Preparation, Characterization, and Drug-Release Properties of PEG-DA-Based Copolymer Hydrogel Microspheres

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ABSTRACT: The research was to design a carrier used in tissue engineering and drug-release system. In this article, a new dispersion polymerization system without organic solvent was described to prepare poly(ethylene glycol) diacrylate/2-hydroxyethyl methacrylate (PEG-DA/HEMA) copolymer hydrogel microspheres as carrier, which introduced chitosan (CS) as reaction medium and costabilizer, polyvinylpyrrolidone (PVP) as steric stabilizer and benzoy peroxide (BPO) as initiator. The effects of mass ratios of monomers, concentration of stabilizer and initiator on partilcle size and polydispersity index (PDI) of microspheres were investigated. Furthermore, bovine serum albumin (BSA) as protein model, the composition of copolymer, size, and polydispersity index of microspheres were employed to study the amount and released ratio of loaded BSA in this article. The results showed that hydrogel microspheres with 3.26 ± 0.24 μm in particle size and 1.03 ± 0.008 in PDI, obtained with mass ratios of PEG-DA 60/HEMA 40, 2 wt % of stabilizer content and 2 wt % of initiator content (relative to total mass of monomers), had 124.57 ± 3.14 mg/g in the amount of loaded BSA and 59.09 ± 1.43% of released ratio. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: microspheres; PEG-DA; HEMA; drug release; dispersion polymerization

INTRODUCTION

Hydrogel materials are crosslinked and three-dimensional polymer networks with a high affinity for water they swell significantly in water, but do not dissolve in it.[1–6] More specifically, PEG-based hydrogel are versatile materials that are highly hydrophilic, nonimmunogenic, and biocompatible.[7–10] Such materials derive their high hydrophilicity from the ethylene oxide linkages in the polymer backbone. The most important properties of PEG-based hydrogel are that PEG chain in it can resist not only cell adhesion but also recognition from the immune system, which allows its use in applications as scaffolds for tissue engineering, as drug delivery carriers, as surgical barriers, in postoperative tissue repair, in cell encapsulation for transplantation, and as coatings for biosensors.[5,8,10–13]

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However, macroscopic PEG-based hydrogel, suffers from the disadvantages of being relatively hard to surface modification, small surface area-to-volume, low encapsulation efficiency of payload in drug-release system, the prolonged response time to external stimulin and so on.[3,8,9] PEG-based hydrogel microspheres fabricated are to overcome the problem metioned above, which maintain characteristics of macroscopic PEG-based hydrogel, such as swelling capacity. To prepare PEG-based hydrogel microspheres, like other polymer microspheres, both the solvent evaporation method and the emulsification/diffusion method have been widely used, which organic solvent or surfactant is needed in these methods, and the resulting microspheres are limited for extensive and further applications in the biomedical field.[14–18] In recent years, a series of techniques were proposed to prepare PEG-based hydrogel microspheres without using organic solvent or surfactant, like An et al.[19] and Zhu and Michael[10] used liposomes and polyelectrolyte multilayer microcapsules respectively, to fabricate PEG-based hydrogel microspheres, PEG-based hydrogel microspheres were obtained by photopolymerizing PEG macromer contained in template, and removing the layer (liposomes and polyelectrolyte multilayer); Zhang and Chu[3] prepared a responsive poly(*N*-isopropylacrylamide)/poly(ethylene glycol) diacrylate hydrogel microsphere at room temperature in an aqueous

