAP microspheres through the chemical reaction between phosphate buffer and LCB glass was confirmed by the XRD analysis. The microstructure characteristics of the hollow, porous microspheres were observed by SEM.

Key words: hollow HAP microsphere; drug delivery vehicle; hydroxyapatite

1 Introduction

Implanting the medicine into a certain part of human body is an economic, effective and low side effect therapy method. A controlled-release drug system avoids the disadvantages of taking medicine repeatedly, which leads to up and down of the medicine concentration in the body (Fig.1). It brings the second revolution of the pharmaceuticals' production. A controlled-release drug system utilizes biocompatible materials as a drug delivery medium. It is aimed to control the drug release at certain speeds in the body, in order to make an improvement of the therapy.The diffusion-control release system is currently the most popular and most-investigated, in which monolith type and reservoir type carriers are used[1], as shown in Fig. 2. Since the diffuse parameter of the reservoir type system could be controlled more easily, this system attracts more attention.

In recent years, there have been lots of reports on organic polymer matrices for drug delivery. For example, poly-aminophenol, non-toxic, can be used as controlled drug delivery [2]. Zhang Wanguo *et al* reported that poly-lactic acid microspheres packing drug Rifampicin could be implanted in the body and treat lung disease [3]. However, the diameter of these microspheres is quiet small, so its drug packing capacity is very low relatively. As a result, it leads to rapid drug release.

Researches on inorganic drug delivery systems are mostly focused on porous β-tricalcium phosphate materials and calcium phosphate bone-cement materials. Few researches on bioactive glasses have been reported for drug delivery system so far [4,5]. Bioglass has a good biocompatibility, since its ion is more active than that of crystal[6]. But the drug delivery systems in current use are rather large dimensional. So it is necessary to investigate controlled drug delivery system with a smaller size. HAP (hydroxyapatite), nontoxic, biocompatible with the body, used as a drug delivery material, is a hot point of research nowadays [7-10]. In this paper, we investigated hollow, porous HAP microspheres in sub-millimeter size as a new controlled-release drug system.

2 Experimental

2.1 Preparation of glass microsphere

The Li_2O-CaO-B_2O_3 ternary system was selected as the precursor. The composition was $10Li_2O•15CaO•75B_2O_3$ (wt%). An appropriate amount of reagent grade Li_2CO_3(CP, Shanghai 2nd Chemical Reagent Factory), $CaCO_3$(CP, Shanghai Silian Chemical Industry Factory)

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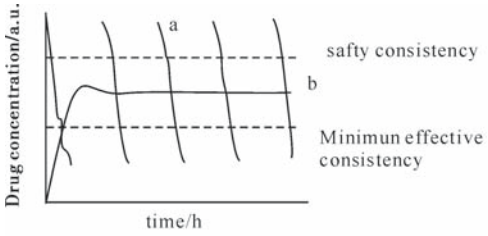


Fig.1 Comparison of routine periodic administration (RPA) with controlled-release drug system(CRDS) line a:RPA line b:CRDS

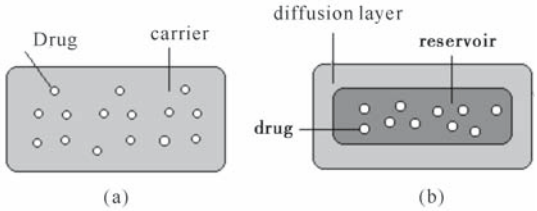


Fig.2 The types of drug carrier a:monolith type b:reservoir type

and H_3BO_3 (AR, Shanghai Yunling Chemical Industry Factory) powders were mixed and melted in a platinum crucible at 1 300 ℃ for approximately 2 hours. The glass melting liquid was quenched between two cold stainless steel plates to prevent crystallization and then the calcium lithium borate (LCB) glass was prepared.

Then the LCB glass was crushed and milled in a agate mortar. Make the glass particles go through a sieve, pick out the particles in the range of 80-100 meshes for future use. Glass microspheres with 80-100 meshes Were prepared through flame spray process, as shown in Fig. 4 (a) and (b).

