J Mater Sci: Mater Med (2006) 17:641–646
DOI 10.1007/s10856-006-9227-5

Preparation of hollow hydroxyapatite microspheres

Qing Wang · Wenhai Huang · Deping Wang · Brian W. Darvell · Delbert E. Day · Mohamed.N. Rahaman

Received: 26 April 2005 / Accepted: 9 August 2005

Abstract The preparation of hollow hydroxyapatite (HA) microspheres as potential drug-delivery vehicles was investigated. A lithium-calcium-borate ($10Li_2O$-$15CaO$-$75B_2O_3$) (mol%) glass, made by fusing the components at 1100°C for 1 h, was ground to a powder and passed through a flame at ~1400°C to spheroidize the particles. The resulting glass microspheres (106–125 μm in diameter) were reacted in 0.25 M K_2HPO_4 solution for 5 days at 37°C and pH 10–12, resulting in the formation of porous, hollow microspheres of a calcium phosphate (Ca-P) material with external diameters similar to those of the original glass particles. Heat treatment at 600°C for 4 h partially converted the Ca-P material to HA, as confirmed by X-ray diffraction, and also increased the strength of the hollow microspheres.

Controlled-release drug systems avoid the disadvantages of conventional repeated doses of pharmacologically-active substances, both in terms of reliability and uniformity of systemic concentration, in that a more constant delivery rate is obtained. An important requirement is that the delivery vehicle is biocompatible, having no or negligible side-effects. Diffusion-control systems are currently the most popular and most-investigated, in which monolithic or reservoir carriers are used [1]. The distinction is that the latter do not depend on structural breakdown as do the former, in which the active agent is uniformly dispersed in a resorbable matrix. An advantage of the reservoir-type is that the diffusion parameters, and thus the release rate, are more readily controlled.

In recent years, there has been much work on organic polymer matrices for drug delivery. For example, derivatives of poly(aminophenol) (PAP) are non-toxic. They often have no side-effects and fair biocompatibility [2]. When used in the human body, the polymers break down into aminophenols through hydrolysis and enzymatic reactions, and can thus be absorbed and excreted by the body. PAPs are widely used for controlled-release drug delivery, surgical sutures, artificial skin and tissue engineering materials [3]. Poly(lactic acid) can be used to make drug-containing microspheres for subcutaneous implantation or venous injection. For example, Zhang *et al*. reported the use of implanted poly(lactic acid) microspheres for the treatment of lung disease with the drug Rifampicin [4]. However, the relatively small size of these microspheres (of the order of a few micrometres in diameter) provides a low drug-carrying capacity and commonly results in very rapid drug release.

Research on inorganic drug-delivery systems has almost exclusively focused on porous β-tricalcium phosphate or calcium phosphate bone-cement materials [5, 6]. The use of glasses for such purposes has not been reported but it is known that certain types, referred to as bioactive glasses, have good biocompatibility. Since their ion-activity is greater

Q. Wang · W. Huang (✉) · D. Wang
Institute of Bioengineering & Information Technology Materials, Tongji University, Shanghai 200092, P.R. China
e-mail: huangwe@umr.edu or whhuang@mail.tongji.edu.cn

W. Huang · D. E. Day
Materials Research Center, University of MIssouri-Rolla, Rolla, MO 65409-1170, USA

B.W. Darvell
Dental Materials Science, Prince Philip Hospital, University of Hong Kong, Hong Kong, P.R. China

D. E. Day · M. N. Rahaman
Department of Matreials Science and Engineering, University of Missouri-Rolla, Rolla, MO 65409-0340, USA