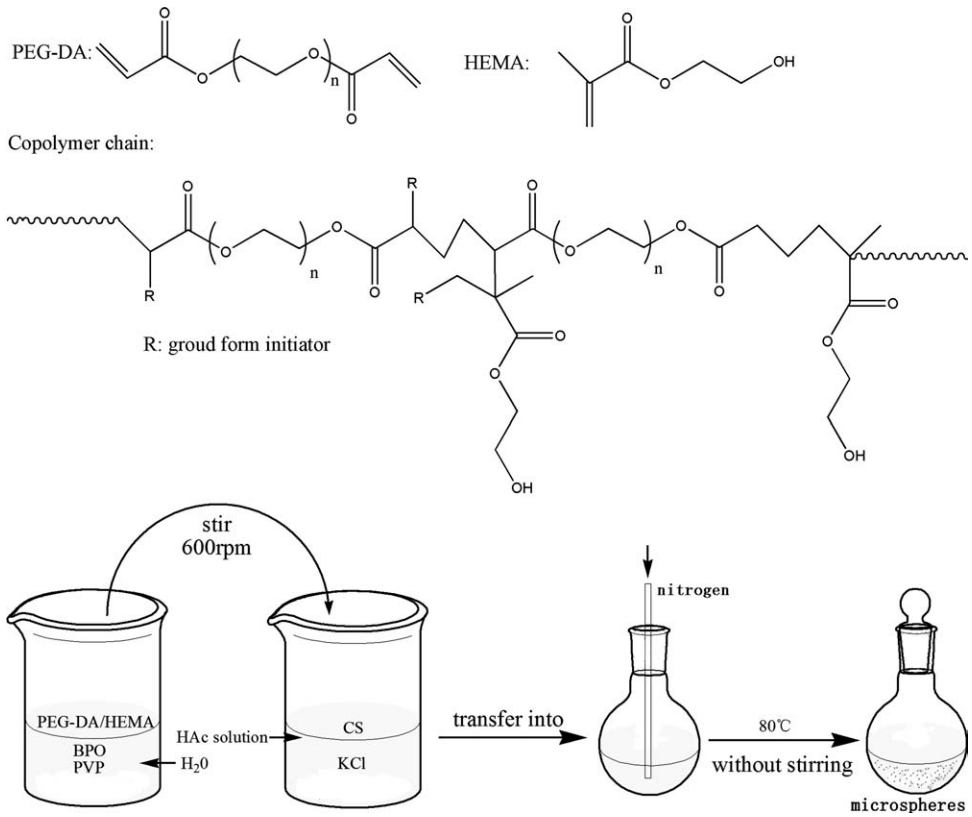

Figure 1 Chemical structure of copolymer and procedure for the preparation of hydrogel microspheres.

two-phase system based on the polymer–polymer immiscibility.

In this article, we employed a water-soluble and biocompatible methacrylate comonomer, 2-hydroxyethyl methacrylate (HEMA), aiming to further improve the adsorption of protein-based drug on hydrogel microspheres surface, its polar properties and small dimensions enhance the wetting properties of material and it is not enzymatically degraded or hydrolyzed by acidic or alkaline solutions.[20,21] We report a method for preparing PEG-DA/HEMA copolymer hydrogel microspheres by dispersion polymerization without stirring in particular media (polysaccharide solution), excluding the use of organic solvents in conventional method and temple mentioned above, which relatively simplify preparation technology and allows its potentiality in tissue engineering and drug-release system. Several polymerization parameters were investigated for preparation PEG-DA/HEMA copolymer hydrogel microspheres. In view of the composition of copolymer, size and size distribution of microsphers having vital influence on drug loading and release, and to evaluate the applied potentiality for target microspheres in drug-release system, we chose bovine serum albumin (BSA) as the model compound for large macromolecular protein-based drug, and several experimentas were performed to discuss the influence mentioned above.

EXPERIMENTAL

Material

PEG-DA with an average molecular weight of 200 was supplied by Sartomer Company. HEMA was purchased from Tokyo Chemical Industry. Polyvinylpyrrolidone (PVP), chitosan (CS), and bovine serum albumin (BSA) were obtained from Shanghai Boao Biological Technology. Benzoy peroxide (BPO) is a product of Shanghai JingChun Reagent, and used before recrystallization in chloroform/methanol. Coomassie brilliant blue staining was obtained from Shanghai Dingguo Biological Technology. Potassium chloride (KCl) was purchased from Luoyang Chemical Reagent Factory.

Preparation of microspheres

The basic recipe for fabrication of PEG-DA/HEMA copolymer hydrogel microspheres by dispersion polymerization were as follows: 1 g of monomer with mass ratio of PEG-DA 60/HEMA 40, 0.02 g of PVP, 0.02 g of BPO, 10 mL 1% CS solution (0.1 g of CS dissolved in 10 mL 2 wt % HAc solution), 0.2 g KCl. Chemical structure of copolymer and procedure for the preparation of hydrogel microspheres were shown in Figure 1, monomer, stabilizer and initiator were dissolved in distilled water (4 mL), KCl were dissolved in CS solution before monomer mixture

solution introduced in with a stirring rate of 600 rpm for 20 min. This solution was transferred into 25-mL round flask, and then nitrogen was bubbled through the solution for 10 min. The polymerization was allowed to proceed without stirring at 80°C for 24 h. The hydrogel microspheres were separated by centrifugation, washed with distilled water for four times and freeze-dried at −50°C for 2 days.

Characterization

FTIR analysis

Fourier transform infrared (FTIR) spectra was used to determine the conversion of acrylate groups after polymerization, and the IR spectra of sample was recorded on FTS-40 FTIR spectrophotometer(Bruker, Germany). Samples were ground with a mortar and pestle and mixed with KBr at a mass ratio of 1 : 100, and then, the mixture was pressed into a pellet and scanned at wavenumber range of 400–4000 cm^{-1}.

SEM analysis

Morphologies of freeze-dried hydrogel microshpheres were examined by using scanning electron micrographs (SEM, Philips-30XLFEG, Holland). The samples on conductive adhesive were sputter coated with gold for 120 s, and were observed at 10 KV accelerating voltage.

Size and size distribution analysis

Particle size distribution was obtained using dynamic light scattering particle size analyzer (Microtrac NanotraTM150, USA), the samples were dispersed and suspended in anhydrous alcohol with a concentration of 1% w/t. The PDI is defined by the following set of eq. (1) (number-average diameter, $\overline{d_n}$; weight-average diameter, $\overline{d_w}$):

$$\overline{d_n} = \frac{\sum_{i=1}^{N} d_i}{N}; \quad \overline{d_w} = \frac{\sum_{i=1}^{N} d_i^4}{\sum_{i=1}^{N} d_i^3}; \quad \mathrm{PDI} = \frac{\overline{d_w}}{\overline{d_n}} \tag{1}$$

The closer PDI is to 1, the more monodisperse the particle is; where N is the total number of microspheres with varying diameter d_i.

