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# Drug Release of Sonochemical Protein Containers

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A new protein container was prepared by introducing gelation into sonochemically prepared protein microsphere. The drug release behaviors of the new container and the previous protein microsphere were first investigated in this paper.

To be effective in treating cancer, chemotherapeutic agents should be presented within the affected tissues at high concentration for a sustained period of time so that it may be taken up by the cancer cells. Obviously, water-soluble molecules can be administrated in this way by continuous infusion and monitoring. For hydrophobic drugs, such as paclitaxel, direct injection may be impossible or highly dangerous and can result in hemolysis, phlebitis, hypersensitivity, organ failure, and death.[1] Some delivery vehicles have to be used to transport them to the therapeutic target. An effective method to deliver hydrophobic drugs is in the form of a dispersion within oil droplets in an emulsion system.[2] The oily droplets can be coated in such a way that they do not interact with plasma protein and are not trapped by the reticuloendothelial system (RES), instead remaining intact in the tissue or blood for hours, days, or even weeks. Since emulsions are thermodynamically unstable, surfactants and co-surfactants are usually used to stabilize the oil droplets, which induce a potential toxicity.[3] Suslick and co-workers have developed a smart and efficient way to produce stable droplets without using surfactants.[4,5] They used a high power ultrasonic device to sonicate oil in an aqueous protein solution, directly forming stable droplets composed of an outer protein shell and an inner oil core.[6] The prepared microspheres are nano- to micro-sized and biocompatible. They are stable at room temperature for several months.

Even though protein microspheres have many advantages as drug carriers, their development in drug delivery is limited. The drug release behavior of protein microspheres has not been reported so far. This is one issue we want to address in this paper. Additionally, we introduce gelation into the protein microsphere to design a new container which has an outer protein shell and an inner gel core. The drug release behavior of the new container is studied and compared with previous protein microspheres. The influence of gel core on the drug release is highlighted.

The preparation of protein microsphere is described in previous publications.[7,8] The new container with gel core is prepared in a similar way. The difference lies in the dissolving of 1.3 wt % HSA gelator (12-hydroxystearic acid) in the silicon oil phase before sonication. After sonication, the HSA gelator is encapsulated in the oil core. When the sample is cooled down from the sonication temperature 45 °C to room temperature about 25 °C, the gelator transforms the oil core to a gel core. The gel core formation is thermally reversible. Increasing temperature to transition temperature leads to the transformation of gel core to liquid core. The transition temperature is dependent on the concentration of HSA gelator. In our study, the 13 wt % concentration corresponds to the transition temperature 40 °C. The new containers are stable at 37 °C for weeks without ripening and accumulation, indicating their possible applications in vivo.

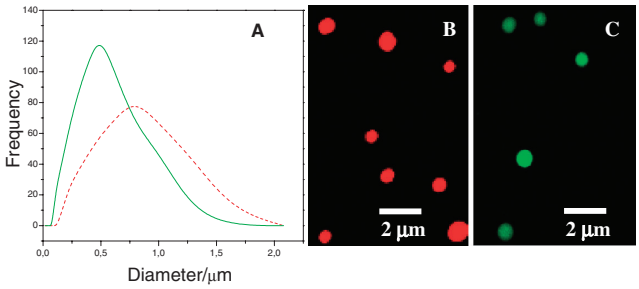


**Figure 1.** Size distribution of the protein microspheres (dash line) and the new containers (solid line) (A), together with the fluorescence confocal micrographs of the protein microspheres loaded with red rhodamine B dye (B) and the new containers loaded with green coumarine 6 dye (C).

The size of the prepared protein microspheres and the new container are measured by dynamic light scattering (DLS), as shown in Figure 1A. Both of them show a rough Gaussian distribution in size; the protein microspheres have an average diameter of approximately 1 μm while the new containers present an average diameter of 0.6 μm. The reduction of the size probably results from the addition of gelator, which leads to the increase of oil viscosity. The high viscosity creates a higher barrier for the coalescence of primary droplets. Therefore, a lot of primary droplets are preserved in the suspension without ripening process, forming small containers. The size reduction brings advantages for biological application. For example, the small containers can help to prevent uptake by the mononuclear phagocyte system (MPS). Confocal images of the prepared containers are shown in Figure 1B and Figure 1C. Figure 1B shows protein microspheres loaded with a red dye (rhodamine B) while Figure 1C shows the new containers loaded with a green dye (coumarin 6). The dyes can be stably carried in both containers as long as the containers are stable.

Rifampicin,[9] a semisynthetic antibiotic widely used for the chemotherapy of tuberculosis, was chosen as a model drug. It was loaded in both protein microspheres and the new containers and released into a drug-free phosphate buffer solution (PBS) at pH 7.4 through a dialysis bag (13500 MW cut-off) which was immersed in a drug-free PBS solution and stirred at 100 rpm.[10] The release as a function of time was analyzed by UV–vis measurements.[11] Figure 2A shows the drug release profile from the protein microspheres, together with a control experiment which was conducted by putting the same amount of drug directly into the dialysis bag and releasing them under the same

  

