J Sol-Gel Sci Technol (2013) 68:31–38
DOI 10.1007/s10971-013-3130-6

REVIEW PAPER

Long-term controlled release of dual drugs from MBG/PLGA composite microspheres

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Received: 3 April 2013 / Accepted: 28 July 2013 / Published online: 4 September 2013

Abstract In this study, a long-term controlled drug release system was designed based on mesoporous bioactive glass coated with poly(lactide-co-glycolide) (MBG/PLGA). In this system ibuprofen (Ibu) and egg white protein were used as the model drugs. Firstly, Ibu was loaded into MBG and MBG/PLGA microspheres were formed after MBG/PLGA. Then the egg white protein was adsorbed outside of the MBG/PLGA because of the interaction between the hydroxyapatite and the protein. The drug release tests indicate that Ibu and egg white protein can release from the long-term controlled dual drugs system at the same time. Notably, the release time of Ibu can reach 18 days, and the release time of egg white protein can reach to 6 days due to the role of PLGA. The release rate of Ibu is 49 % of loading rate (46 %), while the release rate of egg white protein is 47 % of adsorption value (184 μg/mg), indicating that the dual drug release system is highly potential in the practical bone repair application.

Keywords Long-term · Dual drugs release · Mesoporous bioactive glass · PLGA protein

1 Introduction

Up to date, many people have suffered from bone diseases, such as osteoporosis, rheumatism, and fracture [1, 2]. Surgery and drug cure are leading methods for these bone diseases therapy [3–5]. However, most artificially synthesized drugs are proteins and enzymes which have big molecular volume and relative high molecular mass, so that these drugs can't dose by oral administration and the absorbed drug would fluctuate if there isn't a drug location release mechanism. As we know, if large doses of drug are given, side-effect emerging will be increased. At present, one of the controlled drug release methods is to encapsulate drug with a container. Before taking medicine, the drug will ooze from the inside of the container [6, 7]. Another controlled drug release method is drug compounded with polymer, the release of the drug molecule depends on the destruction or degradation of the polymer [8–11], and some applications have been passed by FDA identification [12]. Biodegradable and biocompatible polymers, such as poly(lactide-co-glycolide) (PLGA), offer an advantageous guarantee for long-term drug delivery [13]. Accordingly, PLGA microspheres have been widely used as vehicle materials for drug and gene delivery due to its excellent controllable degradability [14]. Laurencin et al. have developed microspheres based scaffolds on which polymers such as PLGA and polyphosphazene were fabricated via single emulsion technique [15, 16].

Mesoporous bioactive glass (MBG) has attracted much attention in drug release field [17–19]. MBG (80 %SiO_2–15 %CaO–5 %P_2O_5, in mol%) were prepared by the non-ionic block copolymer $EO_{20}PO_{70}EO_{20}$ (P123) surfactant as template and the evaporation-induced self-assembly process using Si, Ca and P sources. The MBG are highly bioactive and non-toxic compared with conventional ones, due to the increased textural characteristics supplied by the template. Due to the structure and property advantages (high specific surface area, high pore volume, uniform pore size, and good bioactivity), MBG shows highly efficient

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immobilization of protein/drug molecules, which are helpful to the bone reparation/regeneration and drug loading. Generally, the best drug release balance time is about 4 weeks, and the release balance time of mesoporous materials can reach 48 h [20]. At present, inorganic material/polymer composites have attracted significant attention in the field of localized drug delivery system because of its combined properties of osteoconductivity of the inorganic material and the flexibility of the polymers [21–23].

In this study, a long-term controlled release of dual drugs system was designed based on mesoporous bioactive glass coated with poly(lactide-co-glycolide) (MBG/PLGA). Ibuprofen (Ibu) and egg white proteins were used as the model drugs. The system were prepared by firstly loading Ibu into MBG, followed by coating a layer of PLGA, then the egg white protein was adsorbed outside of MBG/PLGA. The release properties of Ibu and egg white protein from the designed dual drug systems were investigated in detail.

2 Experiment section

2.1 Materials

All the chemical agents used in this experiment were of analytical grade and without further purification, including triblock polyethylene oxide-propylene oxide block copolymer (P123, average molecular weight: 5,800, Aldrich), PLGA (50:50, 40 kDa, Aldrich), tetraethyl orthosilicate (TEOS, Tiantai Chemical Co., Tianjin), triethyl phosphate (TEP, Xinxi reagent Co., Shenyang), $Ca(NO_3)_2{\cdot}4H_2O$ (Guangfu Industry of Fine Chemicals Institute, Tianjin), ethanol (Yongda Chemical Reagent Company, Tianjin), ibuprofen (Ibu, Tianzhunzezhong Chemical Reagent Company, Nianjing) and egg white protein (Extracted from the eggs).