Analysis of swelling ratio

The swelling ratio of hydrogel microspheres was determined through gravimetrical method. Totally, 20 mg freeze-dried hydrogel microspheres of various mass ratios of monomers were transfered into 2-mL-taper bottom centrifuge tube of known weight (W_1), and added 1.5 mL phosphate buffer solution (PBS, pH 4.9, and 7.4) where microspheres were swelled at given temperature (25 and 37°C) for 2 h (equilibrium state). Taper bottom centrifuge tube was centrifuged at 12,000 rpm for 10 min, about three quarters of supernatant was remove and the weight (W_2) of microspheres after carefully wiping of excess water with filter paper was weighted. The swelling ratio (SR) was calculated from the eq. (2) below:

$$\mathrm{SR}\ (\%) = \frac{(w_2 - w_1 - 20)}{20} \times 100\% \tag{2}$$

- W_1 (mg): the weight of 2-mL-taper bottom centrifuge tube
- W_2 (mg): the weight of taper bottom centrifuge tube and swollen microspheres
- 20: the weight of freeze-dried microspheres

Protein loading and release

A BSA solution was prepared by dissolving 0.12 g of BSA in 100 mL of PBS (pH 4.9). The given freeze-dried hydrogel microspheres were immersed in a BSA solution above at room temperature. At the predetermined time points, protein-loaded hydrogel microspheres were centrifuged at high speed for 10 min to separate the microspheres from supernatant, and the supernatant was collected for next-step analysis. Coomassie brilliant blue staining method was conducted in order to determine the amount of BSA in the supernatant. The amount of loaded BSA in microspheres was calculated from the eq. (3) below:

$$\text{Amount of loaded BSA}\ (\mathrm{mg/g}) = \frac{W_{\text{loaded BSA}}}{W_{\text{micropsheres}}} \tag{3}$$

Protein-loaded hydrogel microspheres above were freeze-dried, and were used for in vitro release study. Protein-loaded hydrogel microspheres were immersed in 5 mL of PBS (pH 7.4) at 37°C for a certain period of time. Coomassie brilliant blue staining method tracked the concentration change of BSA in supernatant before and after release, released ratio stopped increasing after 7 h. The released ratio (7 h) of BSA was calculated from the eq. (4) below:

$$\text{Released ratio}\ (\%) = \frac{W_{\text{released BSA}}}{W_{\text{total BSA}}} \times 100\% \tag{4}$$

Statistics analysis

Results from the study were analyzed using a GraphPad Prism 5.00 software (San Diego, USA). Four experiments were performed for each study, and the results are expressed as mean ±SD. $P < 0.05$ was considered to be statistically significant.

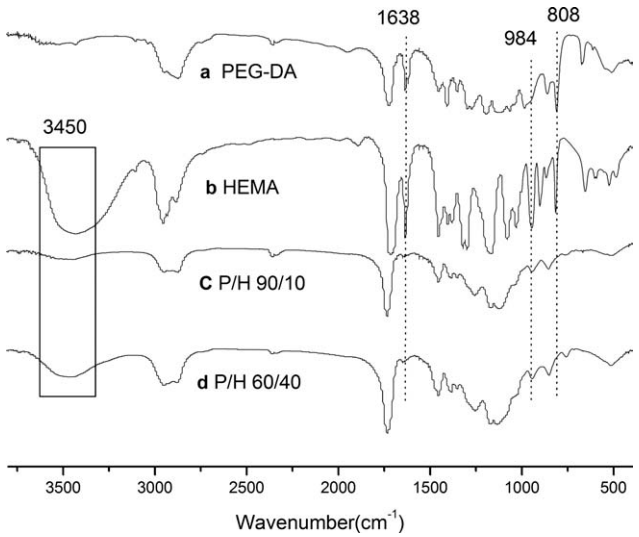

Figure 2 FTIR spectra of PEG-DA (a), HEMA (b), copolymer of PEG-DA/HEMA (90/10) (c), copolymer of PEG-DA /HEMA (60/40) (d).

RESULTS AND DISCUSSION

Characterization

FTIR spectra shown in Figure 2 was used as component analysis of hydrogel microspheres. The sharp band at 1730 cm^{-1} in all spectra attritubed to strectching vibration of —COO— of acrylate group. In Spectra (a) and (b), three characteristic double bond band at 808, 984, 1638 cm^{-1} corresponded to the $=C-H_2$ deformation vibration, terminal alkenes $=C-H_2$ out of plane twist vibration and —C=C— stretching vibration, respectively. Three characteristic double bond band above completely disappeared in spectra(c) and (d), indicating that all or most of C=C of acrylate group underwent polymerization into copolymer. A broad band at 3450 cm^{-1} was assigned to stretching vibration of —OH from HEMA. With the increase in HEMA content, the band at 3450 cm^{-1} increase from Spectra (c) to (d) due to the increasing density of —OH connected to polymer chain.

