Biodegradable Polymer-Based Composite Particles for Targeted Drug Delivery Carriers with Magnetic Response

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ABSTRACT

Core–shell composite particles with biodegradability and magnetism were prepared using a Pickering emulsion for targeted drug delivery based on magnetic guidance. The composite particles were composed of a core of biodegradable polymer and a shell of assembled magnetic iron oxide nanoparticles. It was found that the dispersibility of the nanoparticles is crucial for controlling the core–shell structure, and the addition of a small amount of dispersant facilitated the formation of composite particles with a thin magnetic shell covering a polymeric core. A model drug with fluorescent capability was loaded into the biodegradable core of the composite particles, and was released via hydrolysis of the core under strong alkaline conditions. Because the core can also be biodegraded also by lipase, this result suggests that the slow release of the drug from the composite particles should occur inside the body..

Keywords: drug delivery, drug carrier, iron oxide nanoparticle, biodegradable polymer, polyhydroxyalkanoate

1 INTRODUCTION

Drug delivery carriers are required to achieve pinpoint precision of targeting to avoid side effects and controlled release of drugs to maximize their effect. Magnetic drug carriers composed of magnetic nanoparticles can be delivered to the required area through the use of external magnets [1]. However, drug release from these carriers is often not controlled because it occurs even in a neutral buffer solution. In addition, some carriers are composed of substances that have the potential to be retained inside the body. Therefore, there is a strong need for magnetic drug carriers that enable controlled drug release and are passed from the body after treatment. Polyhydroxyalkanoates (PHAs) are biopolymers with high biocompatibility and biodegradability, and PHA particles that are decomposed by specific proteins have been studied as drug carriers for sustained drug release applications [2,3]. Furthermore, some researchers have reported that the degradation of PHA rarely occurs in the absence of degrading proteins, unlike other biodegradable polymers such as poly(lactic acid) (PLA). For example, the molecular weight of PHA did not change at 37 °C in a pH 7.4 phosphate buffer for a period of more than 84 days [4]. Therefore, PHA particles have the potential to serve as drug carriers that can release drugs only inside the body, because drug release from such drug-loaded PHA particles cannot happen without the decomposition of PHA. Thus, we designed and fabricated a core-shell composite particle composed of a core of the biodegradable polymer and a shell of assembled magnetic iron oxide nanoparticles, which not only have high biocompatibility but are also biodegradable (Fig. 1). The magnetic nanoparticles not only realize magnetic guidance of drug but also facilitate a second drug release mechanism via the melting of the PHA particles using the heat generated when exposed to an applied high frequency magnetic field. Therefore, the composite structure should lead to a magnetic drug carrier possessing two types of drug release mechanism: sustained release by biodegradation and rapid release by melting of the PHA particles.

2 EXPERIMENTAL PROCEDURES

2.1 Composite particles of PHA and iron oxide

Iron oxide nanoparticles (either Fe3O4 or γ-Fe2O3) were synthesized via a co-precipitation of Fe^{2+} and Fe^{3+} aqueous salt solutions using an alkaline NaOH solution according to the method reported by Nedkov et al. [5]. Briefly, an aqueous mixture of Fe^{2+} and Fe^{3+} chlorides was mixed with an aqueous solution of NaOH at 80 °C. The precipitated nanoparticles were separated from the solution by centrifuging and then washed with water. Finally, the nanoparticles were redispersed in water via sonication.

Core–shell composite particles consisting of PHA particles and iron oxide nanoparticles were prepared using a modified emulsification solvent diffusion method that involved the formation of a Pickering emulsion [6,7]. In

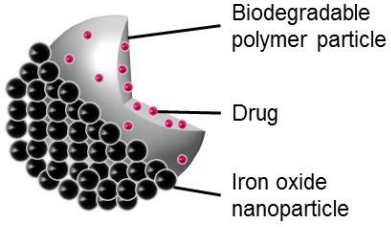


Figure 1: Schematic illustration of composite particles of iron-oxide nanoparticle-assembly shell/PHA core with drug.

this method, a surfactant is used as a stabilizer for the emulsion, and it is thought that this surfactant remains on the surfaces of the resulting particles. Here we used the ironoxide nanoparticles as a surfactant. PHB-co-HV as a PHA polymer was added to dichloromethane (1 mL), and the mixture was stirred to ensure that all of the PHB-co-HV dissolved. Separately, an iron oxide suspension was prepared by adding the above iron oxide suspension to a PVP solution. The PVP acts as a dispersant for the iron oxide nanoparticles. To form the emulsions, each suspension was sonicated, and then the PHB-co-HV dichloromethane solution was rapidly added. After sonication, the mixture was gently stirred for 3 h at room temperature. The resultant composite particles were collected by centrifugation, and then washed with water.