2.2 Synthesis of MBG

Mesoporous bioactive glass was synthesized using P123 as the structure directing agent. In a typical process, 4.0 g P123, 0.73 g TEP, 6.7 g TEOS, 1.4 g $Ca(NO_3)_2{\cdot}4H_2O$, and 1 g HCl solution (0.5 M) were dissolved in 60 g ethanol, and stirred at room temperature for 24 h. The resulting sol was introduced into a Petri dish to undergo an EISA process. The dried gels were calcined at 973 K for 5 h to remove the template. The obtained powders were grinded for a period.

2.3 Synthesis of MBG-Ibu

0.206 g Ibu and equal quantity of MBG were dispersed in 10 mL n-hexane, and magnetically stirred for 6 h. After that the solution was leached by vacuum filter. The Ibu loaded MBG sample were collected and dried at room temperature, and named as MBG-Ibu. The filtrate was collected by centrifugation and properly diluted several times. The loading amount of Ibu is determined by UV–Vis spectrophotometer.

2.4 Synthesis of MBG/PLGA microspheres

MBG/PLGA microspheres were prepared by a solid-in-oil-in-water (s/o/w) emulsion/solvent evaporation technique. Briefly, 1 g PLGA was dissolved in 10 mL $CHCl_3$ to form a solution. Then 0.2 g MBG-Ibu was mixed with the PLGA solution and the mixture was stirred for 20 min. The MBG-Ibu/PLGA mixture was then added dropwise into 200 mL phosphate buffered saline (PBS) solution (pH = 7.4) containing 0.2 % (w/v) polyvinyl alcohol (PVA) as an emulsifier. The mixture was vigorously stirred for 24 h to allow complete solvent evaporation. The resulting microspheres were centrifugated, washed twice with deionized water, freeze dried overnight, and stored in a desiccator before use.

2.5 Synthesis of MBG/PLGA composite microspheres

Modified MBG/PLGA microspheres were prepared by incubating in mSBF at 37 °C and pH of 6.8 for 7 days. Modified simulated body fluid (mSBF) (pH = 6.8, Table 1) possesses inorganic ion concentrations similar to those of human blood plasma with double the concentration of calcium and phosphate ions. Samples were rinsed with distilled water and freeze dried prior SEM, FTIR measurement. In a typical procedure for the adsorption of protein on MBG/PLGA microspheres, modified MBG/PLGA microspheres (0.2 g) were immersed in solutions (50 mL) containing variable protein concentrations (0–2,000 mg/mL) at 37 °C for 12 h. The solution was centrifuged to sediment the

Table 1 Ion concentration of SBF and mSBF

	Na^+ (mM)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	Cl^-	HCO_3^- (mM)	HPO_4^{2-} (mM)	SO_4^{2-} (mM)
SBF	142	5	1.5	2.5	103.0	27	1.0	0.5
mSBF	142	5	1.5	5	103	27	2.0	0.5

microspheres, and the amount of protein in the supernatant was determined at 280 nm by UV–Vis spectrophotometer. The centrifuged microspheres were washed with distilled water and freeze dried. The possible formation process of MBG/PLGA composite microspheres was presented in Fig. 1.