2.2 Preparation of hollow HAP microsphere

The glass microspheres were reacted with 0.25 M K_2HPO_4 phosphate solution, the pH value was controlled in the range of 10 to 12. Microspheres were reacted at temperature 37 ℃ for up to 5 days. At the completion of the reaction period, the microspheres were liberally rinsed three times with distilled water and once with ethanol. Then they were dried at 90 ℃ for at least 24 hours and finally heated at temperature of 600 ℃ for 4 hours.

To verify if the microspheres were hollow, they were punctured with a needle and observed under a scanning electron microscope (SEM)(S-2360, Hitachi, Japan) (Fig. 5(b)).

2.3 XRD

The product microspheres were heat treated at 90 ℃ for 24 hours and 600 ℃ for 4 hours, respectively. Then they were examined by X-ray diffraction (XRD) (DX-2000, Dandong Instruments, Dandong, China) to identify if any crystalline phases formed (Fig. 3).

2.4 SEM

The reaction microspheres during and after the reaction were observed by SEM (S-2360,HITACHI), as shown in Fig.5. After completion of the reaction, the microspheres were also heat treated at 90℃ for 24 hours and 600 ℃ for 4 hours, respectively. Also they were cut into halves to observe the inside microstructure (shown in Fig.6(a) and (b)).

3 Results and Discussion

3.1 Mechanism of reaction between LCB glasses and phosphate solution

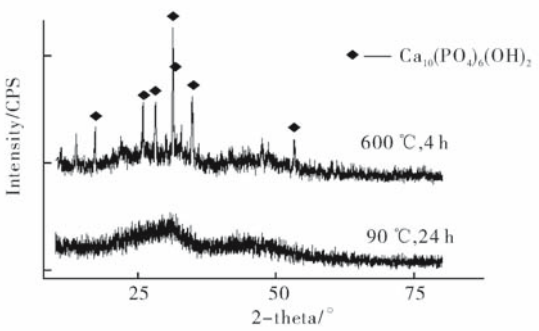


Fig.3 The XRD pattern for the precipitate heat-treated at 90 ℃ and 600 ℃ for 4 h

The XRD analysis for the reaction products proved the reaction between LCB glass and phosphate solution. Fig.3 shows the XRD patterns for Ca-P-OH precipitate heated at 90 ℃ for 24 h and 600 ℃ for 4 h, respectively. The XRD pattern for the former presents a broad dome, which might be amorphous Ca-P-OH deposit. However, the XRD pattern for the latter matches the predominant peaks for HAP peaks and no other crystalline phases were detected. From the XRD patterns, it can be seen that when heated up to a certain temperature (about 600 ℃), the amorphous Ca-P-OH deposit became crystalline, and at last the crystals of HAP formed.

The Li_2O-CaO-B_2O_3 (LCB) ternary system, the precursor of preparing hollow HAP microspheres, is a typical borate glass. The structure of B_2O_3 glass network is of B-O trihedron. Other oxides in the glass can offer quite a lot of dissociative oxygen and transform some B-O trihedrons into B-O tetrahedron, so that the sheet structure of the glass network changes into a three-dimensional one. The Li^+ ions are located in the interstice of the network, and the Ca^{2+} ions in the glass are between the trihedron and tetrahedron, so they can enhance the structure of the network. Thus it is easier to form glass from the chosen ingredients. In

spite of that, the strength of the covalent bond is very weak. The trihedron and tetrahedron of the network can not effectively bind up Li^+ ions. The strength between the trihedron and tetrahedron is also weak, which is far weaker than that of Si-O tetrahedron in the silicate glass. Accordingly, Li^+ ions would get out from the glass network and the B-O trihedrion and tetrahedron would also be eroded in alkaline solution (pH>10). Thus, it can seen be that the LCB glass has a high reactivity in alkaline solution.