The morphological properties of the prepared particles were determined via scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The crystallographic properties of the iron oxide nanoparticles were analyzed using an X-ray diffractometer with Cu Kα radiation (λ = 0.1542 nm). The magnetic properties were measured using a Physical Property Measurement System (Quantum Design, PPMS).

2.2 Loading and release of model drug

Loadong and release of model drug were evaluated in the following way. We used pyrene with fluorescent capability as a model drug. Pyrene-loaded composite particles were prepared by adding pyrene to the PHA/dichloromethane solution used in the above method for preparation of the composite particles. Except for the addition of pyrene, the particles were prepared as described above. The presence of the loaded pyrene was confirmed by excimer fluorescence analysis of the particles after exposing them to UV rays.

PHA degrades in aqueous acidic or alkaline media [8]. Hence, in this study, the release of the model drug was investigated via hydrolysis of the PHA particles using an alkaline solution. The composite particles were dispersed in an aqueous NaOH solution and then placed in a petri dish. They were subsequently collected at the center of the dish using a NdFeB magnet, and then the florescence was observed. Next the composites and NaOH solution were placed in a glass vessel and stirred for 3 days. The decomposition of the polymer was confirmed by the color change of the composite suspension from light brown to dark brown. The suspension was once again placed in a petri dish, and the brown residue was collected at the center of the dish using a magnet. The release of pyrene was confirmed by determining whether fluorescence could be detected at the center of the dish.

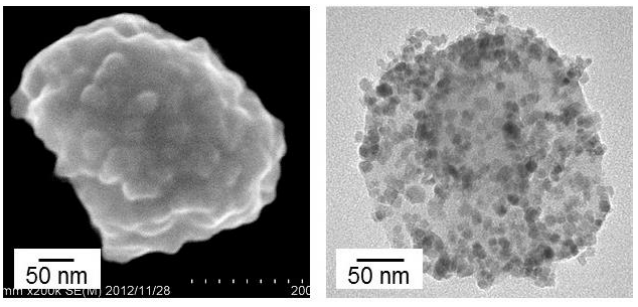
3 RESULTS & DISCUSSION

The particle size of the iron oxide nanoparticles ranged from 7 nm to 20 nm based on TEM observation. The XRD pattern indicated that the synthesized iron oxide nanoparticles had a spinel structure similar to that of magnetite (Fe_3O_4). The mean crystallite size, estimated using the Scherrer equation, was 11 nm. From these results, it was concluded that the nanoparticles were single crystals

PHA and iron oxide composite particles were then fabricated using a Pickering emulsion stabilized by the iron oxide nanoparticles. Since the iron oxide nanoparticles work as a stabilizer of the emulsion and finally cover a single biodegradable particle, dispersibility and surface properties of the nanoparticles strongly influence the structure of the composite particles. Thus, the concentration of PVP, which works as a dispersant agent of the iron oxide naoparticles, is a key parameter. Three types of composite particles were fabricated using three different iron oxide nanoparticle suspensions prepared in PVP aqueous solutions with concentrations of 0, 0.5, and 3 wt.%. When the concentration was 0.5 %, composite particles with a monolayer-assembly of the iron oxide nanoparticles were observed to be approximately 200 nm in size as shown in Fig. 2. A model drug was incorporated in the composite particles seen in Fig. 2. The SEM image (Fig. 2(a)) shows that small particles cover the core particles; the size of the small particles is larger than that of the iron oxide naoparticles. Because an approximately 5-nm-thick osmium conductive metal coating was applied to the composite particles for the SEM observation. The TEM image (Fig. 2(b)) also suggests that iron oxide nanoparticles uniformly covers a core particle. Thus, core-shell composite particles with a monolayer-assembly of iron oxide nanoparticles of approximately 200 nm in size were obtained with the use of the 0.5 wt.% PVP solution. At this PVP concentration, the iron oxide nanoparticles are slightly coated by PVP and adsorb at the oil–water interface but remain dispersed.