Figures 3 and 4, respectivlely, showed the SEM and size distribution of freeze-dried microspheres prepared at PVPconcentration of 2 and 5%. It was clearly observed that freeze-dried microspheres exhibited spherical shape and smooth topography in Figure 3. These microspheres ($n = 4$, $P < 0.05$) had a number-average diameter of 3.26 ± 0.24 and 1.79 ± 0.17 μm with normal distribution, as shown in Figure 4(a,b). Comparing Figure 4 with Figure 3, it seems that dynamic light scattering particle size analyzer was an effective measure to characterize particle size and size distribution.

Effect of polymerization parameters on particle size and size distribution

Amount of stabilizer

Figure 5 listed the particle size (number-average diameter) and polydispersity index (PDI) of hydrogel microspheres prepared upon concentration of PVP, and other reaction conditions stand showed at basic recipe. As the concentration of PVP increased from 2 to 5 wt %, particle size decreased from 3.25 ± 0.24 to 1.79 ± 0.17 μm as well as PDI linearly increased from 1.03 ± 0.008 to 1.17 ± 0.021. In dispersion polymerization, the particle size is determined by the number of nuclei generated in the early stage of polymerization. The nuclei are generated from the precipitation of oligomeric species whose molecular weight exceeds the critical solubility limit in reaction medium, and subsequently grown into primary particle by absorbing oligomers and monomers from medium.[22,23] With respect to polydispersity of particle, it is closely related to the nucleation period, the shorter the nucleation period is, the narrower the size distribution is. At a high

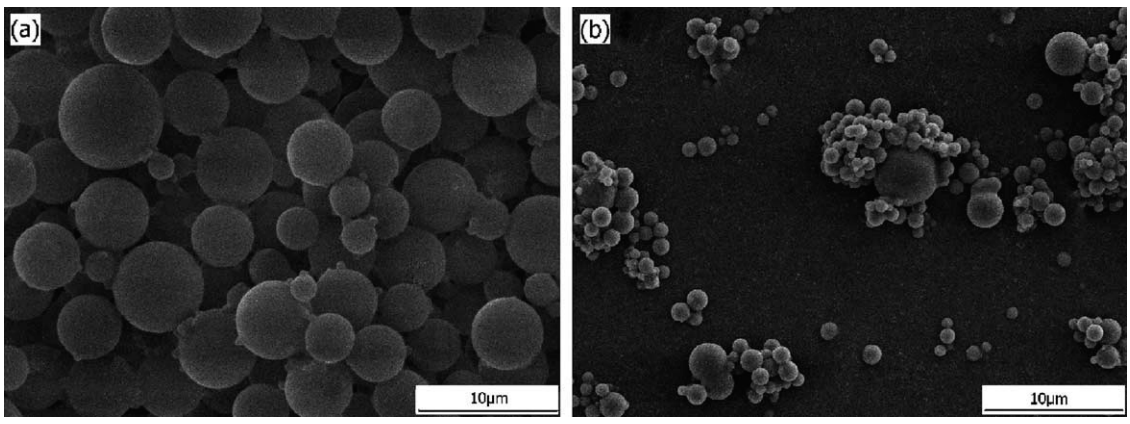

Figure 3 SEM images of freeze-dried microsperes prepared with PVP concentration of 2 wt % (a) and 5 wt % (b) (relative to total mass of monomers).

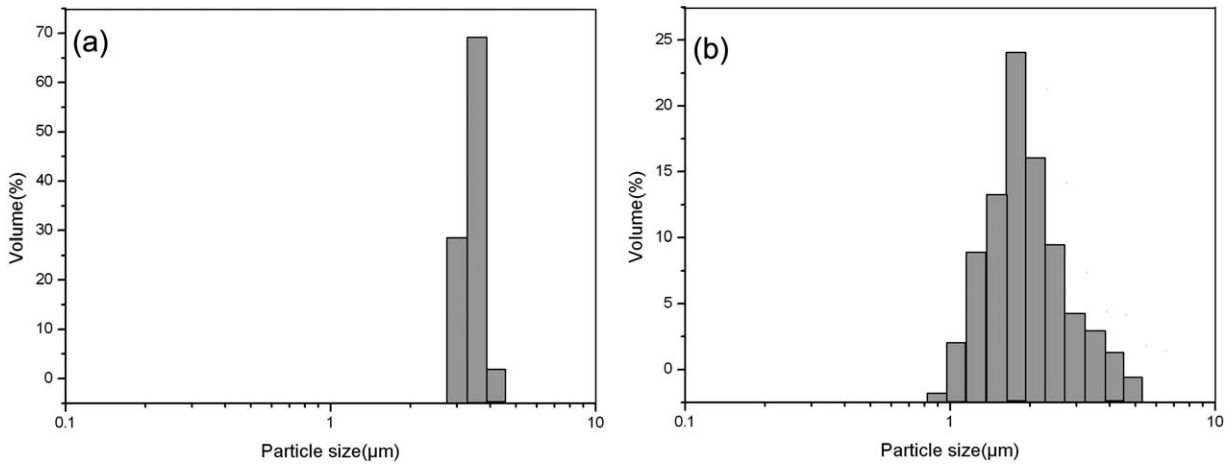

Figure 4 Particle size distribution of microspheres prepared with PVP concentration of 2 wt % (a) and 5 wt % (b) (relative to total mass of monomers).

