Article ID: 1000-324X(2014)07-0776-05 **DOI:** 10.3724/SP.J.1077.2014.14148

Microwave-assisted Solvothermal Synthesis of Calcium Phosphate Microspheres and Polyhedra

CHEN Feng[1,2], SUN Tuan-Wei[2], QI Chao[2], WU Jin[2], CUI Da-Xiang[1], ZHU Ying-Jie[2]

(1. Research Institute of Micro/Nano Science and Technology, Shanghai Jiao Tong University, Shanghai 200240, China, 2. State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, China)

Abstract: Synthetic calcium phosphate (CaP) has been investigated as promising biomaterial due to its excellent biocompatibility. CaP nanosheet-assembled microspheres were prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4•2H_2O$ and PLA-mPEG in aqueous solution by microwave-assisted hydrothermal method at 120℃ for 30 min. Meanwhile, CaP polyhedral were prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4·2H_2O$ and PLA-mPEG in mixed solvent of water and ethylene glycol (EG) by microwave-assisted solvothermal method at 120℃ for 30 min. The products were characterized with X-ray powder diffraction (XRD), transmission electron microscope (TEM), scanning electron microscope (SEM) and thermogravimetric analysis (TG). EG showed a significant effect on the chemical composition and morphology of the product. The hemoglobin protein adsorption properties of the as-prepared products were investigated. The Hb adsorption amount increased with increasing Hb initial concentration, and were decreased with increasing volume of EG.

Key words: biomaterials; calcium phosphate; microwave; self-assembling; protein adsorption

Calcium phosphate (CaP), widely exists in the hard tissue, is main composite of biological mineral apatite growing on collagen fibrils[1-2]. Due to the similar chemical properties, the synthetic nanostructured CaP materials are promising for applications in biomedical fields[3-5]. CaP materials with a variety of morphologies, including nanoparticles, nanorods, nanotubes, nanowires, self-assembled nanostructures, and so on, have been reported[6-11]. The performance and application of CaP materials depends greatly on their morphology, size and chemical composition[12]. For example, protein adsorption is an important property of CaP based materials, which can be used in drug delivery and bone tissue engineering[13]. Many previous studies on the protein adsorption behaviors of CaP materials have indicated that the physical/chemical properties such as structure, surface charge, specific surface area of the CaP materials significantly influence their protein adsorption behaviors[14-15].

Microwave heating has advantages such as rapid heating, high reaction rate and energy saving, comparing with the conventional heating methods[16]. Since the first report of microwave-assisted organic synthesis in 1986, the applications of microwave heating in chemistry and materials science have become a fast growing area of research[17-19]. Various CaP materials including nanorods, nanosheets, flowers and microspheres have been prepared using microwave-assisted methods[20-25].

In this work, hydroxyapatite nanosheet-assembled microspheres were prepared in aqueous solution by the microwave-assisted hydrothermal method. Meanwhile, CaP polyhedra were prepared in mixed solvent of water and EG by the microwave-assisted solvothermal method. The hemoglobin protein adsorption properties of the as-prepared products were investigated.

1 Experimental

1.1 Preparation of calcium phosphate microspheres and polyhedra

In a typical experiment, 0.1762 g $Ca(CH_3COO)_2$ and 0.1000 g polylactide-block-monomethoxy (polyethyleneglycol) (PLA-mPEG) (Molecular weight=8000, Jinan Daigang Biomaterials Co. Ltd., China) was dissolved in 30 mL mixed solvent of deionized water and ethylene