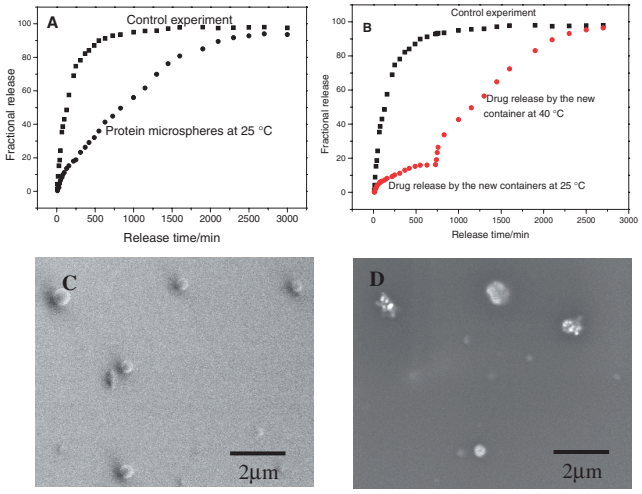


**Figure 2.** Drug release profile of the protein microspheres at 25 °C (A) and the new containers at 25 °C and 40 °C (B). The control experiments are conducted by directly putting the same amount of drug into the dialysis bag and releasing them under the same condition. SEM images of the new containers before drug release (C) and after drug release (D).

condition. The drug loaded in the protein microsphere is released slowly compared with the control experiment. Only 20 wt % of drug is released from the protein microsphere during the first 240 min while more than 80 wt % of drug is released in the control experiment at the same time. Hence, the protein microsphere can be used for sustained release of drugs. After release, we find that most protein microspheres are broken, which is attributed to the shear force by stirring. The big microspheres should be first broken, then the middle microspheres, and finally the small microspheres, resulting in a sustained release of drug. At the same time, we cannot exclude the diffusion mechanism since the drug molecules can penetrate through the oil interface and protein shell. The drug release profile of the new containers is shown in Figure 2B. When the release test is conducted at 25 °C, the loaded drug is released slowly in the beginning. This release is ascribed to the diffusion of the drug located in the outer region of the gel core. The release reaches equilibrium after 300 min and only 16 wt % of drug is released. The remaining drugs cannot be further released at 25 °C due to protection by the gel core. To release the remaining drug, we increase the temperature of buffer solution to 40 °C turning the gel core to the oil core. The remaining drug is completely released at 40 °C, as shown in Figure 2B. Figure 2C shows a SEM image of the new containers before drug release. They have smooth surface and stand on the wafer glass. After drug release, the quantity of the containers in the dialysis bag is dramatically reduced and the remaining containers become smaller and irregular in shape, as shown in the Figure 2D, which indicates a degradation of the containers. The degradation should play an important role for the drug release of the new container at increased temperature. Since the new containers are expected to specifically target and internalize into the disease cells and the degradation should take place inside the cells, the possible cytotoxicity of HSA gelators is limited to the disease cells.

In conclusion, the drug release of protein microspheres prepared by sonication of oil in a protein solution was first investigated in this paper. Additionally, a new container composed of an inner gel core and an outer protein shell was developed by introducing gelation into the protein microspheres. The new container presents a temperature responsive controlled release of drugs. It is expected to develop a multifunctional delivery system on the basis of the new container by incorporating nanoparticles into the gel core[12,13] and coating targeting ligands on the surface of containers.[14,15]

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## References and notes

1 K. J. Lambert, P. P. Constantinides, S. C. Quary, U.S. Patent 6 667 048 B1, **2003**.
2 J. A. Hanson, C. B. Chang, S. M. Graves, Z. B. Li, T. G. Mason, T. J. Deming, *Nature* **2008**, *455*, 85.
3 Z. Pavlić, Z. Vidaković-Cifrek, D. Puntarić, *Chemosphere* **2005**, *61*, 1061.
4 K. S. Suslick, M. W. Grinstaff, *J. Am. Chem. Soc.* **1990**, *112*, 7807.
5 S. Avivi, A. Gedanken, *Ultrason. Sonochem.* **2007**, *14*, 1.
6 F. J. Toublan, S. Boppart, K. S. Suslick, *J. Am. Chem. Soc.* **2006**, *128*, 3472.
7 Y. Han, D. Radziuk, D. Shchukin, H. Möhwald, *Macromol. Rapid Commun.* **2008**, *29*, 1203.
8 Y. Han, D. Radziuk, D. Shchukin, H. Möhwald, *J. Mater. Chem.* **2008**, *18*, 5162.
9 H. Ogata, M. Kubo, K. Tamaki, H. Hirakata, S. Okuda, M. Fujishima, *Nephron* **1998**, *78*, 319.
10 X. Teng, D. G. Shchukin, H. Möhwald, *Langmuir* **2008**, *24*, 383.
11 C. Nadejde, D. E. Creanga, I. Humelnicu, E. Filip, D. O. Dorohoi, *J. Mol. Liq.* **2009**, *150*, 51.
12 A. G. Skirtach, P. Karageorgiev, M. F. Bedard, G. B. Sukhorukov, H. Möhwald, *J. Am. Chem. Soc.* **2008**, *130*, 11572.
13 G. Wu, A. Mikhailovsky, H. A. Khant, C. Fu, W. Chiu, J. A. Zasadzinski, *J. Am. Chem. Soc.* **2008**, *130*, 8175.
14 H. Fuchs, C. Bachran, *Curr. Drug Targets* **2009**, *10*, 89.
15 H. Fuchs, D. Bachran, H. Panjideh, N. Schellmann, A. Weng, M. F. Melzig, M. Sutherland, C. Bachran, *Curr. Drug Targets* **2009**, *10*, 140.

  