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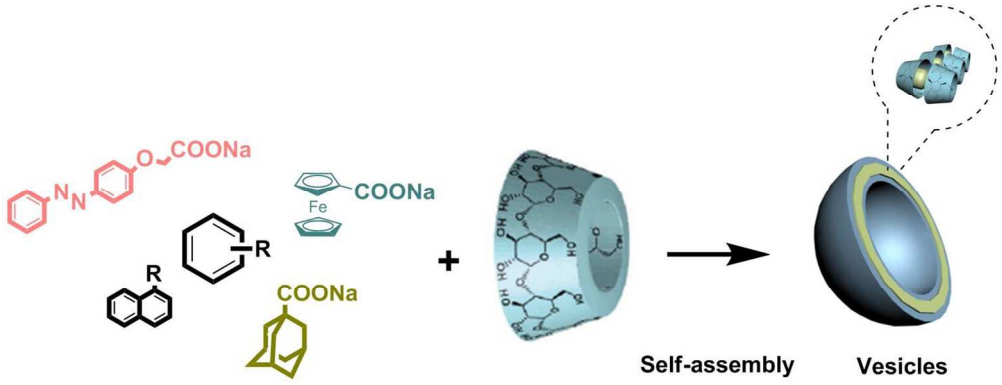

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ARTICLE TYPE

A facile approach to construct vesicles by usual aromatic molecules with β-cyclodextrin

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Vesicles were formed in aqueous solution by β-cyclodextrin (β-CD) complexes with a series of ultra-small aromatic molecules. The vesicles possess an ease of preparation property without complicated synthesis procedure and the structure was identified and characterized by various techniques including transmission electron microscopy (TEM), atomic force microscope (AFM) and dynamic laser light scattering (DLS). Structural factors that caused the self-assembly were evidenced by the ^{1}H Nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD) using a representative example of β-CD/L-phenylalanine system. In addition, the vesicular architectures were endowed with diverse stimuli-responses realized with the addition of different guest molecules. We believe this unprecedented building strategy can be further extended and thus presents new opportunities for the development of nanocarriers and soft materials.

1 Introduction

Over the decades, self-assembled aggregates have received considerable attention owing to the exhibition of their various morphologies and extensive applications.[1] Among the various structures, vesicles, microscopic capsule that enclose a volume with membrane consisting of bilayer or multilayer, are always considered as one of the most important typologies of them.[2] In general, the practical methods for preparing vesicles contain using artificial building blocks such as surfactants, lipid molecules and amphiphilic block copolymers.[3-5] These self-assembled vesicles can mimic the remarkable properties of biological membranes with simple but well-organized structures. In addition, vesicular structures are also considered of promising applications in fields such as drug and gene delivery, nanoreactors, and artificial cell membranes. [6-8]

Being one of the most widely used host molecules, CDs, a class of biocompatible cyclic oligosaccharides which contains hydrophilic outer surfaces and hydrophobic internal cavities[9], are important candidates in self-assembly and can be basal for more ordered structures such as micelles, nanotubes and fibers.[10-17] CD-based vesicles, which have been progressively developed since the 1990s, are conventionally constructed principally from cyclodextrin derivartives with hydrophobic modifications by covalent bonds. Bugler[18] and Ravoo[19] successively synthesized hydrophobically modified CDs to prepare vesicles by attaching calixarenes, alkyl chains onto the primary or second side of cyclodextrin. Since then large amount of this kind of amphiphilic

cyclodextrins were synthesized with various hydrophobic moieties and functions.[20-26]