concentration of PVP, a greater number of unclei was formed, resulting in the smaller particle size and broader size distribution due to secondary nucleation. When no PVP was employed in polymerization, huge particle was obtained with size of about 12 μm and poor polydispersity index. In our system, CS solution was not only working as reaction medium but also functioning as same as PVP solution as stabilizer ingredient. CS is cationic polysaccharide surfactant, and can be used as cocostabilizer to protect particles from coagulation in polymerization system.[24,25] However, CS could not completely substitute PVP, because it was difficult for CS to cater to the particle of large surface area (i.e., small particle size), but the larger particle. In the conventional dispersion polymerization, no particle was fabricated when no stabilizer was used.

Amount of initiator

As seen in Figure 6, the particle size increased from 0.95 ± 0.09 to 6.33 ± 0.19 μm, while PDI varied from 1.02 ± 0.024 to 1.44 ± 0.037 with BPO concentration from 0.5 to 5 wt % (no changes on other conditions at basic recipe). The formation of large particle at a high BPO concentration corresponded to the reduced amount of nuclei in the very early stage of the dispersion polymerization. The increased amount of initiator produced the greater number of oligomeric species due to higher concentration of active radicals, which resultted a low molecular weight or a short polymer chain dissolving in the reaction medium. Consequently, a smaller amount of oligomers were precipitated to generate the decreased number of nuclei. Otherwise, the small number of nuclei adsorbed dissociative oligomers of a high concentration dissolving in medium, which led to a prolonged

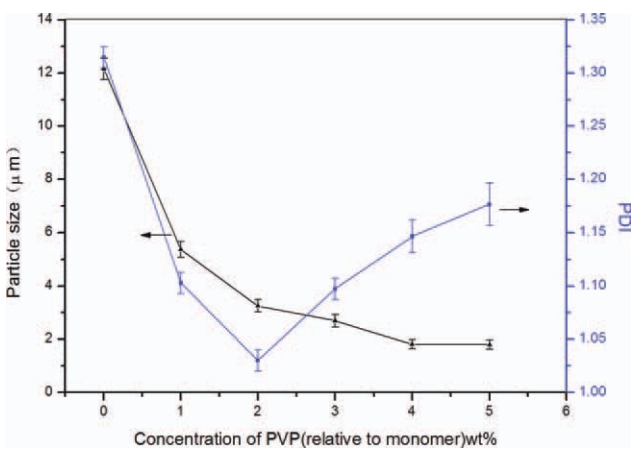

Figure 5 Effect of amount of PVP on particle size and size distribution ($n = 4$, $P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

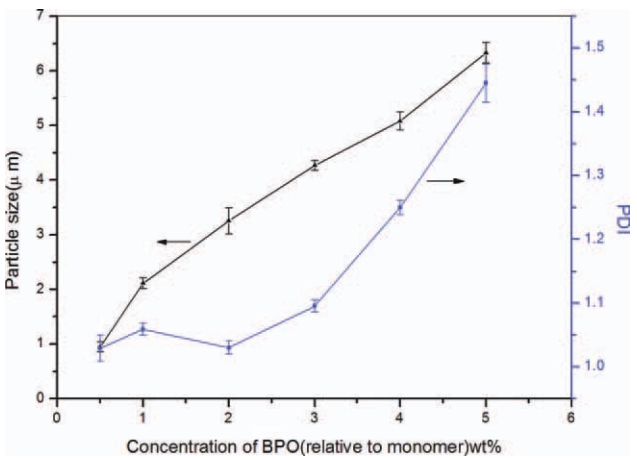

Figure 6 Effect of amount of BPO on particle size and size distribution ($n = 4$, $P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

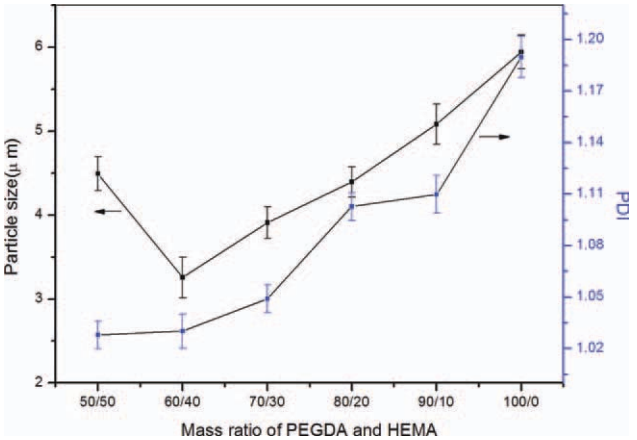

Figure 7 Effect of mass ratio of monomers on particle size and size distribution ($n = 4$, $P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nucleation period, therefore, the particle size distribution became broader. Moreover, lager particles lacked stabilizer or oligomer adsorbing capability due to the small surface area of particles, which also made the polydispersity index increase.