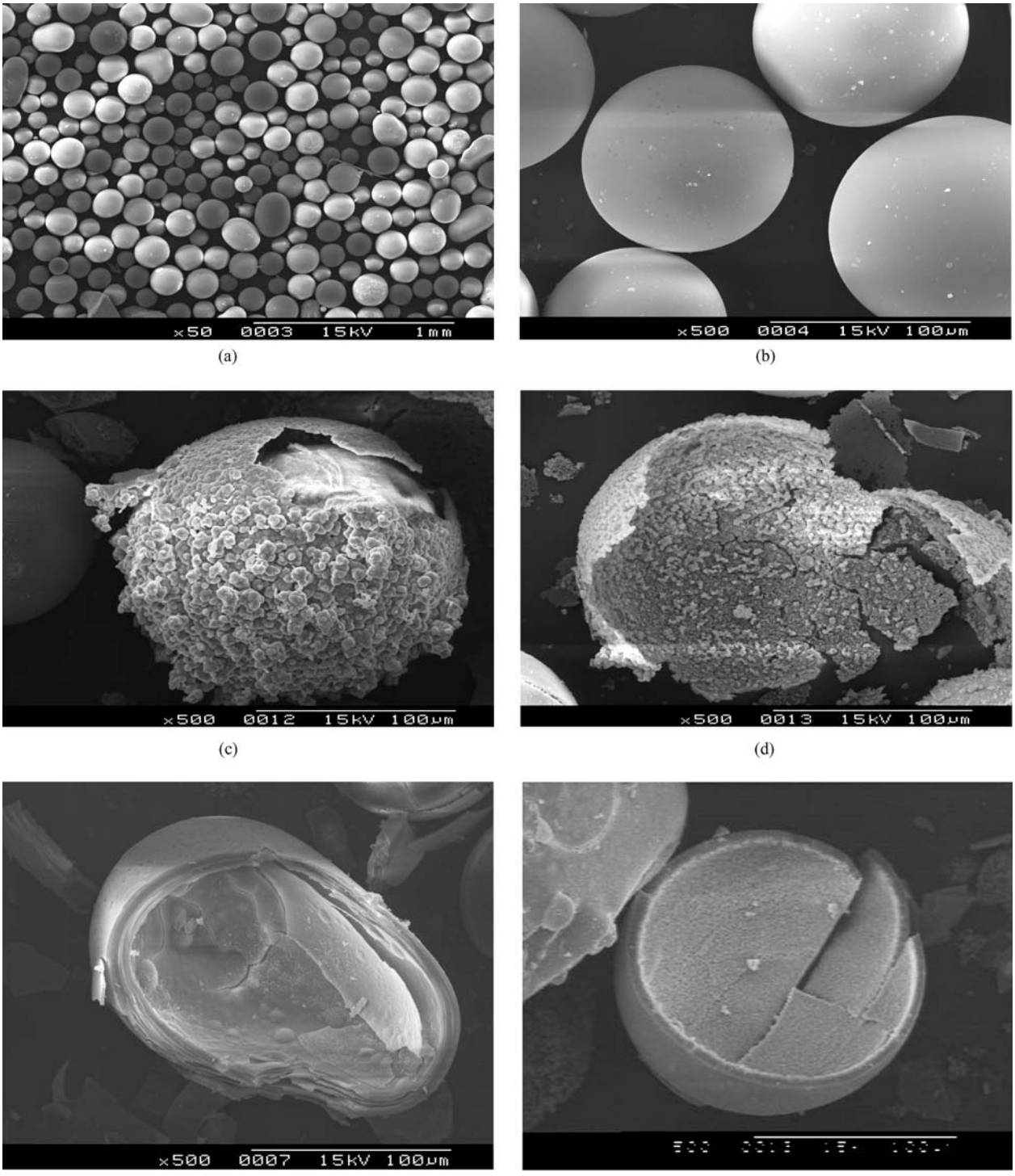


Fig. 1 SEM observations at various stages during the process of forming hollow Ca-P microspheres from lithium calcium borate glass: (a) original LCB glass microspheres after washing (×50); (b) higher magnification image of particles shown in (a) (×500); (c) after 24 h reaction in phosphate solution (×500); (d) after complete reaction at 5 d (×500); (e) after drying at 90°C for 24 h and fracturing to show hollowness and wall structure (×500); (f) after heat-treatment at 600°C for 4 h: fractured microshell showing preservation of sphericity and apparent layering of wall (×500).

than that of crystalline materials, it causes enhanced rates of degradation, making them suitable for this type of application [7, 8]. However, the drug-delivery systems currently in use are rather large-scale (cylinder, 10 mm in diameter and 50 mm in length).

The objective of the present work was to explore a much smaller scale drug-delivery system (*i.e.*, sub-millimeter diameter spheres) based on the formation of hollow HA microspheres from a borate glass. The structure and chemistry of bioactive glasses that can transform *in vivo* into hydroxyapatite (HA) have been widely studied [9, 10] Hydroxyapatite, which is non-toxic and eminently bio-compatible, is of much interest as a drug-delivery medium [11–14]. Accordingly, we report here on the formation of hollow HA microspheres intended for use as a controlled-release drug-delivery system of the reservoir type.