Moreover, CD vesicles based on inclusion have drawn much more attention due to their unprecedented properties, especially stimuli-responsiveness.[27-29] Cyclodextrin not only can be regarded as a "link and interdiction group" to combine the hydrophilic and hydrophobic moieties together, [30-32] but also can play the role of hydrophilic group itself due to the rim covered with polar hydroxyl groups. A typical and first example by Chen[33] is the inclusion complexes of β-CD and 1-naphthylammonium chloride molecule, coupled with an anionic surfactant, and in which 1-naphthylammonium chloride plays the role of linker. Jiang[34] has designed an amphiphile by capping a hydrophobic compound which had an *azo* head group and three tails of 18-carbon alkyl chains with CD. The amphiphilic compound then self-assembled into UV-responsive vesicles in water. We previously reported a series of novel slender hydrophobic ferrocenyl or anthraquinone derivatives which were guest molecules in the formation of inclusion complexes with β-CD.[35, 36] These inclusion complexes formed the one-head and one-tail "tadpole-like supramolecular amphiphiles" which was assumed to assemble into vesicles.

The classic assemblies mentioned above are mainly driven by amphiphilic CDs and most people believe that the nonamphiphilic CDs and its complexes only dispersed in a molecular level in water and ordered aggregates were not taken into account.[37] Until recently Huang's group[38,39] first studied the assembly of β-CD/SDS complex in aqueous solution. Previous reports have proved that β-CD could spontaneously form an inclusion complex with SDS in a 2: 1 stoichiometry, in which one SDS aliphatic chain is embedded into two β-CD cavities. At this case the outer surfaces of the surfactants are completely covered by the hydroxyl groups of CDs and become hydrophilic. Huang found that β-CD /SDS complex with a molar ratio of 2:1 formed vesicles which were different from the conventional ones. From then on more and more follow-up works were carried out, indicating that although these microstructures take the morphology just like amphiphiles, the formation mechanism of the unamphiphilic self-assembled structures is different where hydrogen bonding of CDs is suspected to be the driven force.[40,41] These nonamphiphilic CD vesicles emerging as a new form have propelled us a lot to recognize cyclodextrin in a new light on the role of self-assembly.

In our group, many specific hydrophobic compounds were reported to form complexes with β-CD or simply modified β-CD and to be capable of assembling into vesicles, it is worth to note that among them even some common hydrophobic molecules without intentional modifications (such as ethyl benzoate[42], bromophenol blue[43] and methyl orange[44]) were reported. Implications of these results have inspired us a lot in the building of vesicular structures, and meanwhile brought us more ideas about the effect β-CD played in the constructing nanostructures. Since these molecules are comparatively small, cyclodextrins can almost encapsulate them into their cavities completely. It seems to be different from the classic supramolecular amphiphiles based on host-guest interactions whose guest molecules always contain hydrophobic tails outside the CD cavities after inclusion to maintain a balance between the hydrophobic and hydrophilic effect.

In this paper, we choose a series of ultra-small aromatic organic guests, forming an inclusion complex with native β-CD through noncovalent interaction, in order to give more comprehensive and specific investigation about their supramolecular self-assembly in aqueous solution. There were plenty of theoretical and experimental studies on the stoichiometries, structures, and stabilities of the these inclusion complexes with CDs.[45-47] However, scarcely any of them have involved their morphologies, which means there is still a broad space for development. To our surprise, vesicular structures could be obtained from these aqueous solutions based on cyclodextrin-guests pair assembly. The vesicles were fully characterized by means of TEM, AFM, and DLS while the mechanism of the

vesicle-formation was suggested on the basis of the result of ^{1}H NMR, FT-IR, and XRD. Based on above, we could deduce that β-CD played a role of "nanostructure constructor" which directly assembled the guest molecules into vesicles. In addition, responsive materials with various stimulus factors were also designed by changing the binding ability of guests in accordance with this valid principle. We hope this finding may open up a novel and simple way of vesicles preparation from supramolecular host molecules.

2 Experimental Sections

2.1 Materials

β-CD was purchased from Binzhou Zhiyuan Biotechnology Co. Ltd., China. L-phenylalanine, adamantanecarboxylic acid, ferrocenecarboxylic acid and α-amylase were purchased from Aladdin Chemicals. All the other reagents of AR grade were commercially available from Country Medicine Reagent Co. Ltd., Shanghai, China. All the compounds were used directly without further purification. Water used was doubly distilled and deionized.