When the 0 wt.% PVP suspension was used, the size of the composite particles was greater than 1 μm, and clusters of iron oxide nanoparticles were also observed on the PHA particles (not shown). The use of the 3 wt.% PVP solution induced the formation of clusters of composite particles (not shown).

Loading pyrene into the composite particles was readily achieved by simply adding pyrene to the PHA/dichloromethane solution during the composite fabrication process. The difference in the morphological



(a) (b)

Figure 2: SEM and TEM images of composite particles.

properties of the composite particles was not observed from SEM and TEM observations after loading of the pyrene. The magnetization curve for the pyrene-loaded composites was obtained, and the results are shown in Fig. 3(a, ii). The magnetization of the loaded composite particles was found to be 7 emu/g at 8 T, which is one tenth that of the pure iron oxide nanoparticles (Fig. 3(a, i)) because the composite particles include nonmagnetic PHA. Even so, the composite particles were strongly attracted to a NdFeB magnet. Figure 3(b) shows optical photographs of a suspension of the composites. The color of the initial suspension was brown, but it became transparent when exposed to a magnet because the composites were all drawn to the side of the bottle. Notably, the particles could be readily redispersed by removing the magnet and shaking the bottle.

Next, to confirm that the pyrene was in fact loaded into the prepared composite particles, a fluorescence analysis was carried out. Figure 4(a) shows an optical photograph of the composite particles collected at the center of a petri dish. In the fluorescence image presented in Fig. 4(c), the white area at the center of the dish indicates that the pyrene was loaded in the composite particles collected at the center of the dish.

Release of the pyrene from the loaded composite particles via the decomposition of PHA under strong alkaline conditions (hydrolysis) was then investigated. Figure 6(b) shows an optical photograph after exposure to an alkaline solution for 3 days. It was observed that the color of the center area in Fig. 6(a) changed from light brown to dark brown in Fig. 6(b) after 3 days. These results indicate that only the iron oxide nanoparticles remained and the PHA was completely decomposed under the alkaline conditions. The release of pyrene with decomposition of the PHA was then confirmed via fluorescence analysis. The fluorescence observed at the center of the dish (Fig. 6(c)) vanished after exposure to the alkaline conditions, as can be seen in Fig. 6(d). Figure 6(e) shows a schematic illustration of the release of pyrene due to the decomposition of PHA. If we use lipase as a decomposing agent in the body, the release due to the decomposition will last a few months, achieving sustainable release of drug.

4 CONCLUSIONS

In the present study, pyrene-loaded core–shell composite particles composed of PHA particles and assembled iron oxide nanoparticles were successfully prepared using a Pickering emulsion stabilized by the iron oxide nanoparticles. The composite structure depended on the dispersibility of the iron oxide nanoparticles, and the use of a 0.5 wt.% aqueous PVP solution led to the formation of core-shell composite particles with a thin shell composed of a monolayer of iron oxide nanoparticles. Notably, the composite particles were attracted to a NdFeB magnet. In addition, fluorescence analysis confirmed that the model drug pyrene was incorporated into the composite particles and then released upon decomposition of the PHA core. Therefore, the drug-loaded composite particles designed in the present study have potential for use in magnetically guided drug delivery systems that allow slow and sustained drug release via biodegradation.