1. Materials and methods

A lithium calcium borate (LCB) glass of composition $10Li_2O$-$15CaO$-$75B_2O_3$ (mol%) was prepared by mixing and melting the appropriate amounts of reagent-grade Li_2CO_3 (CP, batch 20030423, Shanghai 2nd Chemical Reagent Factory, Shanghai, China), $CaCO_3$ (CP, batch 20020701, Shanghai Silian Chemical Industry Factory, Shanghai, China), and H_3BO_3 (AR, batch 20020712, Shanghai Yunling Chemical Industry Factory, Shanghai, China) in a platinum crucible at 1100°C for ∼ 1 h. The melt was poured onto a stainless plate and immediately covered with another cold stainless steel plate to chill the melt rapidly to prevent crystallization and to shatter the resulting glass. The glass fragments were then crushed and ground in an agate mortar and pestle, and sieved to a particle size range of 106–180 μm (80–150 mesh). This powder was then spheroidized by passing through a flame, using equipment described elsewhere [15]. A stream of nitrogen gas was used to carry the glass particles into a natural gas–air flame (∼1400°C), whereupon they were re-melted, becoming spherical as a result of surface tension. The resultant glass microsphere powder was collected in a stainless steel container, then wet-sieved to 115–150 mesh (106–125 μm) using ethanol to obtain a more uniform particle size.

One gram portions of the resulting LCB glass microspheres were soaked in 100 mL of 0.25 M K_2HPO_4 solution at pH 9–12 in a borosilicate glass beaker in a water bath at 37°C. The mixture was stirred at 10 rpm with a PTFE-coated magnetic bar for up to 5 d. The change in pH value of the solution resulting from the ions concentration change during the reaction was monitored by a pH meter (Model PHS-3C, Shanghai Precision and Scientific Instrument Co. Ltd, Shanghai, China) to display the procedure of the reaction (with measurement deviation of ± 0.01). The solid material remaining was recovered by filtration on a 25 μm-pore filter paper, rinsed three times with distilled water and once with ethanol, and dried at 90°C for 24 h. Finally, the dried powder was heat-treated at 600°C for 4 h in an alumina crucible in air.

Both the dried and heat-treated microspheres were examined by X-ray diffraction (XRD) (DX-2000, Dandong Instruments, Dandong, China) to identify any crystalline phases present. Microstructural changes occurring during soaking were observed at various stages using scanning electron microscopy (SEM) (S-2360, Hitachi, Tokyo, Japan).

2. Results

The general appearance of the spheroidized glass powder is shown in Fig. 1(a) and (b). The particles are smooth and featureless, with a near-uniform diameter of ∼100 μm. After 24 h of reaction in the phosphate solution, an irregular reaction-product layer was apparent, distinct and separate from the solid glass core [Fig. 1 (c)]. After 5 d, the particles were found to be hollow [Fig. 1 (d), (e)], a condition that was preserved after heat-treatment [Fig. 1(f)].

The XRD pattern of the dried, reacted powder showed no distinct peaks, with only a broad background indicating a non-crystalline structure [Fig. 2, bottom] After heat treatment, a number of sharp peaks were found which corresponded well to those of HA (JCPDS 72-1243, Table 1), although a broad background still remained (Fig. 2, top). No other peaks were detected.

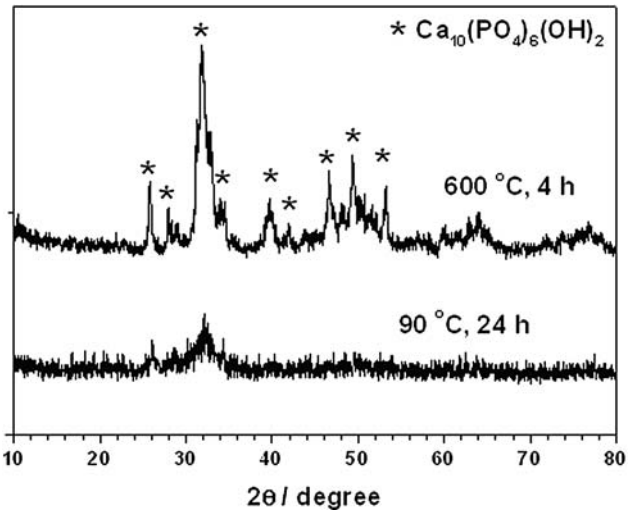


Fig. 2 X-ray diffraction patterns for the phosphate-soaked glass microspheres after reaction for 5 d. Bottom: after drying at 90°C for 24 h; top: after heat-treatment at 600°C for 4 h. The identified HA peaks are marked.