Mass ratios of monomers

Particle size and size distribution with respect to the mass ratio of PEG-DA/HEMA from 50/50 to 100/0 (w/w) were shown in Figure 7, without any differenerce from other conditions at basic recipe. The particle size was found to increase from 3.25 ± 0.24 to 5.94 ± 0.20 μm as the PEG-DA content increases from 60 to 100%. In typical dispersion polymerization, if a comonomer (like HEMA in this article) is more hydrophilic than another, with the increase of the comonomer content, the length of critical oligomeric chain increase due to the oligomer owning a higher solubility in the reaction medium, which generates the decreased number of nuclei, and the particle size increase as effect of BPO concentration on size. Obviously, the typical dispersion polymerization was hard to explain what showed in Figure 7, excepted from 50 to 60%. In our system, PEG-DA, was working as a macromonomer, also a self-crosslinking agent with two acrylate groups. At a higher PEG-DA concentration, PEG-DA/HEMA copolymer chains (oligomers) were crosslinked by PEG-DA before the nuclei generated from the precipitation of oligomers, which relatively increased the length of critical oligomeric chain, thus, larger particles were fabricated from the precipitation of oligomers. Quite a few PEG-DA on the surface of primary particle were working as crosslinking agent, resulting in aggregation of primary particle, which make particle size distribution deteriorated from 1.03 ± 0.008 to 1.19 ± 0.012 with the increase of PEG-DA content.

Protein loading

Particle size and polydispersity index

A series of hydrogel microspheres with varying particle size and size distribution, which remained the same composition of polymer (mass ratio of PEG-DA 60/HEMA 40), smooth surface and spherical form, were employed to load BSA were studied. Figure 8 represented the particle size and size distribution dependent on the amount of loaded BSA. As particle size increased from 0.35 to 12.15 μm with an ruleless change of polydispersity index, the amount of loaded BSA correspondingly decreased from 159.59 ± 4.83 to 38.58 ± 2.75 mg/g. The reason for this variation in the amount of loaded BSA was attributed to the continuously decrease of surface area; however, such a variation is almost independent of size distribution even though the mean particle size was the same.

Mass ratio of monomers

As depicted in Figure 9, various mass ratioes of monomers for preparing hydrogel microspheres led to a variation in the amount of loaded BSA. The amount of loaded BSA and swelling ratio(pH 4.9, 25°C) decreased from 122.15 ± 2.43 to 58.38 ± 2.65 mg/g and 315.73 ± 10.63 to 105.66 ± 6.93% with PEG-DA content from 50 to 100%. The result about the amount of loaded BSA was forced by rhree powers as follow. First, higher content of hydroxyl groups linked in copolymer from higher HEMA content (lower PEG-DA content), maked more BSA encapsulated within the particle due to the hydrogen bond between hydroxyl groups and BSA molecule. Second, as illustrated in Figure 7, the increase in PEG-DA content led to an increase in particle size,

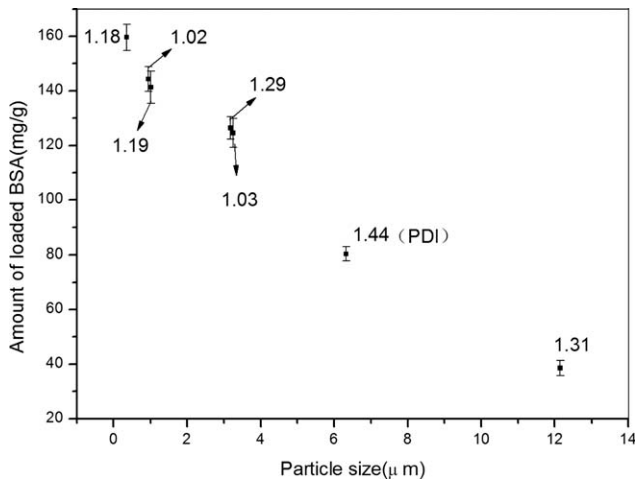

Figure 8 Effect of particle size and polydispersity index on amount of loaded BSA (the numbers near spots represent PDI, $n = 4$, $P < 0.05$).

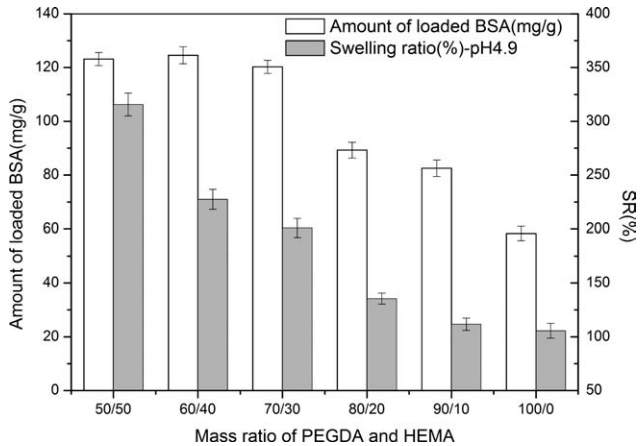

Figure 9 Effect of mass ratio of monomers on amount of loaded BSA and swelling ratio ($n = 4$, $P < 0.05$).

i.e., a decrease in total surface area of particle, which formed a smaller amount of loaded BSA. Third, in Figure 8, more PEG-DA resulted in higher swelling ratio, which was favorable motivation for microspheres to contain more BSA in their polymer networld space within microspheres.

