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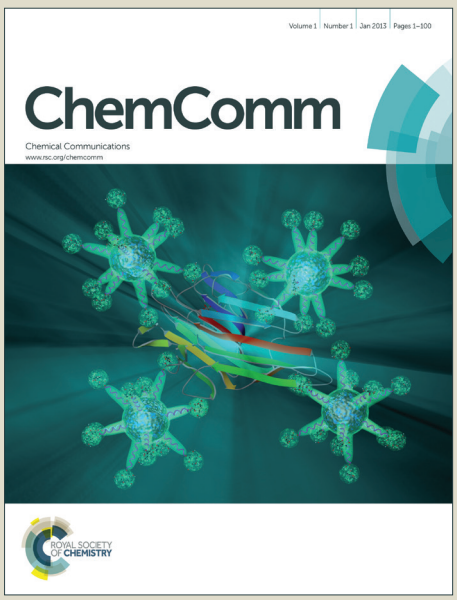

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Facile Fabrication of Diphenylalanine Peptide Hollow Spheres Using Ultrasound-assisted Emulsion Templates

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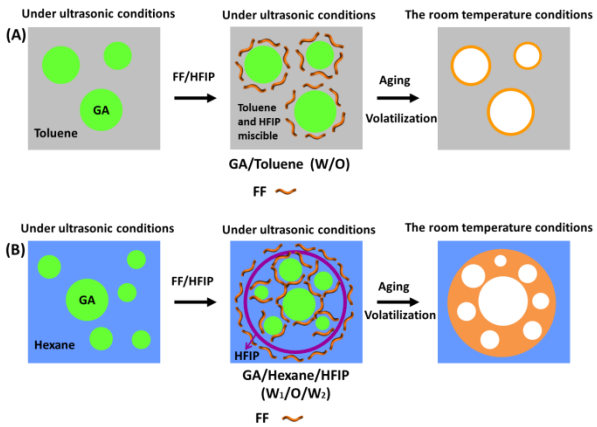
Controlled self-assembly of diphenylalanine (FF) into unilocular and multilocular hollow spheres were successfully achieved by ultrasound-assisted emulsion droplet template method. This novel surfactant-free emulsion droplet template method is envisaged to be applicable to other biomolecules and materials.

Designed self-assembly of building blocks into supramolecular structures is a powerful method to prepare novel functional nanomaterials.[1] Up to now, a great many of nano/micro-structures with one dimensional (1D), two dimensional (2D) and three dimensional (3D) structures possessing diverse functions have been synthesized using this method.[2] Among them, hollow spheres have attracted widely attention due to their excellent properties such as large surface area, low density and well-defined morphology.[3] Various strategies such as layer-by-layer assembly, solvothermal and template-induced self-assembly have been employed to synthesize functional hollow spheres.[4] Recently, the emulsion soft template has been widely used to prepare hollow structures due to its environment-friendly property and its convenience to use.[5] Thus a great many of hollow structures with versatile functions have been fabricated through this method and applied in drug carriers, catalysis and sensors.[6]

Diphenylalanine peptide (FF), the key recognization motif of Alzheimer's disease's polypeptide, is an excellent candidate to prepare bio-functional nanomaterials.[7] To date, a great deal of ordered assemblies including nanotubes, nanorods, nanobelts, nanofibers and nanoflowers have been synthesized by self-assembly of FF and its derivatives.[8] Recently, researchers have been attempting to design experiments for controlling the self-assembly of FF-based peptides to prepare novel bio-functional materials and smart devices. For example, by diluting and concentrating the concentration of a cationic diphenylalanine peptide in aqueous solution, reversible structure transition between nanotubes and vesicles has been observed.[9] Besides, our group found that sulfonic-azobenzene with tiny differences in structure could manipulate the cationic diphenylalanine peptide to assemble into urchin-like, flower-like and plate-like structures.[10] However, it still remains a great challenge to develop new strategies for controlling the self-assembly of FF-based peptide to obtain supramolecular assemblies with novel structures and functions.



Scheme 1. Illustration of formation of hollow spheres.

Herein, we have successfully achieved controlled self-assembly of FF into unilocular and multilocular hollow spheres by using emulsion droplet as template and glutaraldehyde (GA) as crosslinking agent. Ultrasound and different miscibility of HFIP with toluene and hexane were ingeniously exploited to prepare different types of emulsion droplet templates, avoiding the addition of extra surfactant. We hope our approach could help to construct new strategies to prepare novel bio-functional materials and shed light on designing and expanding peptide-based assembly structures.