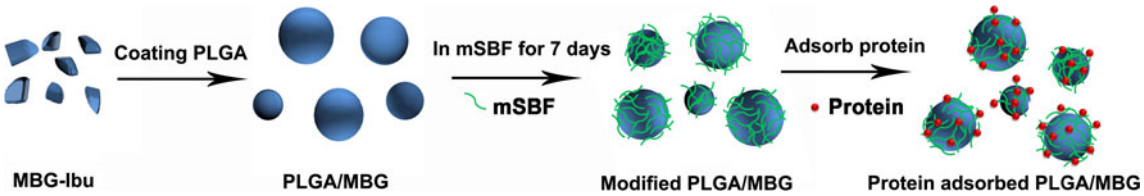

Fig. 1 The possible formation process of PLGA/MBG composite

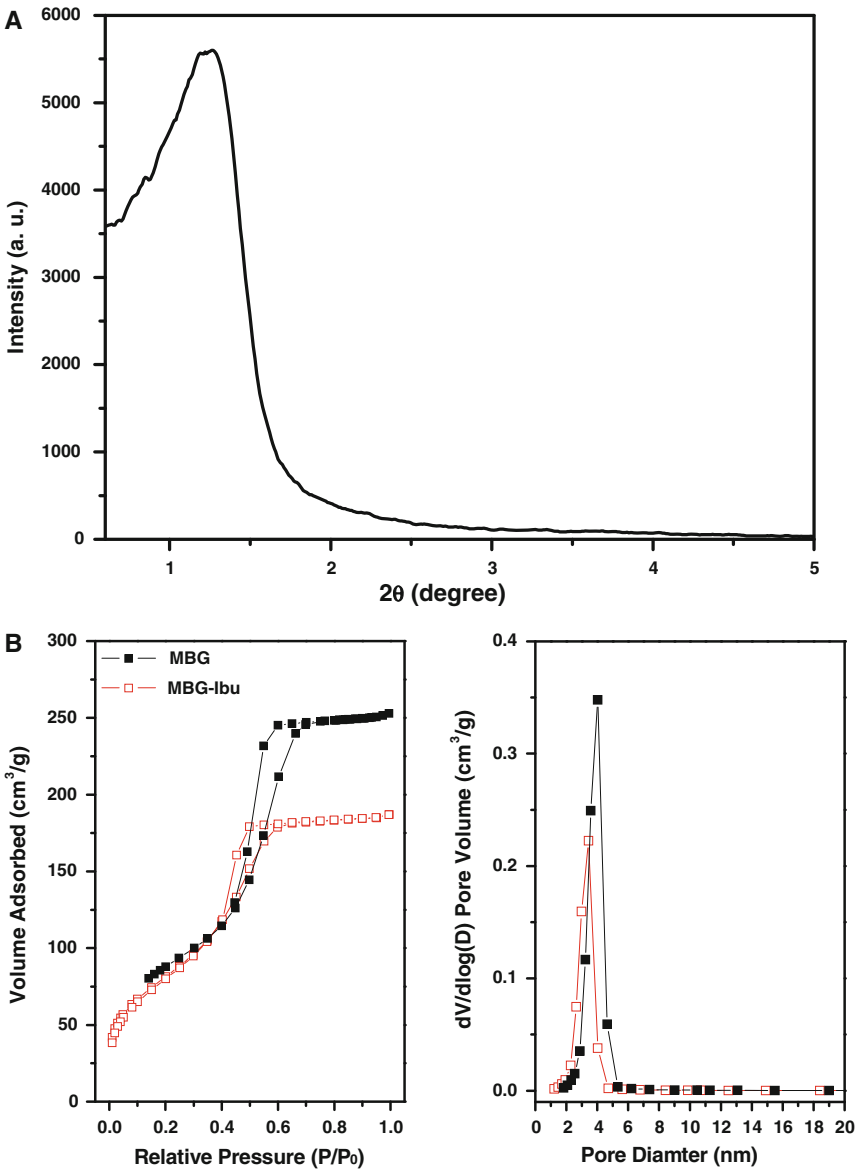

Fig. 2 **a** Small-angle XRD pattern of MBG, and **b** nitrogen adsorption–desorption isotherms (*left*) and pore size distributions (*right*) of MBG and MBG-Ibu

2.6 In vitro ibuprofen and protein release

The release of Ibu and protein were obtained by soaking MBG/PLGA composite microspheres into 300 mL simulated body fluid (SBF) (pH = 7.4, Table 1) solution at 310 K [24]. At predetermined time intervals, 3 mL solution was withdrawn and immediately replaced with an equal volume of dissolution medium to keep the volume constant. Then the 3 mL solution properly diluted and monitored by UV–Vis spectrophotometer. According to the quantitative determination method of overlap of UV absorption spectroscopy between two binary mixtures, the concentration of Ibu and protein content were determined. Experiments were repeated three times and results were presented as means and standard deviations from the three replicates.