Released ratio

Particle size and polydispersity index

Hydrogel microspheres in Figure 7 were collected, freeze-dired, and employed to study for release. Figure 10 represented the particle size and size distribution of microspheres dependent on released ratio of loaded BSA. As particle size increased, the released ratio increased to 70.11 ± 3.02%. It could be explained that larger particle with lower total surface area, had a growing tendency for releasing larger amount of BAS held in particle, which has been verified in quite a few of studies. In Figure 8, it was known that the amount of loaded BSA was almost independent of size distribution even though the particle size was the same. What about released ratio of loaded BSA? Two varying polydispersity indexs of particle, with almost the same particle size, like about 1.0 μm or 3.2 μm in size shown in Figure 10, owning different polydispersity indexs, 1.17 and 1.02, and 1.29 and 1.03, it could be observed that released ratio decreased with the increase in polydispersity index. Briefly, released ratio was not only determined by the particle size, but also determined by polydispersity index when the particle size was the same.

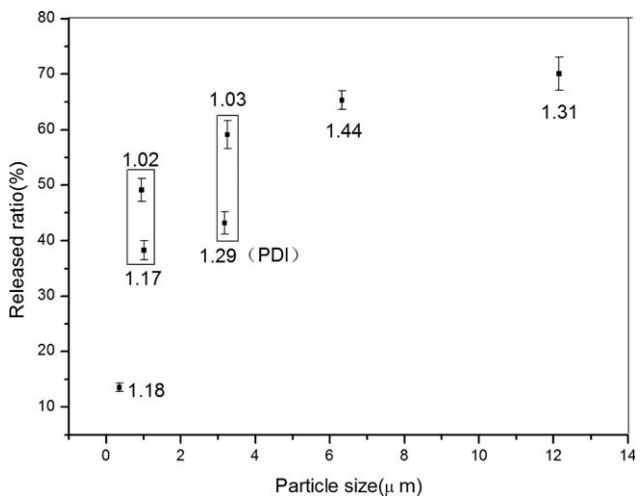

Figure 10 Effect of particle size and polydispersity index on released ratio (the numbers near spots represent PDI, $n = 4$, $P < 0.05$).

Mass ratio of monomers

Hydrogel microspheres in Figure 8 were collected, freeze-dired, and employed to study for release.The effect of mass ratio of monomers for preparing hydrogel microspheres on released ratio and swelling ratio (pH 7.4, 37°C) was summarized in Figure 11. with an increase in PEG-DA content from 50 to 100%, released ratio of loaded BSA unsteadily increased from 55.24 ± 2.07 to 69.02 ± 2.92% and swelling ratio decreased from 212.71 ± 5.54 to 80.60 ± 2.43%. Comparing with Figure 9 (pH 4.9), at the same composition, swelling ratio in Figure 11 (pH 7.4) was much smaller. In neutral environment, —OH was uncharged, which make space among polymer chains of hydrogel microspheres shrink through hydrogen bonds, and led to a small swelling ratio. The reason for this variation in the released ratio of loaded BSA was attributed to four factors. First, as hydrogen bond between hydroxyl groups and BSA molecule is firmer than electrostatic force and hydrophobic interactioer between microsphere material and BSA molecule, low content of

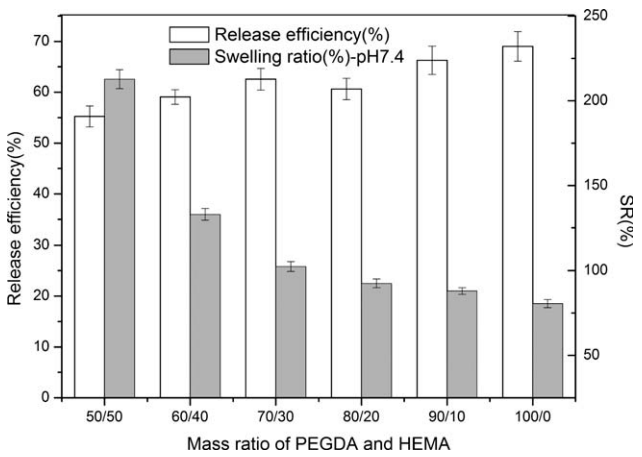

Figure 11 Effect of mass ratio of monomers on released ratio and swelling ratio ($n = 4$, $P < 0.05$).

hydroxyl groups from HEMA facilitated BSA's release from loaded particle. Second, according to Figures 7 and 10, released ratio increased in direct proportion with the increase in PEG-DA content or particle size. Third, with the combination of Figures 7 and 10, released ratio decreased with the increase in PEG-DA content or polydispersity index. Fourth, in Figure 10, more PEG-DA resulted in higher swelling ratio, which was favorable to release BSA through polymer netword space. Aforementioned four factors working together resulted in an unsteadily increase in released ratio with an increase in PEG-DA content.