Table 1 Comparison of the XRD spectrum of a sample heat-treated at 600°C for 4 h with that of $Ca_{10}(PO_4)_6(OH)_2$ (JCPDS 72-1243)

Sample heat-treated 600°C		$Ca_{10}(PO_4)_6(OH)_2$ (JCPDS 72–1243)				
2θ	$I/I_x \times 1000$	2θ	$I/I_x \times 1000$	h	k	l
25.84	326	25.89	378	0	0	2
28.00	254	28.14	103	1	0	2
29.08	185	28.92	152	2	1	0
31.87	1000	31.77	999	2	1	1
32.32	681	32.21	457	1	1	2
32.74	602	32.90	564	3	0	0
34.09	279	34.07	223	2	0	2
39.64	275	39.78	193	1	3	0
46.57	409	46.70	267	2	2	2
48.07	275	48.08	130	1	3	2
49.39	488	49.50	294	2	1	3
50.53	271	50.48	170	3	2	1
51.61	267	51.25	114	4	1	0
52.87	244	52.81	122	3	0	3
53.14	338	53.25	140	0	0	4

3. Discussion

It had been found that if the crushed glass powder was greater than ∼300 μm in diameter spheroidizing was difficult, while if less than ∼80 μm the anticipated drug-carrying capacity would be too small. The size range chosen here therefore represents a practical compromise from both points of view.

The LCB glass composition used had a cation charge to boron ratio of 1:3, a rather high value which suggests a low-connectivity network and, thus, relative ease of hydrolysis, despite the presence of the doubly-charged calcium ions. However, borate glasses are notable for their complexity, with ring and cluster structures common [16]. Here, the B : O ratio was 3 : 5, giving on average a network unit (shared oxygen) which may be described as $\sim[BO_{3.3}]$, or equivalently $BO_3 : BO_4 = 2 : 1$, as implied by the charge ratio. Borate glasses are, in general, more readily hydrolysed in acid or base than silicate glasses, and even in deionized water [17].

On immersion in the phosphate solution, the glass appeared to be completely reacted by 5 d, producing only a calcium-phosphate (Ca-P) precipitate. That was confirmed by the change in pH value of the solution during the reaction (shown in Fig. 3). The pH value increased with reaction time due to the higher basicity of Li^+ than the acidity of $[BO3]^{3-}$ in the solution. The pH value eventually reached a nearly constant, which indicated the completion of the reaction.

The conjecture that reaction product was calcium-phosphate (Ca-P) is based on consideration of the solubilities of all other possible compounds from the components present, supported by the absence of other identifiable diffraction peaks. Elemental analysis of the Ca-P material is currently in progress.

The solution chemistry of the conversion process is likely to be particularly complex and is the subject of current study. Here, the technique for preparing hollow HA microspheres from the borate glass particles is of primary interest. The key issue is that the precipitation of the initial Ca-P material is probably nucleated heterogeneously on the glass surface such that a coherent shell is formed, aided by maintaining the particles in suspension. If the nucleation were homogeneous, no shell would form. It is possible that calcium ions in the glass surface provide nucleation sites. Even so, this shell appears to be porous, and to remain so, given that the glass continues to react and eventually dissolves completely.