Table 2 Structure parameters of the samples

	S_{BET} (m^2/g)	V_p (cm^3/g)	Pore size (nm)
MBG	312	0.39	4.0
MBG-Ibu	237	0.26	3.4

2.7 Characterization

The morphologies of the samples were characterized by Scanning Electron Microscope (SEM, Hitachi S-4800). X-ray diffraction (XRD) data were collected on a SIEMENSD5005 diffractometer with CuKa, using a radiation at 40 kV and 30 mA. The nitrogen adsorption/desorption was measured using a Micromeritics ASAP 2,010 M sorptometer. Before measured at 77 K, the samples were degassed at 373 K for 12 h. Specific surface areas and pore size distributions were calculated using the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) models from the adsorption branches, respectively. An FT-IR spectrometer (JASCOFT/IR-420) was used to record the infrared spectra of the samples by the KBr method. The powder samples were pressed into a tungsten mesh grid and installed in an in situ FT-IR transmission cell, and the samples were outgassed in a vacuum system with a residual pressure of less than 3×10^{-4} Torr at ambient temperature. UV–Vis spectra were taken on a Lambda 45 spectrophotometer.

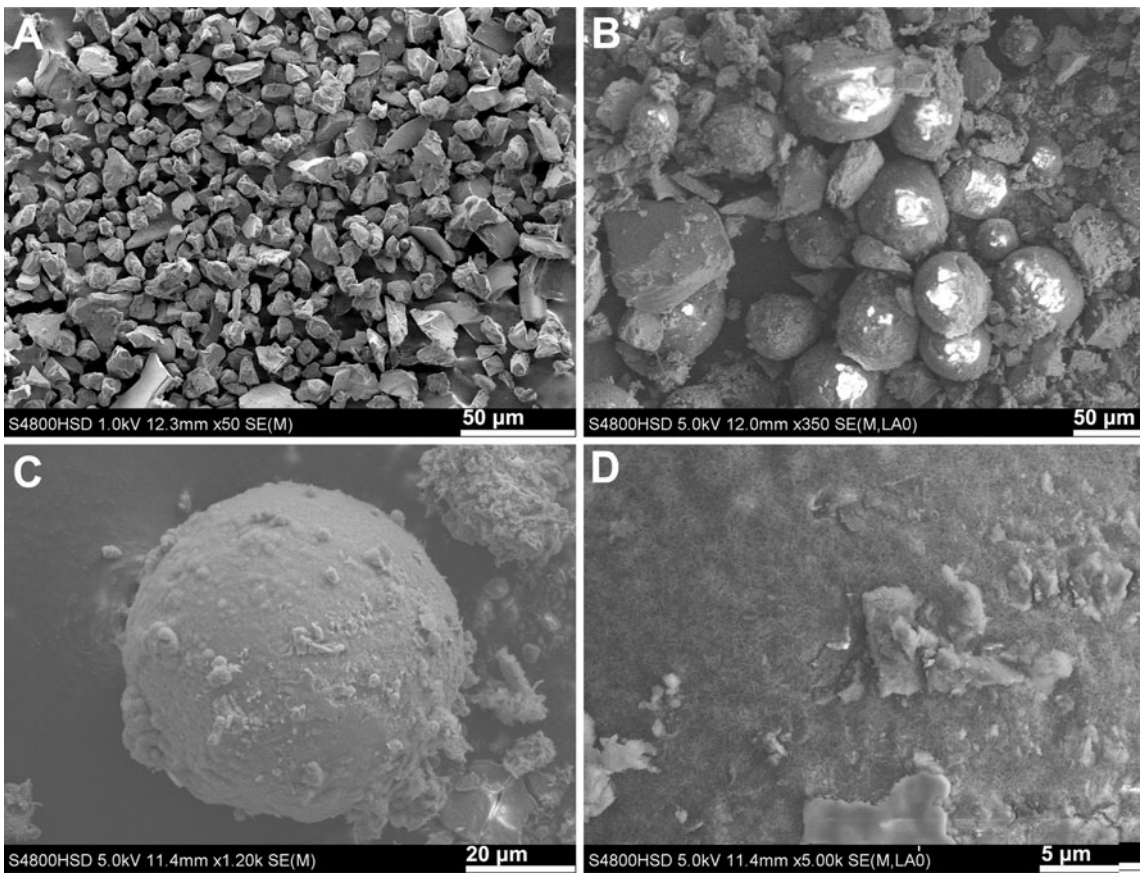

Fig. 3 SEM images of **a** MBG, **b** PLGA/MBG, and **c**, **d** PLGA/MBG in mSBF for 7 days

3 Results and discussion

3.1 Phase, morphology, composition and structure of the samples

The small-angle XRD pattern of MBG, N_2 adsorption–desorption isotherms and the pore size distributions of MBG and MBG-Ibu are shown in Fig. 2. In Fig. 2a, a diffraction peak at about 1.2° indicates the ordered mesoporous structure of MBG. The nitrogen adsorption–desorption isotherms (Fig. 2b, left) show a type IV isotherm with H4-type hysteresis loops, revealing the mesoporous nature of the samples. All the two samples show narrow size distribution curves (Fig. 2b, right). The corresponding textural parameters are list in Table 2. The BET surface area of MBG is 312 m^2/g, and the pore volume is 0.39 cm^3/g. As expected, the BET surface area and the pore volume of the Ibu loaded MBG are decreased to 237 m^2/g and 0.26 cm^3/g, respectively. And the pore size is decreased from 4.0 to 3.4 nm accordingly. The results indicate that MBG has obvious mesoporous structure and Ibu loading has not altered the mesoporous structure of the sample.