With pH of phosphate solution ranging from 10 to 12, the LCB glass network would break into ions in K_2HPO_4 solution. The reaction equation is as follows:

$$(Li_2O\cdot B_2O_3\cdot CaO)_{(glass)}+(PO_4^{3-}{}_{(aq)})+OH^-_{(aq)} \rightarrow Li^+_{(aq)}+(B^{3-}_{(aq)})+Ca\text{-}P\text{-}OH_{(s)} \quad (1)$$

In this solution it is mostly the reaction compound of Ca^{2+} ions with PO_4^{3-} groups comes out as precipitation, due to its low solubility. Since pH of the solution ranges from 10 to 12, the reaction compound of Ca^{2+} ions with OH^- groups can also come out, so the precipitation is hydroxide calcium phosphate, marked as Ca-P-OH in Equation (1).

The Li^+ and B^{3-} ions dissolved from the LCB glass and went to the solution. The leaving of Li^+ and B^{3-} ions left pores in the LCB glass. Ca^{2+} ions react with the PO_4^{3-} groups, the Ca-P-OH compound formed. Since the Ca-P-OH compound has a precipitation trend for low energy, it precipitated and adhered onto the surface of LCB glass. But the crystal did not grow perfectly, so the Ca-P-OH compound was very loose and porous. Thus, the solution can easily pass through the pores and enter inside the LCB glass microsphere, as a result new reaction occurs between LCB glass microsphere and phosphate solution. So Ca-P-OH compound precipitated layer by layer on the LCB glass microshpere. When LCB glass was run out completely, hollow, porous HAP microshperes formed.

3.2 Microstructure of HAP microshpere

Fig.4 shows an SEM image for the LCB glass microspheres. The LCB glass microshperes were made through flame spray process. It can be seen that their surfaces were glazed in the image.

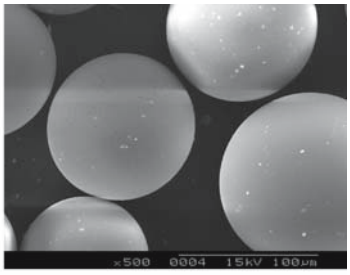
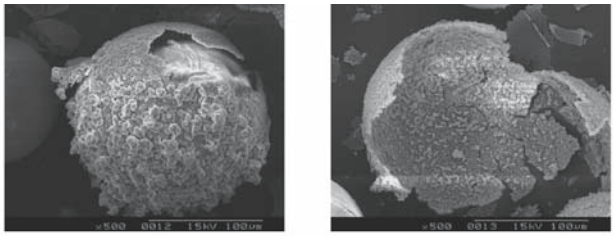
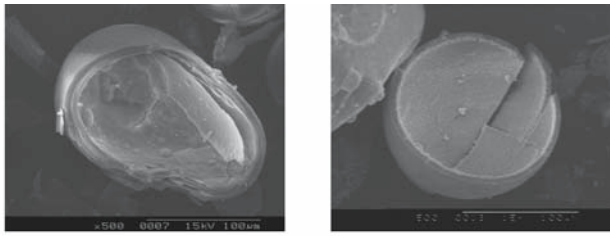


Fig.4 SEM image for LCB glass microspheres (×500)



a b

Fig.5 SEM images for LCB glass microsphere during reaction(a) and completed reaction(b)



a b

Fig.6 SEM images for cross-section of hollow microsphere dried for 4 h

Fig.5 shows the SEM image for preparing hollow, porous HAP microspheres in different reaction stages. After the 5 day reaction between the LCB glass microshperes and K_2HPO_4 solution, the surface of glass microspheres turned to be gray in color. In Fig.5(a) the microsphere was still in reaction process. One thin layer of Ca-P-OH compound was formed on the surface. Under the surface LCB glass still exist. Thus, it shows that the microsphere reacted with the solution from exterior to interior, which left pores on the wall.

The phosphate solution passed through the pipe in the wall and it reacted with the interior part of the microsphere. The new layer with pores kept on depositing onto the old, finally the LCB glass run out and hollow HAP microspheres formed. As shown in Fig. 5(b) the reaction product microsphere is hollow indeed. We can also see there are many particles deposited inside the wall. The particles were also amorphous Ca-P-OH deposits by XRD analysis. They loosely conglutinated together.

The fresh reaction product microsphere has a low strength. It can be easily punctured with a pin as shown in Fig.5(b). The strength of the microsphere can be improved through heat treatment.