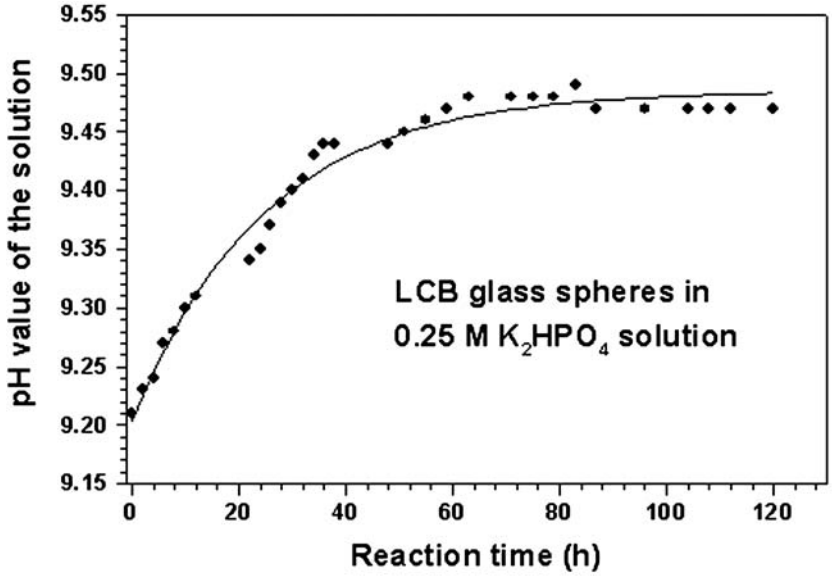
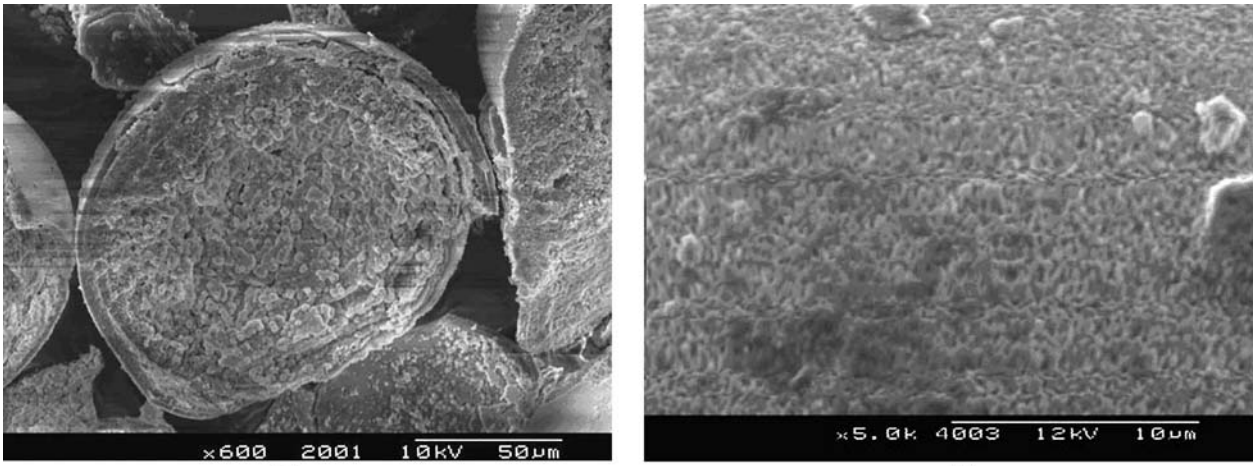


Fig. 3 pH value of the K_2HPO solution reacted with LCB glass spheres in different reaction time.



(a) (b)

Fig. 4 SEM observations of the wall of deliberately-broken microshell after filling with drug: (a) cross-section of microshell showing the thickness of the wall (× 600); The particles in the shell are drug which enter inside by impregnation and dry processes. (b) microstructural morphology of the microshell surface showing micropores presented on the surface (×5000).

However, the non-equivalence of the volume of the Ca-P precipitated with respect to the glass dissolved means that the initial shell could only grow inwards by a relatively small proportion of the initial diameter. Hence, and crucially, hollow particles are formed. It cannot be determined from the present observations if any outward growth from the original particle surface occurs.

The presence of a substantial amorphous background in the XRD pattern after heat treatment suggests that attention will need to be paid to the stoichiometry of the Ca-P precipitate. The effects of reaction time and temperature on the degree of crystallinity, and the influence of the solution composition will need to be studied to provide further control of the process.

Prior to the heat treatment, the hollow HA microspheres had low mechanical strength, as indicated by the ease with which the shells could be broken [Fig. 1(d)]. Some shape-deformation during fracture is apparent in Fig. 1(e), showing that they are not completely brittle, even after drying. The wall also appears to be laminated, perhaps indicating variation in the dissolution process with time. Indeed some layering is still apparent after heat treatment [Fig. 1 (f)], suggesting that diffusion gradients result in compositional variation across the thickness of the shell. This layering may be linked to a similar variation in crystallinity after heating at 600°C. Even so, the heat treatment appears to make the shells more brittle as there is no evidence of deformation in the deliberately-broken microshell shown in Fig. 1(f). Control of strength by the heat-treatment regime would be worth investigating, especially as this strength may be crucial to subsequent processing to load the microspheres with a drug formulation. The speed of drug release could be designed through the control of the thickness of the microshell wall and the average of diameter of the micropores on the surface of microsphere. In the present study, the cross-section of the deliberately-broken microshell after filling with drug (with 10–15 μm in thickness) and microstructural morphology of the wall surface covered with micropores (with hundreds nanometer in size) are shown in the Figs. 4(a) and (b). Future work will address the means of doing this, and the subsequent drug-delivery behavior.