Figure 3 shows SEM images of the samples in the different stages. From Fig. 3a, it can be seen that the size of MBG block is about 20–50 μm. After coating a layer of PLGA, the MBG/PLGA sample becomes to spherical microsphere with the diameter of about 50 μm (Fig. 3b). After immersing in SBF solution, the surface of the microspheres as covered with a large amount of flowers (Fig. 3c, d). EDS (Fig. 4) curves give the component information of MBG, PLGA/MBG microspheres and modified MBG/PLGA microspheres. From Fig. 4a, we can see that the main composition of MBG is Si, P, O and Ca, and the Ca/P (atomic ratio) is 2.29 for MBG as synthesized. From EDS of MBG/PLGA microspheres (Fig. 4b), the present high amount of carbon (53 %) confirms the successful coating of PLGA for MBG/PLGA microspheres. In addition, comparing the EDS of MBG/PLGA with MBGs, the atomic ratio of P increased obviously that is ascribing to the use of PBS, a phosphate buffer, as the medium to form MBG/PLGA. When MBG/PLGA was prepared in

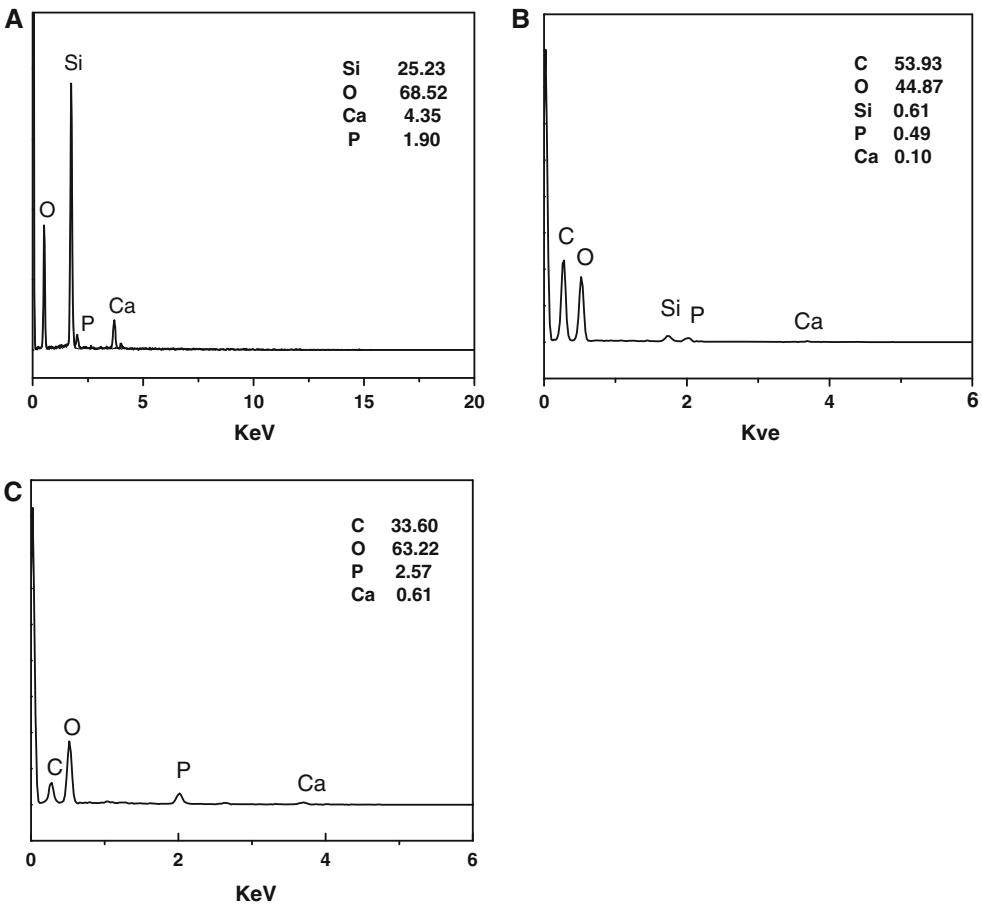

Fig. 4 EDS spectra of **a** MBG, **b** PLGA/MBG microspheres, and **c** modified PLGA/MBG microspheres