We cut the microspheres heat-treated at 90 ℃ and 600 ℃ into half repectively.Fig.6(a) shows the semi-microsphere dried at 90℃ for 4 hours. Its strength became larger to an extent. It also has some toughness since it correspondingly changed from spherical to elliptical under cutting. It can also be seen that the wall

was precipitated layer by layer. However, its strength was still low and the binding force between layers was very small due to the low temperature heat-treatment.

When heat-treated at 600 ℃ for 4 h, the strength was largely improved. It can keep being spherical after cutting, which also shows it has a higher strength and brittleness. Heat-treated at higher temperature, the amorphous Ca-P-OH deposits became crystallized (proved by XRD analysis). It resulted in the disappearance of the interspaces between layers mentioned in Fig 6(a). Therefore, the wall became more compact and thinner. Due to that its strength increased greatly. It is concluded the strength of hollow, porous microspheres can be improved through heat treatment at 600 ℃.

3.3 Mechanism of forming the hollow HAP microsphere

By analysis of XRD and SEM the mechanism that hollow, porous HAP microsphers were formed is shown in Fig.7:

In alkaline solution the LCB glasses release Li^+, B^{3-} , Ca^{2+} ions, among which Li^+ and B^{3-} can not precipitate in the solution so they still keep in solution during the reaction. However, Ca^{2+} ions react with PO_4^{3-} ions and form the compound of Ca-P-OH. And the Ca-P-OH compound deposites on the exact place formerly inhabited by the Ca^{2+} ions in the LCB glasses. The Li^+ and B^{3-} ions dissolve into solution and leave pores on the wall of microsphere, which makes it possible for PO_4^{3-} ions in the solution to keep on reacting with the inner part of the glass sphere. The initial precipitated compound of Ca-P-OH plays a role of base. The new reacted product of PO_4^{3-} ions and fresh Ca^{2+} ions successively deposit on its inner surface. While the LCB glass mircospheres react from the external surface to the interior part, the fresh Ca-P-OH compound always deposites onto the inner surface of the base precipitations. In this way hollow, porous microspheres finally form layer upon layer.

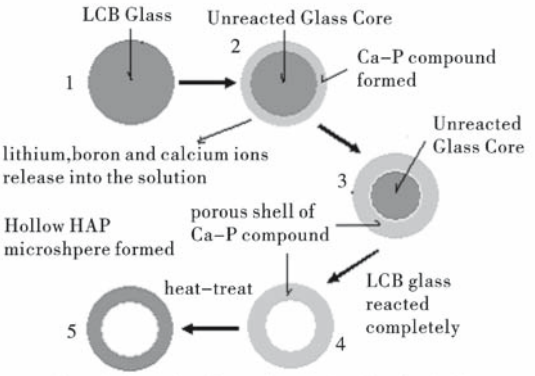


Fig.7 Schematic of reaction mechanism for hollow, porous HAP microsphere formation

The fresh reaction products of LCB glass and K_2HPO_4 solution are amorphous compound of calcium phosphate. The micro-particles of the compound gradually grew up and aggregated during heat-treatment. After heated at about 600 ℃ for 4 h the amorphous calcium phosphate was crystallized and the hollow, porous HAP microspheres were successfully prepared.

4 Conclusions

The lithium-calcium borate (LCB) glasses are quietly active in phosphate solution, which makes it possible for preparing hollow, porous HAP microspheres. During the reaction the produced compound of Ca-P-OH precipitation can precipitate on the initial place of Ca^{2+} ions. The LCB glass microspheres gradually dissolve, during which the Li^+ and B^{3-} ions are released into the solution. Thus the phosphate solution passes through the pores left by the dissolved Li^+ and B^{3-} ions and reacts with Ca^{2+} ions in the glass continuously. The fresh reacted precipitation is amorphous calcium phosphate, but after heat-treated at 600 ℃ for 4 h it is crystallized and finally the hollow, porous HAP microspheres with a certain strength are formed. Such hollow, porous microspheres may provide a valuable means for controlled-release drug delivery.

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