2.2 General procedures

Samples for transmission electron microscopy (TEM) were prepared by the phosphotungstic acid staining technique and measured on a JEM-100CX II electron microscope (100 kV). Cryo-TEM samples on copper grids were prepared in a controlled environment vitrification system (CEVS) at 298K and quickly plunged into a reservoir of liquid ethane (cooled by nitrogen) at -165□. The vitrified samples were then stored in liquid nitrogen until they were transferred to a cryogenic sample holder (Gatan 626) and examined at about -174□. Atomic force microscopy (AFM) images were recorded under ambient condition by using a Veeco Nanoscope Multimode III SPM, operated in tapping contact mode. Light scattering measurements were carried out with a Wyatt Qels Technology Dawn Heleos instrument set at constant room temperature by using a 12-angle replaced detector in a scintillation vial and a 50 mW solid-state laser (labda = 658.0 nm). All solutions for DLS were filtered through 0.45 μm filters before detection. The radius of gyration (R_g) was obtained from static light scattering data at different concentrations. Samples for powder X-ray diffraction (XRD) were loaded on a rectangular sample holders respectively, and then scanned by a German Bruker/D8 ADVANCE diffrac-tometer with Cu Ka radiation (λ= 0.15406 nm, 40 kV, 40 mA). Solid samples of supramolecular complex for FT-IR were prepared by UNICRYO MC2L lyophilizers at -60℃ and measured on an Avatar 370 FT-IR spectrometer. ^{1}H NMR spectra were measured on a Bruker AM-300 spectrometer at room temperature with TMS as the reference. UV-irradiation experiments used a CHF-XM35-500W ultrahigh pressure short arc mercury lamp with optical filter of 200–400 nm.

2.3 Preparation of aggregates

The typical preparation method of vesicles is as follows: 0.1 mL of the selected hydrophobic guest in methanol solution at a concentration of 100 mmol L^{-1} was slowly injected into 10 mL of β-CD (1 mmol L^{-1}) in water. Existence of the small amount of methanol does not affect the system because the amount of water is much greater. To be mentioned, n-dodecylbenzene is slightly soluble in methanol so we replaced with another common used organic solvent DMSO. Then the solution was dealt with a 20min-sonication treatment for three times to form a homogeneous mixture. Bluish opalescence appeared during this procedure, indicating the formation of assembled nanoparticles. Preparation of hydrophilic guests can directly mix equal amounts of the corresponding guest and β-CD in water. All the samples were incubated at 298K for 48 hours before detection.

2.4 Encapsulation experiments

Encapsulation experiments were prepared as follows: 50μL of 10 mmol L^{-1} RHB was injected into 10ml vesicle solution at a concentration of 1 mmol $L^{-1}$and then treated with a sonication for 20 min. A blank controlled trial was carried on by replacing the

solution with distilled water. After stabilized for 48h the samples were poured into dialysis tubes with molecular weight cut off of 3500, purified by 500 ml water replaced every 12 hours. After 24 h, the process was considered to achieve a balance because the water outside the dialysis tube exhibited negligible UV absorption.