Received date: 2014-03-27; **Modified date:** 2014-04-22; **Published online:** 2014-05-12
Foundation item: National Natural Science Foundation of China (51172260, 51102258, 81171727); Science and Technology Commission of Shanghai (11nm0506600, 13JC1407300)
Biography: CHEN Feng (1981–), male, PhD candidate. E-mail: fchen@mail.sic.ac.cn
Corresponding author: ZHU Ying-Jie, professor. E-mail: y.j.zhu@mail.sic.ac.cn; CUI Da-Xiang, professor. E-mail: dxcui@sjtu.edu.cn

glycol (EG). The volumes of EG used in the experiments were 0, 10, 15, and 20 mL, respectively. Then, 0.1560 g $NaH_2PO_4{\cdot}2H_2O$ was dissolved in 10 mL deionized water, and added dropwise to the above solution under magnetic stirring at room temperature. The pH value of reaction solution was maintained at 4–4.5 by slow addition of 1 mol/L HCl aqueous solution. Thereafter, the resulting solution was loaded into a 70 mL autoclave, sealed and microwave-heated in a microwave oven (MDS-6, Sineo, China) to 120℃ and maintained at that temperature for 30 min, and then cooled down to room temperature naturally. The product was washed with deionized water and ethanol several times, and dried to powder at 60℃.

1.2 *In vitro* protein adsorption

For the protein adsorption experiments, hemoglobin (Hb) was used as a model protein. The sample (5 mg) was dispersed in the Hb solutions (4 mL) with concentrations of 100 and 500 μg/mL, respectively. Each solution was shaken at a constant rate of 130 r/min for 4 h at 37℃. Then, the solution was centrifuged, and the concentration of Hb protein in the supernatant was measured by the UV-Vis absorption at 406 nm.

1.3 Characterization of samples

The samples were characterized by scanning electron microscope (SEM, JEOL JSM-6700, Japan), transmission electron microscope (TEM, Hitachi H-800, Japan), X-ray powder diffraction (XRD, Rigaku D/max 2550V, Cu Kα, λ=0.154178 nm), Fourier transform infrared (FTIR, FTIR-7600, Lambda Scientific, Australia), thermogravimetric (TG) analysis (Netzsch, Germany, 10 ℃/min in flowing air) and surface area and pore size analyzer (V-Sorb 2800P, Gold APP, China).

2 Results and discussion

The morphologies of the samples prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4{\cdot}2H_2O$ and PLA-mPEG in mixed solvent of water and EG by the microwave-assisted solvothermal method at 120℃ for 30 min were characterized by SEM (Fig. 1) and TEM (Fig. 2). EG has an obvious influence on the morphology of the product. The product prepared in the absence of EG was consisted of nanosheet-assembled microspheres (Fig. 1(a)-(b) and Fig. 2(a)). However, polyhedra were obtained in the presence of EG (Fig. 1(c)-(h) and Fig. 2(b)-(d)). When the valume of EG increased to 20 mL, irregular nanosheets were observed in addition to the polyhedra (Fig. 1(g)-(h) and Fig. 2(d)).