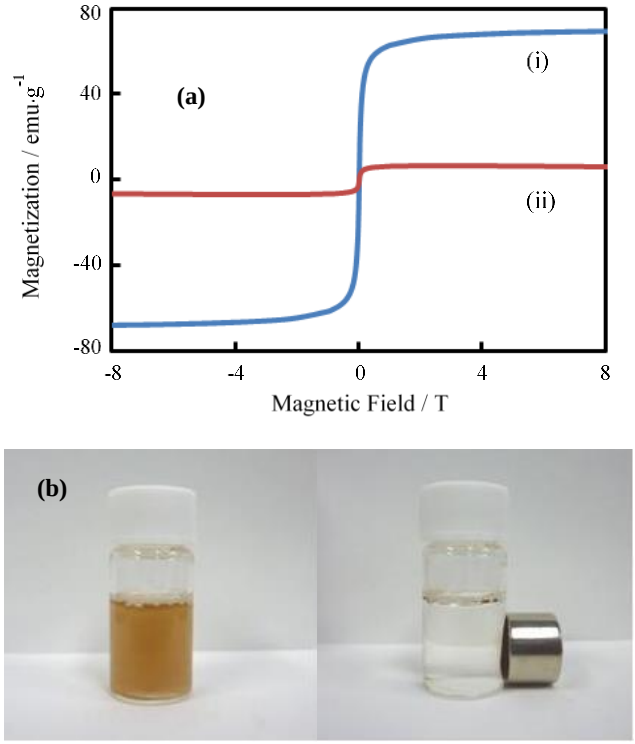


Figure 3: (a) Magnetization curves of composite particles and (b) optical photographs of their suspension before and after magnetic separation.

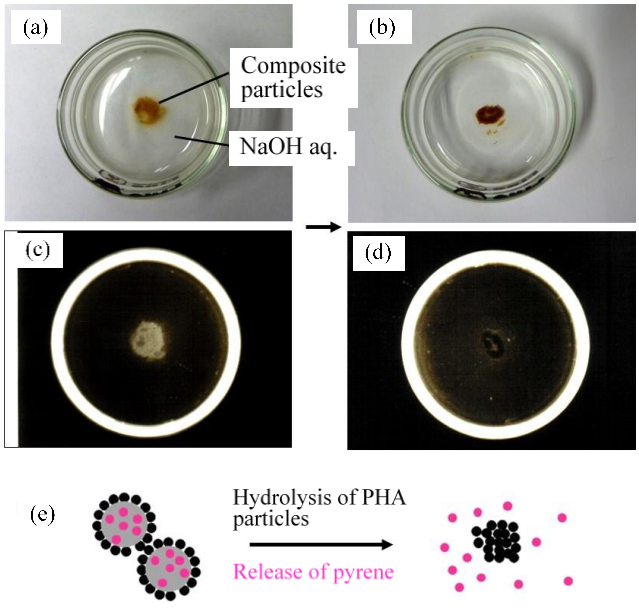


Figure 4: Optical and fluorescence photographs of pyrene-loaded composite particles (a, c) before hydrolysis of PHA, (b, d) after hydrolysis, and (e) a schematic illustration of pyrene release.

REFERENCES

[1] T. Fuchigami, R. Kawamura, Y. Kitamoto, M. Nakagawa, Y. Namiki, "A magnetically guided anti-cancer drug delivery system using porous FePt capsules," Biomaterials, 33, 1682-1687, 2012.

[2] Y.J. Hu, X. Wei, W. Zhao, Y.S. Liu, G.Q. Chen, "Biocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) with bone marrow mesenchymal stem cells," Acta Biomater, 5, 1115-1125, 2009.

[3] Y.C. Xiong, Y.C. Yao, X.Y. Zhan, G.Q. Chen, "Application of polyhydroxyalkanoates nanoparticles as intracellular sustained drug-release vectors," J Biomater Sci, 21, 127-140, 2010.

[4] M.I. Artsis, A.P. Bonartsev, A.L. Iordanskii, G.A. Bonartseva, G.E. Zaikov, "Biodegradation and medical application of microbial poly(3-hydroxybutyrate)," Mol Cryst Liq Cryst, 523, 21-49, 2010.

[5] I. Nedkov, T. Merodiiska, L. Slavov, R.E. Vandenberghe, Y. Kusano, J. Takeda, "Surface oxidation, size and shape of nano-sized magnetite obtained by co-precipitation," J Magn Magn Mater, 300, 358-367, 2006.

[6] S. Sacanna, W.K. Kegel, A.P. Philipse, "Thermodynamically stable pickering emulsions," Phys Rev Lett, 98, 158301, 2007.

[7] W.H. Binder, "Supramolecular assembly of nanoparticles at liquid-liquid interfaces," Angew Chem Int Ed, 44, 5172-5175, 2005.

[8] E.I. Shishatskaya, T.G. Volova, S.A. Gordeev, A.P. Puzyr, "Degradation of P(3HB) and P(3HB-co-3HV) in biological media," J Biomater Sci Polym Ed, 16, 643-657, 2005.