3 Results and Discussion

3.1 Vesicles directly assembled by β-CD with diverse aromatic derivatives.

Generally, the principle applied in the bilayers fabrication of cyclodextrin vesicles is considered as follows: after the formation of inclusion complexation, guest molecules usually contain hydrophobic "tails" which are directed inward, while cyclodextrins acting as the hydrophilic macrocycle "head groups" tend to face water[48] (Fig. 1). This kind of orientation simulates the packing mode of traditional surfactants and thereby encloses an aqueous interior. Based on this theory, *n*-dodecylbenzene, which had a benzene ring head group and a tail of 12-carbon alkyl chain, was selected as the guest molecule and it could convert into an amphiphile by capping it with β-CD. As expected, spherical structures of the micro-aggregates with vesicular shells were observed by negatively stained TEM, indicating the self-assembly of vesicle in aqueous solution and this phenomenon verifies the theory mentioned above (Fig. 2a). Nonetheless, most people profoundly believe that "hydrophobic tails" provide the hydrophobic parts of amphiphilic molecules to keep a balance but in fact the necessity of their existence has been rarely reported. To confirm the effect of the "tail" on vesicle formation, alkylbenzene derivatives with different alkyl length were then tested (Fig. 1). The result needs to be emphasized that with the length of the alkyl chain decreases, similar structure of vesicles could still be detected by TEM all the time even if using toluene as the hydrophobic guest. The nanoaggregates of about 150 nm in diameter show a contrast between the center and the periphery, which is a typical characteristic property of vesicular structure (Fig. 2b, c and d). Many reports have confirmed that the stability of CD inclusion complexes with long chains is stronger than that of the inclusion complexes of the same CD with short chains.[49] Although we cannot distinguish the difference of the series of vesicles up to now, a reasonable assumption could be deduced that the stability of vesicles is consistent with the stability of their inclusion complexes. At room temperature, these vesicles could survive for about two weeks with no morphological and size changes observed by TEM. Notably, the stability of vesicles is also sensitive to temperature. The increase of temperature will weaken the H-bonds of CDs and the binding constant of the inclusion complex will also decrease. As a result, the stability of the vesicles decreased and became difficult to form. So preparing, incubating and characterization of the vesicles were carried out at room temperature at 298K. Higher temperature is thus not recommended for vesicles' preparation. DLS was further applied to confirm the size and size distribution of the vesicles and the data indicates an average hydrodynamic radius (R_h) from 50 nm to 100 nm for the vesicles in the aqueous solution, which signified a diameter of about 100-200nm (Fig. 3).

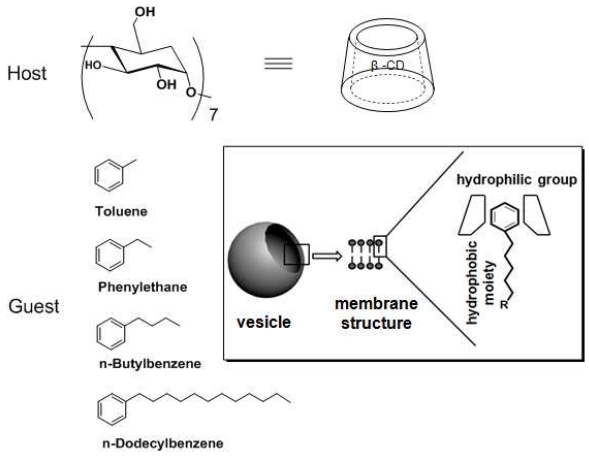


Fig. 1 Chemical structure of β-CD, guest molecules and general mechanism illustration of vesicles formation based on cyclodextrin.