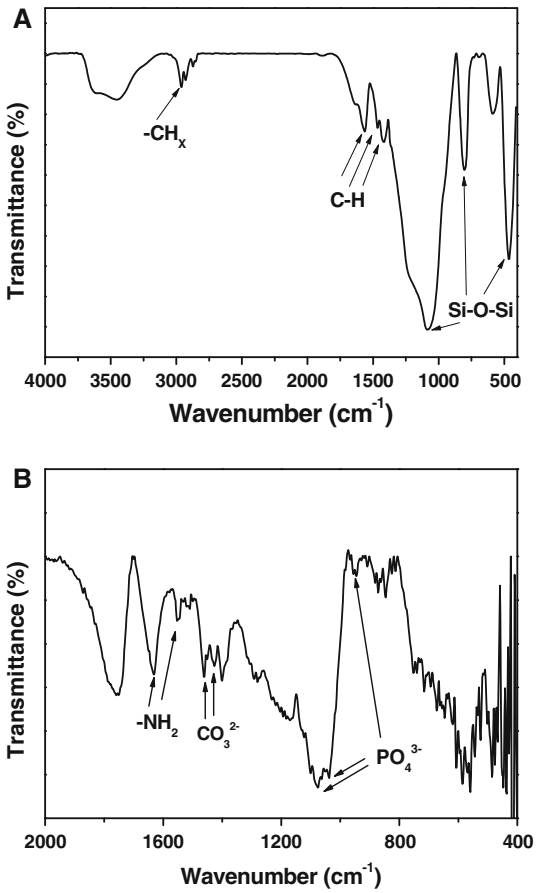

Fig. 5 FT-IR spectra of MBG-Ibu and modified PLGA/MBG composite microspheres

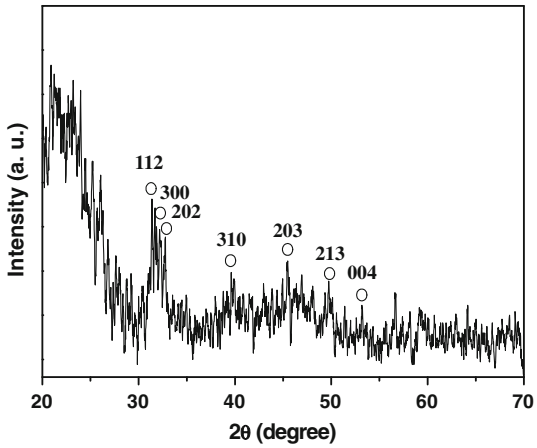

Fig. 6 XRD pattern of PLGA/MBG composite microspheres

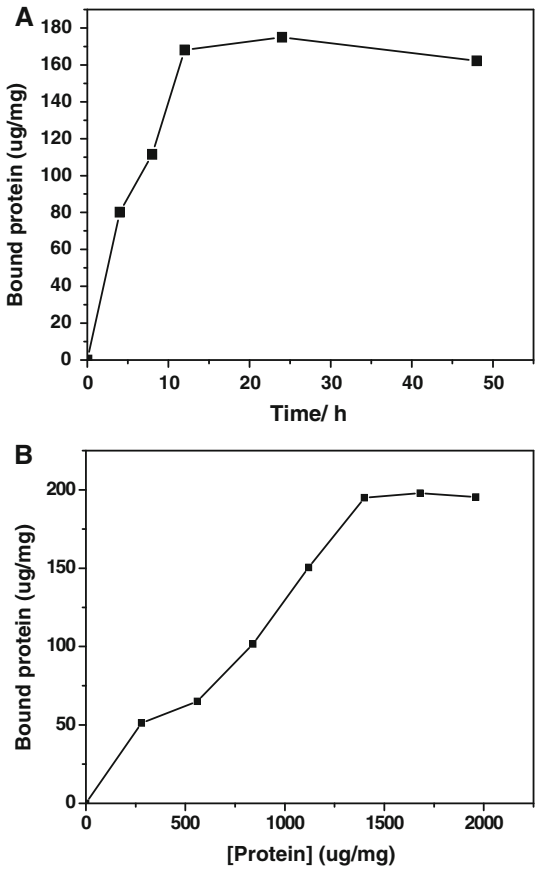

Fig. 7 Influence of reaction time (**a**), and protein amount (**b**) on the bound protein amounts on the modified PLGA/MBG

PBS, a lot of of PO_4^{3-} can be adsorbed outside MBG/PLGA microspheres, making the P surface mount increase. Thus, the Ca/P atomic percent increases from 0.2 (Fig. 4b) to 0.23 (Fig. 4c), revealing the mineralization of hydroxyapatite (HA) (Ca/P = 1.67) after incubation in an mSBF solution for 7 days.