CONCLUSION

The PEG-DA/HEMA copolymer hydrogel microspheres were prepared by dispersion polymerization with the complete absence of any organic solvents. In our system, CS solution was not only working as reaction medium but also functioning as same as PVP solution as stabilizer ingredient. Then, the effects of mass ratios of monomers, concentration of stabilizer and benzoy peroxide on the size and polydispersity index (PDI) of microspheres were investigated. The results showed that the particle size and polydispersity index increased with BPO content and PEG-DA content of monomers; the lower PVP content down to 0%, the larger the particles were obtained, while the particle size distribution was the best at the PVP content of 2%. BSA as the protein model, the amount and released ratio of loaded BSA were affected by the particle size and mass ratio of monomers: as particle size and PEG-DA content increased, the amount of loaded BSA correspondingly decreased and released ratio of loaded BSA increased, respectively. Interestingly, the amount of loaded BSA was almost independent of size distribution when the mean particle size was the same, while released ratio decreased with the increase in polydispersity index. These data suggested that PEG-DA/HEMA copolymer hydrogel mcicrospheres could be useful in drug-release system and tissue engineering.

References

1. Wang, K.; Zhi, F. S.; Gu, Y. C.; Xu, X.; Dong, P. W.; Guo, G.; Zhao, X.; Wei, Y. Q.; Qian, Z. Y. Polym Degrad Stab 2009, 94, 730.
2. Wang, K.; Xu, X.; Wang, Y. J.; Guo, G.; Huang, M. J.; Luo, F.; Zhao, X.; Wei, Y. Q.; Qian, Z. Y. Int J Pharm 2010, 389, 130.
3. Zhang, X. Z.; Chu, C. C. Colloid Polym Sci 2008, 284, 1415.
4. Mehrad, H.; Amir, A.; Padram, R. Adv Drug Rev 2008, 60, 1639.
5. Tan, G. X.; Wang, Y. J.; Li, J.; Zhang, S. J. Polym Bull 2008, 61, 91.
6. Tasdelen, B.; Kayaman-Apohan, N.; Zülal, M.; Olgun, G. J Appl Polym Sci 2005, 97, 1115.
7. Wei, X. W.; Gong, C. Y.; Gou, M. L.; Fu, S. Z.; Guo, Q. F.; Shi, S.; Luo, F.; Guo, G.; Qiu, L. Y.; Qian, Z. Y. Int J Pharm 2009, 381, 2.
8. Wu, Y. H.; Park, B. H.; Kai, T.; Freeman, B. D.; Kalika, D. S. J Membr Sci 2010, 347, 197.
9. Lilian, E. V.; Tushar, K. V.; Mansoor, M. A. Pharm Res 2007, 24, 1406.
10. Zhu, H. G.; Michael, J. M. Chem Commun 2006, 153.
11. Xu, X.; Fu, S. Z.; Wang, K.; Jia, W. J.; Guo, K.; Zheng, X. L.; Dong, P. W.; Guo, Q. F.; Qian, Z. Y. J Microencapsulation 2009, 26, 7, 642.
12. Ram, M. K.; Prithiviraj, C.; Tang, L. P.; Kytai, T. N. Biotechnol Prog 2006, 22, 118.
13. Smoak, E. M.; Henricus, M. M.; Banerjee, I. A. J Appl Polym Sci 2010, 115, 2562.
14. Freiberg, S.; Zhu, X. X. Int J Pharm 2004, 282, 3.
15. Yamamoto, T.; Sugimoto, T.; Suzuki, T.; Mukai, S. R.; Tamon, H. Carbon 2002, 40, 1345.
16. Shi, M.; Yang, Y. Y.; Chaw, C. S.; Goh, S. H.; Moochhala, S. M.; Ng, S.; Heller, J. J Control Release 2003, 89, 167.
17. Shen, R.; AKIyama, C.; Senyo, T.; Ito, K. C R Chim 2003, 6, 1329.
18. Sah, H. J Pharm Sci 1999, 88, 1321.
19. An, S. Y.; Bui, M. N.; Nam, Y. J. J Colloid Interface Sci 2009, 311, 99.
20. Sheena, A.; Sean, B.; Kazuhiko, I.; Anthony, G. E. Biomaterials 2005, 26, 4767.
21. Van, L. K.; Snauwaert, J.; Peumans, M.; De, M. J.; Lambrechts, P.; Van, M. B. Dent Mater 2008, 24, 1412.
22. Kim, O. H.; Lee, K.; Kim, K.; Lee, B. H.; Choe, S. Polym 2006, 47, 1959.
23. Lee, J.; Ha, J. U.; Choe, S.; Lee, C. S.; Shim, S. E. J Colloid Interface Sci 2006, 298, 665.
24. Pepic, I.; Filipivic-Grcic, J.; Jalsenjak, I. Colloids Surf A: Physicochem Eng Aspects 2009, 336, 135.
25. Elsabee, M. Z.; Morsi, R. E.; Al-Sabagh, A. M. Colloids Surf B: Biointerfaces 2009, 74, 1.