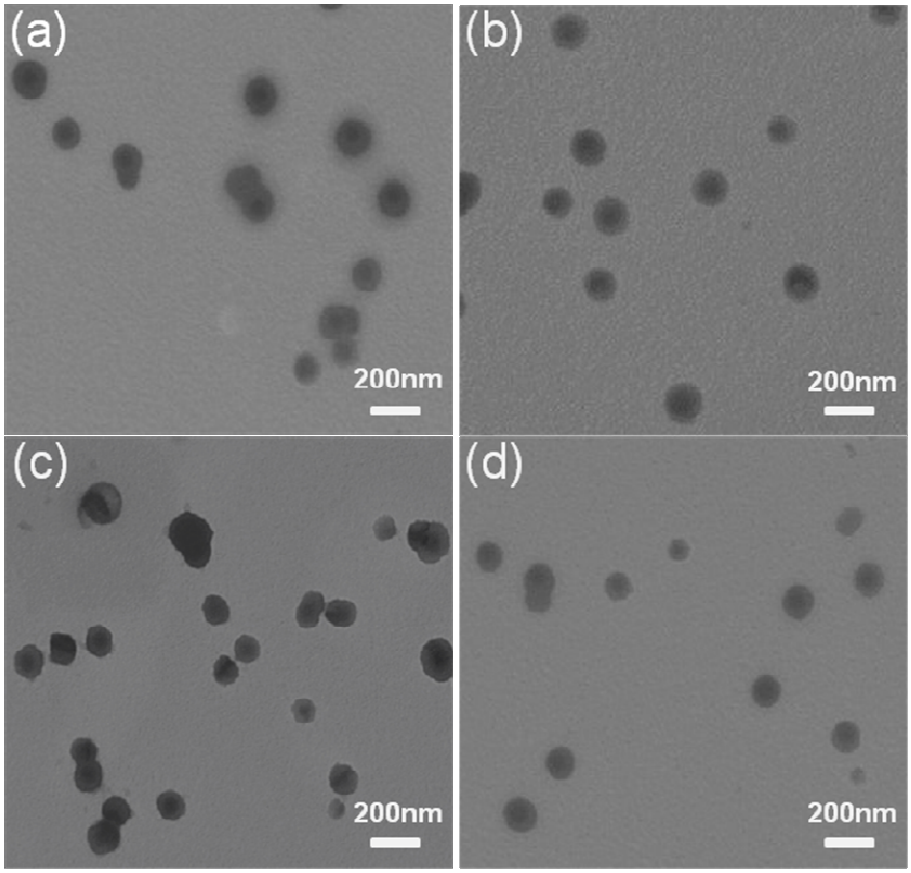


Fig. 2 TEM images of the micromorphology of the vesicular samples formed by (a) n-dodecylbenzene (b) n-butylbenzene (c) phenylethane (d) toluene with β-CD in water with a concentration of 0.5 mmol L^{-1}. Phosphotungstic acid as the negative staining agent.

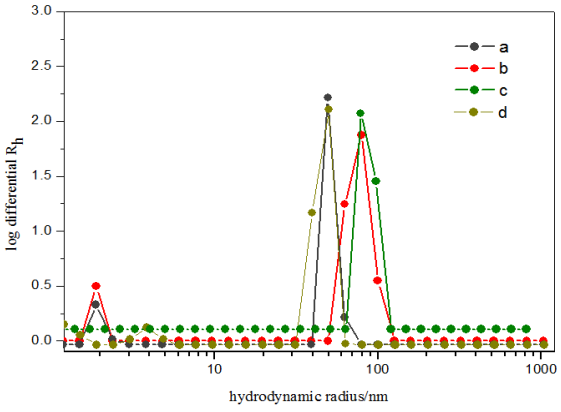
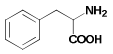


Fig. 3 DLS results of the number-averaged size distribution of vesicular samples formed by (a) n-dodecylbenzene (b) n-butylbenzene (c) phenylethane (d) toluene with β-CD in water with a concentration of 0.5 mmol L^{-1} solution at 300 K.

The characterized results get us to question more about this research, including the proposed mechanism and whether can we introduced more types of guests into this system. More kinds of frequently used aromatic compounds possessed good cooperation ability with β-CD were tested as listed in table 1, including diverse benzene derivatives modified with different functional groups. Both hydrophobic molecules (phenoxymethane, nitrobenzene, benzyl chloride, etc.) and water-soluble molecules such as phenol, phenylamine and L-phenylalanine were involved in. The experimental results were favorable that β-CD was just like a "nano-vehicle" which could directly include these simple molecules and further assemble into vesicular structures in aqueous solution (Fig. S1). Molecules with broader range of species were also tested. In a summary, guests possess strong binding ability with β-CD like naphthalene derivatives, biphenyl derivatives and adamantane compounds possess the vesicular features detected by TEM, while the ones with low association

constants such as alkanes could not be limited by the instability of the inclusion complexes. It is worth mentioning that although a number of vesicle architectures based on CDs host–guest pairs have been reported, most of them involved complicated design and synthesis and such an approach contains both universality and ease of operation properties has been rarely reported. We regard this finding a feasible way to present an approach for the fabrication of vesicles which have great potential applications in many fields.