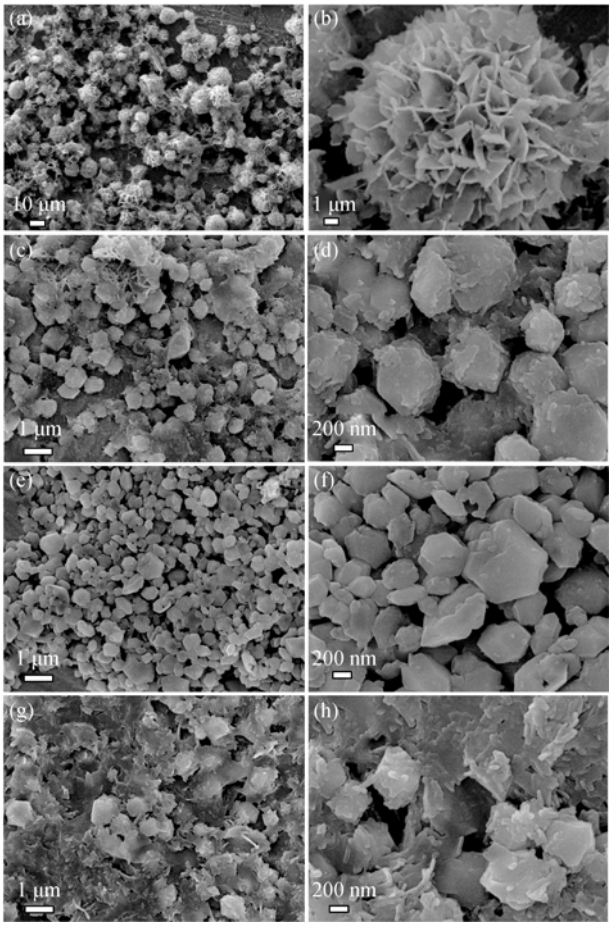

Fig. 1　SEM images of CaP products prepared using Ca $(CH_3COO)_2$, $NaH_2PO_4{\cdot}2H_2O$ and PLA−mPEG in mixed solvent of water and EG by the microwave-assisted solvothermal method at 120℃ for 30 min

The volume of EG: (a,b) 0 mL; (c,d) 10 mL; (e,f) 15 mL; (g,h) 20 mL

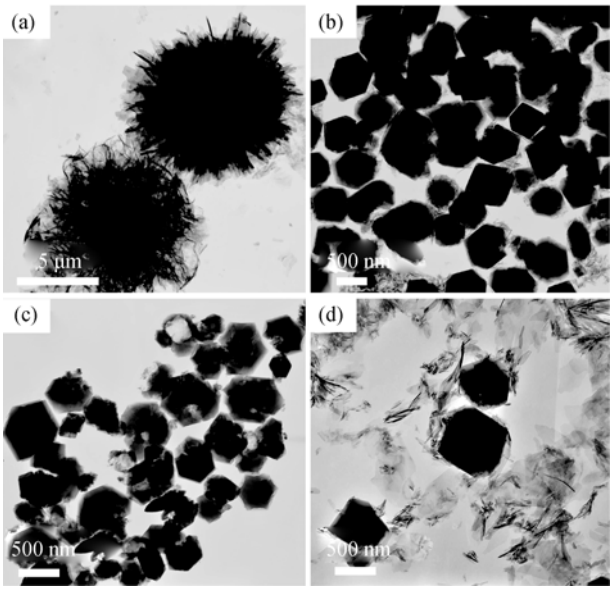

Fig. 2　TEM micrographs (a)-(d) of the CaP products prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4{\cdot}2H_2O$ and PLA-mPEG in mixed solvent of water and EG by the microwave-assisted solvothermal method at 120℃ for 30 min

The volume of EG: (a) 0 mL; (b) 10 mL; (c) 15 mL; (d) 20 mL

The formation of CaP nanosheet-assembled microspheres and polyhedra may follow the formation process depicted in Fig. 3. Under the microwave-assisted hydrothermal conditions in the absence of EG, the CaP nanosheets formed through the reaction between Ca^{2+} and PO_4^{3-} ions and primary crystal growth process in the water environment. Then, the newly formed nanosheets self-assembled to form microspheres for reducing their surface energy in aqueous solution. As for the formation of CaP polyhedra, the EG liquid added in the reaction solution played a key role as a structural control reagent. The EG liquid can significantly inhibit the formation of CaP nanosheet- assembled microspheres, which can be explained by the special properties of EG. Compared with water, EG (with two hydroxyls in each molecule) is polar and viscous. EG molecules can influence the reaction between Ca^{2+} and PO_4^{3-} ions through the interaction with Ca^{2+} ions, and can decrease the diffusion rates of Ca^{2+} and PO_4^{3-} ions. Therefore, CaP polyhedra can be prepared in the presence of EG. On the other hand, with further increasing the ratio of EG, the formation of polyhedra was also inhibited. As shown by the results (Fig. 1(c)-(h) and Fig. 2(b)-(d)), one can see that the amount of CaP polyhedra decreases accompanying the formation of some irregular CaP particles. These results indicate that EG plays an important role in mediating the crystal phase and morphology of the CaP product.

The XRD pattern indicates the formation of hydroxyapatite (JCPDS 09-0432) in the absence of EG (Fig. 4(a)). However, EG has a significant effect on the crystal phase of the product. The products obtained in the presence of EG are consisted of calcium hydrogen phosphate ($CaHPO_4$, JCPDS 09-0080) and a minor crystal phase of tricalcium diphosphate ($Ca_3(PO_4)_2$, JCPDS 09- 0169).