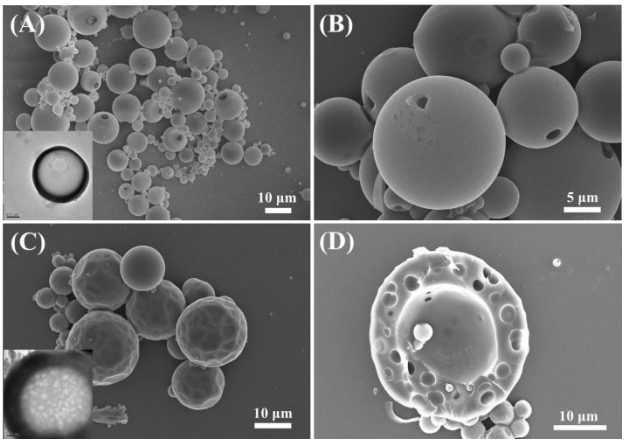


Figure 1. SEM images of unilocular (A, B) and multicompartmental hollow spheres (C, D). The insets are TEM images.

Figure 1 shows the scanning electron microscope (SEM) and transmission electron microscopy (TEM) images of the FF hollow spheres. As shown in Figure 1A, when using toluene as the organic phase in the emulsion droplets, smooth-faced spheres with the sizes ranging from 2 μm to 15 μm were obtained (Figure S1). The enlarged image (Figure 1B) shows that these spheres possess an open hole on their surface, indicating their hollow nature. The TEM image (inset of Figure 1A) also demonstrates the hollow structure of the obtained sphere, and further indicates that the hollow sphere is unilocular. However, after changing hexane as the organic phase, spheres with wrinkled surface were formed (Figure 1C). According to the TEM image (inset of Figure 1C), these wrinkled spheres, which are about 10 μm in size, also possess hollow nature. The detailed SEM image of a broken sphere shown in Figure 1D reveals that the wrinkled hollow spheres are multicompartmental with one big cavity in the center and many small cavities embedded in the wall. It is quite obvious that hollow spheres with completely different structures were formed when using toluene and hexane as the organic phase, respectively. Considering that all the rest experimental conditions are identical, it is reasonable to suppose that different solubilities of toluene and hexane with 1,1,1,3,3,3,-hexafluor-2-propanol (HFIP) (Figure S2, toluene is miscible with HFIP while hexane is immiscible with HFIP) may be the key condition for the formation of hollow spheres.

To further understand the formation of the as-prepared hollow spheres, Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectra (XPS) were employed to investigate the both kinds hollow structures at molecular level. Figure 2A(a-c) show the FTIR spectra of FF powder, unilocular and multilocular hollow spheres, respectively. A number of differences could be observed comparing the FTIR spectra of FF powder with these two kinds of hollow spheres. In Figure 2A(b), a weak peak at 1633 cm^{-1} shows up, which belongs to stretching vibration of C=N group.[11] Similar peak which located at 1636 cm^{-1} could be observed in Figure 2A(c). The appearance of these peaks indicate the generation of C=N bond during the formation of the both kinds hollow spheres. Another weak peak at 1733 cm^{-1} which both shows up in Figure 2A(b) and 2A(c) belongs to the symmetric vibrational band of free aldehyde, revealing the existence of free aldehyde in the hollow spheres.[12] FTIR spectrum of FF (Figure 2A(a)) shows stretching vibrations of –NH- and $–NH_2$ at 3402 cm^{-1} and 3264 cm^{-1}, respectively.[13] After assembled into hollow spheres, these bands shifted to 3428 cm^{-1} and 3220 cm^{-1}. The shift of these bands may be due to the formation of hydrogen bond between these groups and water during the assembling of hollow spheres. Moreover, the peak at 3216 cm^{-1} in Figure 2A(b) is weaker than that at Figure 2A(a), which may contribute to the decrease of $–NH_2$ group because of the formation of C=N bond. Similar peaks which appear in Figure 2A(c) are contributed to the same reason. Figure 2C(a) and 2C(b) show the N1S spectra of unilocular hollow spheres and multilocular hollow spheres, respectively. In Figure 2C(a), four clearly resolved peaks for the N 1S band can be observed. The peak centered at 401.0 eV can be assigned to protonation of amine groups in FF ($C-NH_3^+$).[14] While the peaks located at 399.4 eV and 399.9 eV belong to amine groups ($C-NH_2$) and O=C-N groups, respectively.[15] The peak at 398.8 eV attribute to C=N groups, which is consistent with the FTIR result.[16] Similar peaks which located at 401.4 eV ($C-NH_3^+$), 399.9 eV ($C-NH_2$) , 399.3 eV (O=C-N) and 398.8 eV (C=N) are also found in the XPS spectrum of multicompartmental hollow spheres in Figure 2C(b). Taken together, the FTIR and XPS results indicate the existence of $C-NH_2$ and C=N groups in both kinds hollow spheres, revealing partially crosslinking of the $C-NH_2$ groups by GA during the formation of hollow spheres. This kind of partially crosslinking may be another key condition for the formation of hollow spheres.