Table 1 Examples of diverse benzene derivatives introduced in this system.

Guest of the inclusion complex	Structural formula	Guest of the inclusion complex	Structural formula
Phenol		Phenoxy-methane	
Phenylamine		Nitrobenzene	
Benzaldehyde		Phenylethanone	
Chlorobenzene		Benzylchloride	
Benzoic acid		L-phenylalanine	

3.2 Characterized in detail and mechanism study by using β-CD/L- phenylalanine sample as the template.

It is known that the stoichiometries and stabilities of CD inclusion complexes in aqueous solution vary markedly with the shape, volume, and polarity of organic guest molecules. Surpriseingly, all these factors did not affect the formation of vesicles. Considering the universality of the phenomenon and the wide range options of the selected guest molecules, a reasonable hypothesis can be deduced that CD may play a leading role and is more critical in the nanostructure fabrication. It is also rational to choose one of the guests mentioned above as a typical model for further study of the phenomenon and the related mechanism. As a consequence, *L*-phenylalanine (*L*-phe**)**, a kind of essential amino-acids listed in Table 1, was selected due to its great significance and good solubility in water as to give a comprehensive and in-depth analysis.

Firstly, TEM images clearly verified the membrane structure as regular spheres with diameter of 100–150 nm (Fig.4a). Up to now, by using the negative staining TEM, spherical structures with dark cores and vignetted shells with weaken color are always considered as hollow vesicles as well. In order to convince this data, cryogenic transmission electron microscopy (cryo-TEM) was used to directly and wholly reflect them from solid sphere and reflect the real environmental morphologies. In Fig. 4b, hollow spheres with a thin layered structures were doubtless found with diameter of 100–200nm. Compared with TEM vision field, vesicles are less concentrated, possibly because no drying process is required in the sample preparation procedure for cryo-TEM observations.

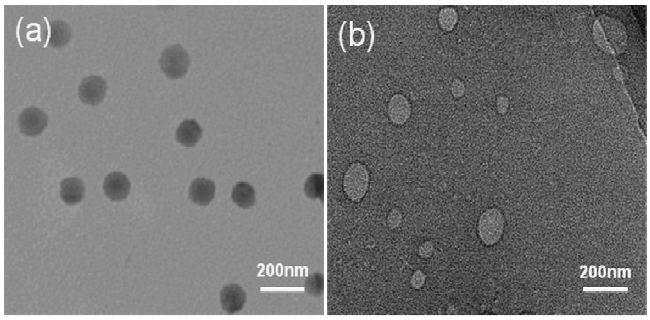


Fig.4 TEM and Cryo-TEM images of β-CD/L- phenylalanine sample with a concentration of 1 mmol L^{-1}.

The sample was also visualized by AFM. The AFM image was prepared by depositing aqueous solution on silicon surface. As shown in Fig. 5a, well-defined spherical structure of the sample

was detected and the diameter is in accordance with the results from TEM. The height of AFM can be seen as the thickness of two closely stacked membranes fit together after dry and collapse of the vesicles. So we think one second of it can be considered as the membrane thickness of the vesicle and the height of 3.9nm corresponds to a membrane thickness of 1.95nm (Fig. 5c). The radius of gyration (R_g) and hydrodynamic radius (R_h) of this complex solution were also measured using light scattering studies. The ratio $\rho=R_g/R_h$ is a highly structure sensitive property, representing the radial density distribution of the particle. In general, $\rho=0.78$ represents solid sphere and the ρ value of hollow sphere usually keeps around 1.0.[50] The experimental data showed Rg=62.9 nm and Rh=62.6 nm, respectively. Rg/Rh= 1.0 is characteristic of hollow sphere, thus also precisely proves the vesicular structure.