The FT-IR spectra of MBG and MBG/PLGA microspheres are given in Fig. 5. For MBG, the three characteristic absorption bands at 1,085, 815, and 465 cm^{-1} can be attributed to the Si–O–Si asymmetric stretching vibration, Si–O–Si symmetric stretching vibration, and Si–O bending vibration, respectively [25, 26]. For the drug loading sample, the absorption bands at 1,560, 1,475, and 1,421 cm^{-1} can be ascribed to the bending vibrations of C–H, and the absorption bands at 2,974, 2,930, and 2,870 cm^{-1} are assigned to vibrations of –CH_x [27]. Particularly, the characteristic carbonate peaks (1,464 and 1,429 cm^{-1}) and phosphate peaks (1,079, 1,033, 950 cm^{-1}) indicate the formation of HA on the surface of modified MBG/PLGA [27]. Moreover, the characteristic absorption peaks (1,653 and 1,548 cm^{-1}) assigned to the NH_2 groups are associated with the introduced egg white protein bound to the surface of modified MBG/PLGA [28]. In the XRD pattern (Fig. 6), the diffraction peaks corresponding to HA (JCPDS No.09-0432) can be found with low peak intensity, suggesting the low crystal degree of the HA.

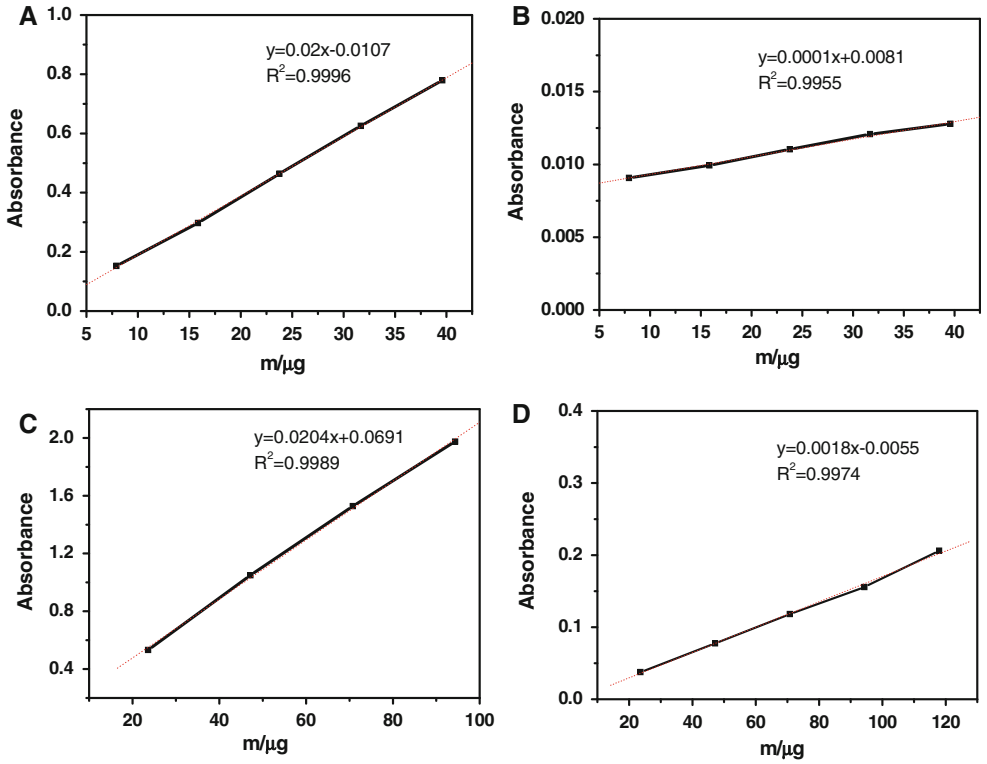

Fig. 8 Standard curves of UV absorption: (**a**, **b**) ibuprofen at 220 and 280 nm; (**c**, **d**) protein at 220 and 280 nm

3.2 Release of ibuprofen and protein

Influence of reaction time and protein amount on the bound protein amounts on the modified MBG/PLGA is given in Fig. 7. From Fig. 7a, we can find that the adsorption amount can reach maximum after 12 h. After 24 h, the adsorption amount decrease due to desorption of the protein molecules. As shown in Fig. 7b, the adsorption capacity increases with the protein content, and reaching saturation until the maximum absorption capacity of 184 μg/mg.