In order to clarify the molecular arrangement in these assemblies, fluorescence spectra of the hollow spheres were recorded (Figure 2B). As shown in Figure 2B(a), two peaks with one located at 400 nm and the other one at 450 nm were observed in the emission spectrum of unilocular hollow spheres. The one located at 400 nm belongs to the arrangement of FF. Previous reports have demonstrated that the free FF in solution shows a peak centered at about 306 nm.[17] The significant red shift from 306 nm to 400 nm suggests the effective π-π stacking between the aromatic residues of FF molecules after assembled into hollow spheres, which indicates that FF may use J-aggregate arrangement during the formation of hollow spheres.[17] The other emission peak at 450 nm may attribute to the formation of C=N bond.[18] Peaks in the same position could be observed in the emission spectrum of the multicompartmental spheres (Figure 2B(b)), revealing the similar molecular arrangement in these assembly structures.

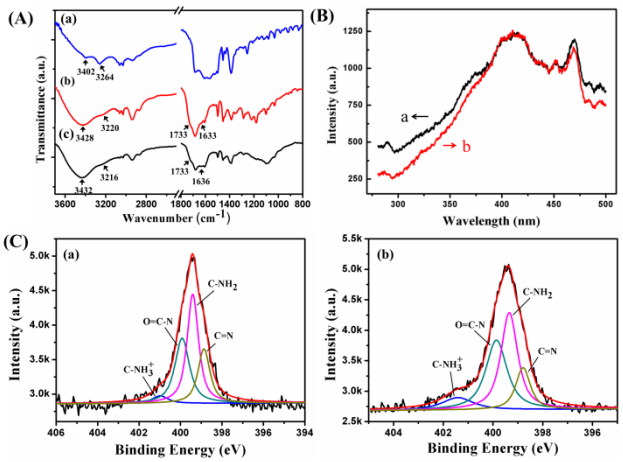


Figure 2. FTIR spectra (A) of FF (a), unilocular hollow spheres (b) and multicompartmental hollow spheres (c); FL (B) and XPS spectra of unilocular hollow spheres (a) and multicompartmental hollow spheres (b).

Based on the above results, we propose a possible assembly mechanism for the formation of the unilocular and multilocular hollow spheres (Scheme 1). As shown in Scheme 1A, when using toluene as the organic phase, w/o (water in oil) emulsion were firstly formed when adding GA aqueous solution to toluene with the assistant of ultrasound. After adding FF/HFIP solution to the above emulsion, FF/HFIP solution would be miscible with toluene. Then

FF molecules would adsorb on the surface of the emulsion droplet due to the generation of Schiff's base bond formed by -CHO group of GA and $–NH_2$ group of FF. The changing of emulsion color from colorless to brown could also indicate the formation of Schiff's base bond (Figure S3A). Further aging, volatilization and washing gave the final product with unilocular hollow spheres. When changing the organic phase into hexane (Scheme 1B), GA/Hexane/HFIP ($W_1/O/W_2$) multiple emulsion would form due to the immiscible property of HFIP and hexane. Then similar to the formation of unilocular structure, FF molecules would adsorb on the surface of the emulsion droplet due to the generation of Schiff's base bond between FF and GA. After aging, volatilization and washing, multilocular structures were obtained. It should be mentioned that ultrasound was applied in the process of whole assembly, which contributed to the formation of emulsion droplet template without adding extra surfactants. It is clearly observed that the mixed Hexane/HFIP liquid immediately separated into two layer once stopping the ultrasounic treatment (Figure S3B).

In conclusion, hollow spheres with unilocular and multilocular structures were controlled fabricated through ultrasound-assisted emulsion droplet template method. The different solubilities of toluene and hexane with HFIP, ultrasound-induced emulsion droplet template and partial crosslinking of FF molecules by GA are considered to be the key factor for the formation of these two different kinds of hollow spheres. We hope this novel surfactant-free emulsion droplet template method could help to develop new strategies to fabricate peptide-based materials with novel structures and functions.

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Notes and references

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