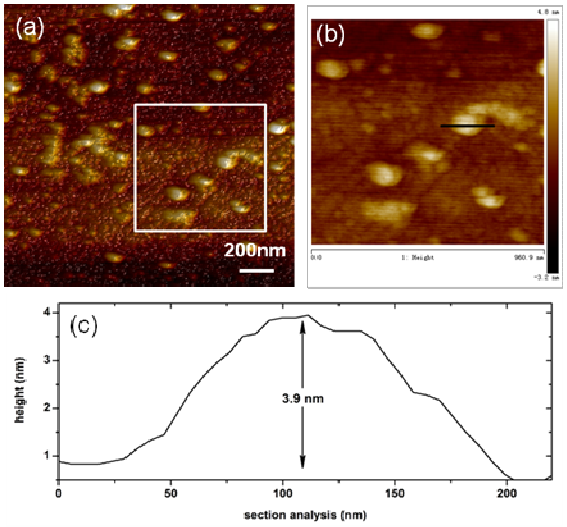


Fig. 5 (a) and (b) AFM images of the vesicle samples prepared on silicon plate after the solvent was evaporated. (c) Height profile plot of vesicles (line marked in b).

To confirm the critical concentration of vesicles formation, β-CD samples (10^{-3} mmol L^{-1}, 10^{-2} mmol L^{-1}, 0.1 mmol L^{-1} and 1 mmol L^{-1}) with the addition of equal amount of *L*-phenylalanine were prepared and investigated by means of DLS. We have already known that β-CD in water can self-assemble only at concentrations as high as 3×10^{-3} mol/L, whereas no large aggregates are present in solution at lower concentrations.[51] As shown in Fig. 6, the main difference between these samples with different concentration is the single size distribution centered at about 50-100nm radius which is in accordance with the size of the vesicles. The dynamic radius of aggregates increases as a function of β-CD and significantly changes until a certain concentration is reached. Based on this result, we can conclude that the critical concentration of vesicle formation is approximately around 0.1mmol L^{-1}. This process can also be confirmed by naked eyes. When a laser pointer was used to light the sample, typical tyndall phenomenon of the vesicle solution was clearly detected while the solution with low a concentration was not.

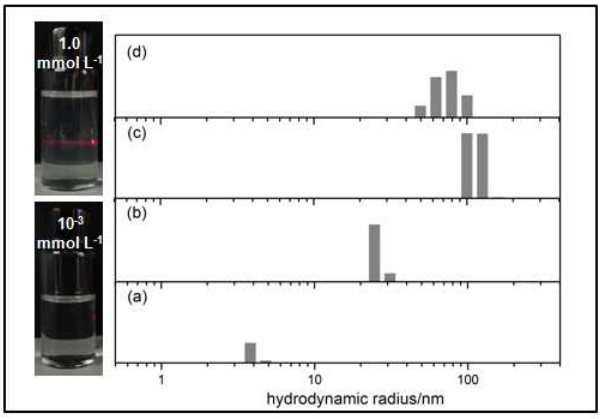


Fig.6 DLS results of the number-averaged size distribution of β-CD/L-Phe self-assemblywith different concentrations: (a) 10^{-3}mmol L^{-1}; (b) 10^{-2}mmol L^{-1}; (c) 0.1 mmol L^{-1}; (d) 1 mmol L^{-1}.