The FTIR spectra of the as-prepared samples are shown in Fig. 4(b). The absorption bands peaks at 1031, 890, 601 and 561 cm^{-1} are attributed to PO_4^{3-}. Among these absorption peaks, the absorption at 1031 cm^{-1} is assigned to the ν_3 vibration of the P–O bond of the phosphate group. The absorption peak at 890 cm^{-1} is due to the ν_1 stretching mode of the P−O bond of the phosphate group. The absorption peaks at 601 and 561 cm^{-1} refer to the ν_4 bending mode of O–P–O bonds. Compared with the FTIR spectrum of hydroxyapatite microspheres obtained in the absence of EG, the absorption bands of PO_4^{3-} for the samples obtained in the presence of EG shift to some extent, implying different chemical properties of the products.

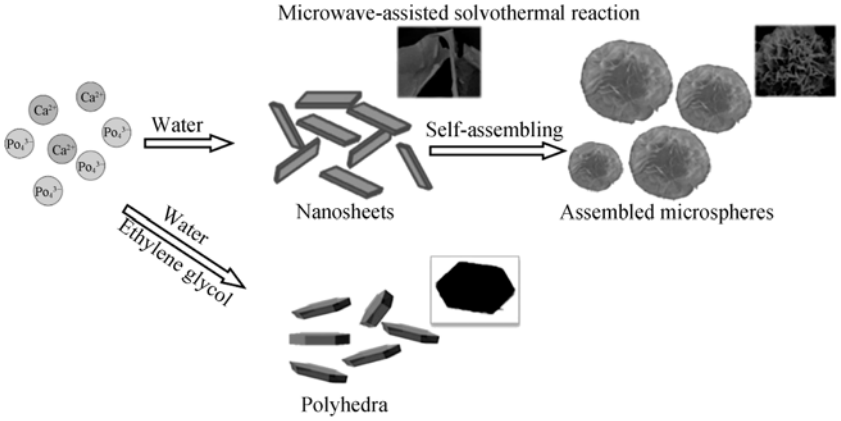

Fig. 3 Schematic illustration of the formation process of the CaP microspheres and polyhedra by the microwave-assisted hydrothermal/solvothermal methods

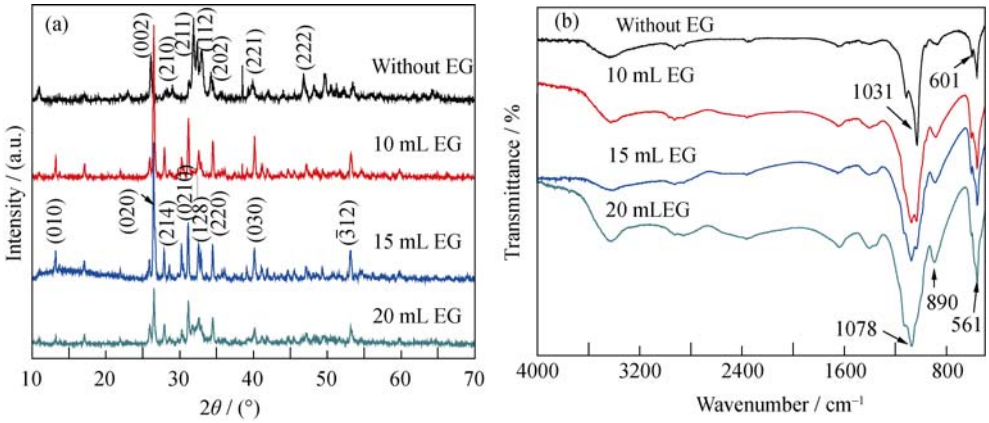

Fig. 4 XRD patterns (a) and FTIR spectra (b) of the products prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4{\cdot}2H_2O$ and PLA-mPEG in mixed solvent of water and EG with different volumes of EG by the microwave-assisted solvothermal method at 120℃ for 30 min