4. Conclusion

Hollow, porous, amorphous calcium phosphate microspheres can be prepared by precipitation *in situ* by dissolution of particles of a lithium-calcium-borate glass in an alkaline phosphate solution**.** Subsequent heat treatment can convert part of the amorphous calcium phosphate precipitate to a material having X-ray diffraction peaks corresponding to hydroxyapatite and also hardens the microshells. Such hollow microspheres are expected to be biocompatible and resorbable, and may provide a valuable means of controlled-release drug delivery.

References

1. D. WANG and W. HUANG, Advancement in research on source-antibacterial ceramic materials, *J. Building Mat* **2**(1) (2000) 73–79 (in Chinese).
2. Y. ZHANG and J. LIU, The research and development of nano-particle of polyalkyl-cyanoacrylate (PACA), *Chinese Modern Medicine* **13**(4) (2003) 31–35 (in Chinese).
3. G. ZHANG, Q. LI and A. HUANG, Determination of biodistribution of galactose- polyhydroxyethylglutamine in mice with isotope 99mTc labeling, *J. Instrumental Analysis* **22**(3) (2003) 49–53 (in Chinese).
4. W. ZHANG, X. JIANG and C. ZHU, Study on the rifampicin polylactic acid microspheres for lung targeting, *Acta Pharm.* **33**(1) (1988) 57–61 (in Chinese).

5. M. OTSUKA, Y. MATSUDA, J. L. FOX and W. I. HIGUCHI, A novel skeletal drug-delivery system using self-setting calcium Phosphate cement, *J. Pharm. Sci.* **84**(6) (1995) 733–736.
6. Q. ZHENG, Y. YAN and C. LIU, The preparation and drug release research of ciprofloxaxin/tricalcium phosphate delivery capsule (CTDC), *J. Wuhan University of Technol.* **3**(2) (1995) 175–179 (in Chinese).
7. J. GI and W. XIE, Process in the study of liposome as drug carrier, *J. Zhengzhou Institute of Techno.* **23**(4) (2002) 14–18 (in Chinese).
8. D. WANG, W. HUANG and N. ZHOU, In vitro drug release behavior of carrier made of porous glass ceramics, *Chinese J. Reparative and Reconstructive Surgery* **16**(5) (2002) 322–324 (in Chinese).
9. D. E. DAY, J. E. WHITE, R. F. BROWN and K. D. MCMENAMIN, Transformation of borate glasses into biologically useful materials. *Glass Technol.* **44**(2) (2003) 75–81.
10. S. D. CONZONE, R. F. BROWN, D. E. DAY and G. J. EHRHARDT, In vitro and *in vivo* dissolution behavior of a dysprosium lithium borate glass designed for the radiation synovectomy treatment of rheumatoid arthritis, *J. Biomedical Materials Res.* **60** (2002) 260–268.
11. C. M. SCHWARZ, Solid liquid nanoparticles for controlled drug delivery. *Micro-encapsulation* **16**(2) (1998) 205–213.
12. C. X. SONG, V. LABHASETWAR, H MUROHY, X. QU., W. R. HUMPHREY, R. J. SHEBUSKI and RJ. LEVY, Formulation and characterization of biodegradable nano-particles for intravascular local drug delivery, *J. Controlled Release* **43**(1) (1997) 197–212.
13. S. KANO, A. YAMAZAKI, R. OTSUKA, M. OHGAKI, M. AKAO and H. AOKI, Application of hydroxyapatite-sol as drug carrier, *J. Bio-materials and Engineering* **4**(4) (1994) 283–290.
14. M. B. YAYLAOGLU and P. KORKUSUG, Development of a calcium phosphate-gelatin composite as a bone substitute and its use in drug release, *J. Bio-materials and Engineering* **12**(2) (1998) 195–201.
15. W. HUANG, S. C. RAY and D. E. DAY, Color and selected properties of $PbO\text{-}BiO_{1.5}\text{-}GaO_{1.5}$ glass, *J. Am. Ceram.* **77**(4) (1994) 1017–1024
16. D. L. GRISCOM, Borate glass structure, In L. D. PYE, V. D. FRECHETTE and N. J. KREIDL (eds) Borate glasses : Structure, Properties, Applications (Materials Science Research, Plenum Press, New York and London, 1977) Vol. 12, 11–138.
17. P. B. ADAMES and D. L. EVANS,: Chemical durability of borate glasses, In: *ibid*, 525–537.