Figure 8 presents the UV absorption standard curves of Ibu and egg white protein at 220 and 280 nm, respectively. Absorptivity (K_1, K_2, K_3, K_4) can be calculated from the standard curves. The experimental concentration of Ibu and egg white protein concentration (C_I, C_P) can be calculated based on the formula 1.

$$\begin{cases} A220 = K1 \times CI + K2 \times CP \\ A280 = K3 \times CI + K4 \times CP \end{cases} \quad (1)$$

Figure 9 shows the release profiles of Ibu and egg white protein from MBG/PLGA composite microspheres. It can be seen dual drugs can release from the system at the same time. As shown in Fig. 9a, Ibu release time is up to 18 days, and the release rate is 49 % of drug loading (46 %). Due to the inorganic/organic composite compound, the release time of Ibu is markedly extended and the concentration of the initial release is also reduced obviously. The low side effects and the long-term drug effect provide great application potentials in bone regeneration. Furthermore, protein release can be maintained for 6 days, 47 % of adsorption value release from MBG/PLGA composite microspheres (Fig. 9b). The results indicate that Ibu and protein can effectively release into SBF solution from this dual release system. This dual drug release can meet the need of long-term anti-inflammatory in the bone repair treatment, and provide nutrients in the early stages of bone repair, such as growth factor.

4 Conclusions

In this study a controlled long-term release of dual drugs system was designed based on MBG/PLGA. In this system Ibu and egg white proteins were used as the model drugs. The results show that Ibu and egg white protein can be successfully loaded and released from the controlled long-term dual drugs system at the same time. Significantly, the release time of Ibu can reach to 18 days, and that of egg white protein can reach to 6 days. The dual drug release system has the potential development space in the practical bone repair application.

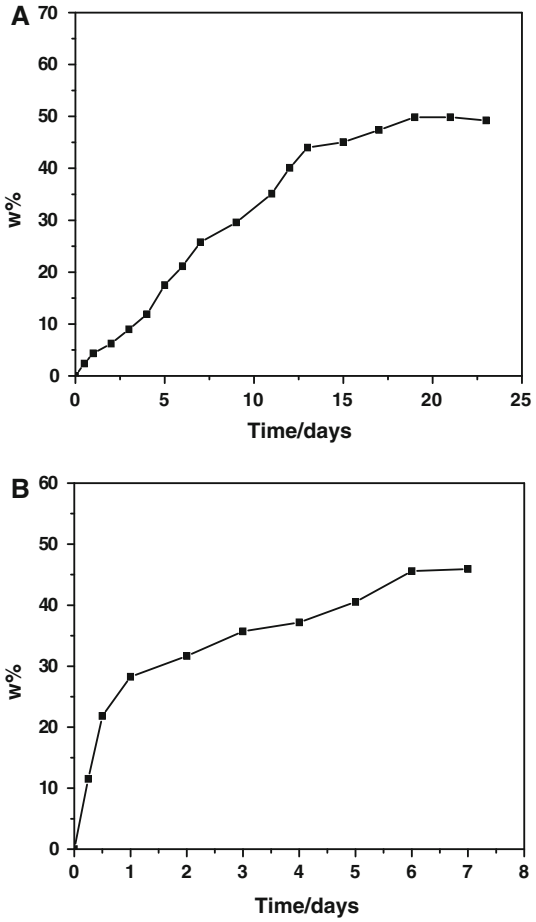

Fig. 9 Release profiles of ibuprofen (**a**), and protein (**b**) from PLGA/MBG composite microspheres

Acknowledgments Financial support for this study was provided by the National Natural Science Foundation of China (21171045, 21101046), Natural Science Foundation of Heilongjiang Province of China (ZD201214, B201206), Program for Scientific and Technological Innovation team Construction in Universities of Heilongjiang province (2011TD010), Specialized Research Fund for the Doctoral Program of Higher Education of China (20102329110002), Technology development pre-project of Harbin Normal University (12XYG-11).

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