The thermal stability and phase transformation of as-prepared products were studied by TG analysis (Fig. 5). The weight loss (about 7.6wt% at 1000℃) of hydroxyapatite microspheres obtained in the absence of EG is attributed to the decomposition of the adsorbed block copolymer PLA-mPEG and dehydroxylation. Compared with the TG curve of hydroxyapatite microspheres obtained in the absence of EG, the TG curves of the samples obtained in the presence of EG show different profiles with obvious weight losses at temperatures between 400–500℃. The weight losses of the three samples obtained using EG of 10, 15 and 20 mL are 2wt%, 3.4wt% and 5.5wt%, respectively. The obvious weight losses at around 460℃ are resulted from the thermolysis reaction of the products. With further increasing the ratio of EG, the formation of CaP polyhedra is inhibited, due to the influence of EG on the reaction between Ca^{2+} and PO_4^{3-} ions, and some irregular CaP particles with low crystallization are observed in the product.

The Hb adsorption properties of the as-prepared samples have been investigated and the results are shown in Fig. 6. The Hb adsorption amount increased with increasing Hb initial concentration, and the highest adsorption value reached 163 mg/g at a Hb concentration of 500 μg/mL for hydroxyapatite microspheres obtained in the absence of EG. The Hb adsorption amount decreased with increasing volume of EG. These results may be explained by the influence of EG on the chemical composition, morphology and surface properties of the products. For instance, the Brunauer-Emmett-Teller (BET) specific surface areas of the samples prepared in the presence of EG (0, 10, 15 and 20 mL) were 28.8, 8.9, 4.9 and 3.0 m^2/g, respectively.

3　Conclusions

In this work, hydroxyapatite nanosheet-assembled microspheres are prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4 \cdot 2H_2O$ and PLA-mPEG in aqueous solution by the microwave-assisted hydrothermal method at 120℃ for 30 min. In addition, using mixed solvent of water and EG, CaP polyhedra are prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4 \cdot 2H_2O$ and PLA-mPEG by the microwave-assisted solvothermal method at 120℃ for 30 min. The experimental results indicate that the solvent of EG has a significant effect on the chemical composition and morphology of the product. The Hb adsorption amount increased with increasing Hb initial concentration, and decreased with increasing volume of EG. The as-prepared products have potential applications in the biomedical fields.

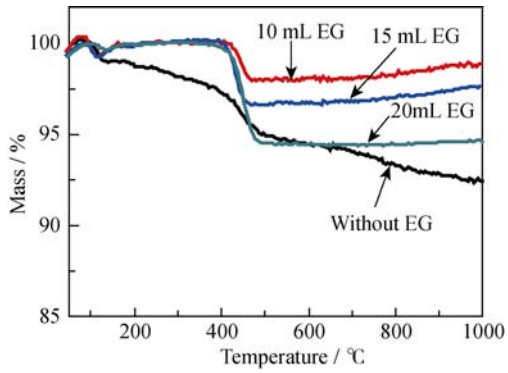

Fig. 5　TG curves of the products prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4 \cdot 2H_2O$ and PLA-mPEG in mixed solvent of water and EG with different volumes of EG by the microwave-assisted solvothermal method at 120℃ for 30 min

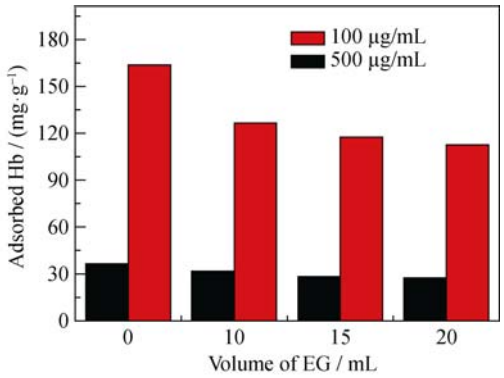

Fig. 6　Hb protein adsorption properties of the products prepared using $Ca(CH_3COO)_2$, $NaH_2PO_4 \cdot 2H_2O$ and PLA-mPEG in mixed solvent of water and EG with different volumes of EG by the microwave-assisted solvothermal method at 120℃ for 30 min

References:

[1] FRATZL P, GUPTA H S, PASCHALIS E P, *et al*. Structure and mechanical quality of the collagen-mineral nano-composite in bone. *J. Mater. Chem.*, 2004, **14(14):** 2115–2123.

[2] TZAPHLIDOU M. Bone architecture: collagen structure and calcium/phosphorus maps. *J. Biol. Phys.*, 2008, **34(1/2):** 39–49.

[3] ONG J L, CHAN D C N. Hydroxyapatite and their use as coatings in dental implants: a review. *Crit. Rev. Biomed. Eng.*, 2000, **28(5/6):** 667a–707a.

[4] YANG S B,WANG J, LIU C S. Research on calcium phosphate cement bone adhesive. *J. Inorg. Mater.*, 2013, **28(1):** 85-90.

[5] CHEN X Q, CHEN X N, ZHU X D, *et al*. Effect of surface topography of hydroxyapatite on human osteosarcoma MG-63 cell. *J. Inorg. Mater.*, 2013, **28(8):** 901-906.

[6] LAI C, TANG S Q, WANG Y J, *et al*. Formation of calcium phosphate nanoparticles in reverse microemulsions. *Mater. Lett.*, 2005, **59(2/3):** 210–214.

[7] LIN KL, CHANG J, ZHU Y J, *et al*. A facile one-step surfactant-free and low-temperature hydrothermal method to prepare uniform 3D structured carbonated apatite flowers. *Cryst. Growth Des.*, 2009, **9(1):** 177–181.

[8] CHEN F, ZHU Y J, WANG K W, *et al*. Surfactant-free solvother-

mal synthesis of hydroxyapatite nanowire/nanotube ordered arrays with biomimetic structures. *CrystEngComm*, 2011, **13(6):** 1858–1863.

[9] CHEN F, TANG Q L, ZHU Y J, *et al*. Hydroxyapatite nanorods/poly(vinyl pyrolidone) composite nanofibers, arrays and three-dimensional fabrics: Electrospun preparation and transformation to hydroxyapatite nanostructures. *Acta Biomater.,* 2010, **6(8):** 3013–3020.

[10] CHEN X, TANG Q L, ZHU Y J, *et al*. Synthesis and antibacterial property of zinc loaded hydroxyapatite nanorods. *Mater. Lett.*, 2012, **89:** 233–235.

[11] LUO H T, ZHI W, LU X, *et al*. Research on preparation and biological properties of dense hydroxyapatite spheres. *J. Inorg. Mater.*, 2013, **28(1):** 40–44.

[12] CHEN F, ZHU Y J, WU J, *et al*. Nanostructured calcium phosphates: preparation and their application in biomedicine. *Nano. Biomed. Eng.*, 2012, **4(1):** 41–49.

[13] WANG K F, ZHOU C C, HONG Y L, *et al*. Review article: a review of protein adsorption on bioceramics. *Interface Focus*, 2012, **2(3):** 259–277.

[14] ZENG H, CHITTUR K K, LACEFIELD W R. Analysis of bovine serum albumin adsorption on calcium phosphate and titanium surfaces. *Biomaterials*, 1999, **20(4):** 377–384.

[15] WASSELL D T H, HALL R C, EMBERY G. Adsorption of bovine serum albumin onto hydroxyapatite. *Biomaterials*, 1995, **16(9):** 697–702.

[16] BAGHBANZADEH M, CARBONE L, COZZOLI P D, *et al*. Microwave-assisted synthesis of colloidal inorganic nanocrystals. *Angew. Chem. Int. Edit.*, 2011, **50(48):** 11312–11359.

[17] GEDYE R, SMITH F, WESTAWAY K, *et al*. The use of microwave-ovens for rapid organic-synthesis. *Tetrahedron Lett.*, 1986, **27(3):** 279–282.

[18] GIGUERE R J, BRAY T L, DUNCAN S M, *et al*. Application of commercial microwave-ovens to organic-synthesis. *Tetrahedron Lett.*, 1986, **27(41):** 4945–4948.

[19] ZHU Y J, WANG W W, QI R J, *et al*. Microwave-assisted synthesis of single-crystalline tellurium nanorods and nanowires in ionic liquids. *Angew. Chem. Int. Edit.*, 2004, **43(11):** 1410–1414.

[20] ZHAO J, ZHU Y J, ZHENG J Q, *et al*. Microwave-assisted hydrothermal preparation using adenosine 5'-triphosphate disodium salt as a phosphate source and characterization of zinc-doped amorphous calcium phosphate mesoporous microspheres. *Micropor. Mesopor. Mater.*, 2013, **180:** 79–85.

[21] ZHAO X Y, ZHU Y J, QI C, *et al*. Hierarchical hollow hydroxyapatite microspheres: microwave-assisted rapid synthesis by using pyridoxal-5 '-phosphate as a phosphorus source and application in drug delivery. *Chem. Asian J.*, 2013, **8(6):** 1313–1320.

[22] QI C, ZHU Y J, LU B Q, CHEN F, *et al*. Hydroxyapatite hierarchically nanostructured porous hollow microspheres: rapid, sustainable microwave-hydrothermal synthesis by using creatine phosphate as an organic phosphorus source and application in drug delivery and protein adsorption. *Chem. Eur. J.*, 2013, **19(17):** 5332–5341.

[23] QI C, ZHU Y J, ZHAO X Y, *et al*. Highly stable amorphous calcium phosphate porous nanospheres: microwave-assisted rapid synthesis using ATP as phosphorus source and stabilizer, and their application in anticancer drug delivery. *Chem. Eur. J.*, 2013, **19(3):** 981–987.

[24] CHEN F, HUANG P, ZHU Y J, *et al*. The photoluminescence, drug delivery and imaging properties of multifunctional Eu^{3+}/Gd^{3+} dual-doped hydroxyapatite nanorods. *Biomaterials*, 2011, **32(34):** 9031–9039.

[25] WANG K W, ZHU Y J, CHEN X Y, *et al*. Flower-like hierarchically nanostructured hydroxyapatite hollow spheres: facile preparation and application in anticancer drug cellular delivery. *Chem. Asian J.*, 2010, **5(12):** 2477–2482.

微波辅助溶剂热法制备磷酸钙微球和多面体

陈 峰[1,2], 孙团伟[2], 漆 超[2], 吴 进[2], 崔大祥[1], 朱英杰[2]

(1. 上海交通大学 微纳科学技术研究院, 上海 200240; 2. 中国科学院 上海硅酸盐研究所, 上海 200050)

摘 要: 磷酸钙材料具有良好的生物相容性, 被广泛应用于生物材料领域。本研究以 $Ca(CH_3COO)_2$、$NaH_2PO_4{\cdot}2H_2O$ 和双亲嵌段共聚物 PLA-mPEG 为原料, 通过微波辅助 120℃水热反应 30 min, 合成了自组装结构磷酸钙微球。以相同的反应原料, 在水和乙二醇混合溶剂中, 通过微波辅助 120℃溶剂热反应 30 min, 制备了具有多面体结构的磷酸钙。通过 X 射线粉末衍射(XRD)、透射电子显微镜(TEM)、扫描电子显微镜(SEM)和热重分析(TG)对所制备样品的物相和形貌进行了表征。研究发现乙二醇的加入对磷酸钙的结构和形貌具有显著的影响。以牛血红蛋白为模型, 研究了所制备的两种不同磷酸钙材料的蛋白吸附效果。磷酸钙材料的牛血红蛋白吸附量随装载溶液中牛血红蛋白浓度的增加而增大, 随样品制备过程中的乙二醇加入量的增加而减小。

关 键 词: 生物材料; 磷酸钙; 微波; 自组装; 蛋白吸附

中图分类号: TQ174 **文献标识码:** A