Inclusion information and molecular interactions in solutions of this system can be provided by using ^{1}H NMR spectra. CD molecules are presented as toroids where the H-3 and H-5 protons are located inside the cavity, while the H-2 and H-4 protons are outside the cavity. It is known that in absence of proper guest molecules, the CD cavity is usually occupied by water molecules in the solution. Upon the addition of guest molecules, water molecules were released from the cavity and replaced by more apolar guest molecules of suitable sizes. As shown in Fig. 7a, the hydrogen resonances of H-3 and H-5 protons show clear

chemical shifts upfield compared to H-2 and H-4 protons, indicating the complexation of β-CD with *L*-phenylalanine in aqueous solution (Fig. 7a and Table 2).

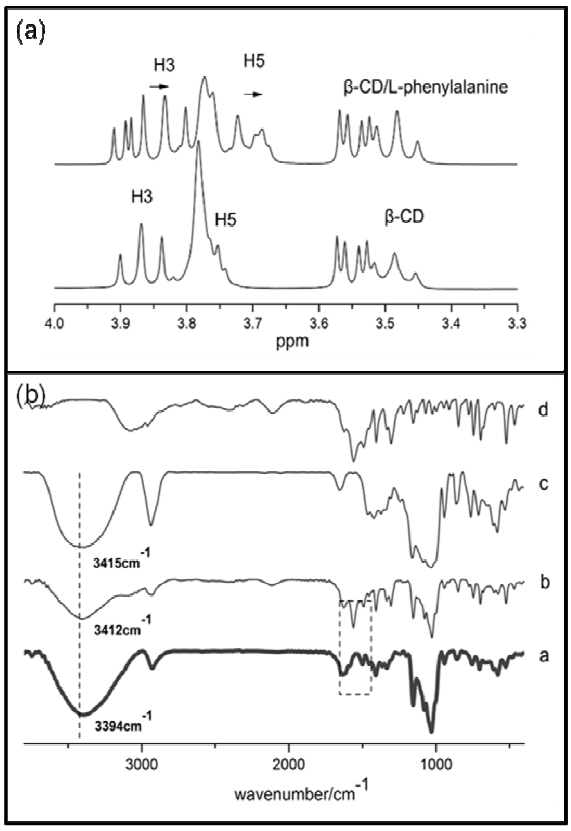


Fig. 7 (a) ^{1}H NMR spectra (300 MHz) of individual compound β-CD in comparison with the complex of L-phenylalanine/β-CD (D_2O, T = 300 K). (b) FT-IR comparison of d: L-phenylalanine; c: β-CD; b: their physical mixture; a: their supramolecular complex at 300K.

Table 2 ^{1}H NMR: chemical shifts and chemical shift differences of β-CD and its complex with *L*-phenylalanine.

	H_2	H_3	H_4	H_5	H_6
δ(β-CD)	3.567	3.869	3.533	3.754	3.782
δ(complex)	3.563	3.832	3.530	3.723	3.773
$\Delta\delta^a$	0.004	0.037	0.003	0.031	0.009

a Δδ=δ(β-CD)-Δδ(complex).

The binding stoichiometry of β-CD with *L*-phenylalanine could be acheieved by the Job's plot method, exhibited a maximum value at a molar fraction of 0.5, corresponding to 1:1 stoichiometry (Fig. S2). ESI-MS is a useful technique to support the formation and composition of an inclusion complex. Fig. S3 depicts the ESI-MS spectrum of *L*-Pheeylalanine/β-CD (m/z 1300.4462) and the result also confirms the quantitative formation of the 1:1 inclusion complex between host and guest even in the case of ESI experiments.

It has been demonstrated that FT-IR is widely used in studying the molecular interactions, especially important to the change in hydrogen-bonding in material science. In figure 7b, the characteristic peaks of *L*-phenylalanine (1561cm^{-1}) can be found in the spectra of the physical mixture but changed to 1589cm^{-1} in the supramolecular complex, indicating the existence of L-phenylalanine in an independent condition, possibly complexed by cyclodextrin. The spectrum of β-CD shows stretching vibration peaks of the hydroxyl group at 3415 cm^{-1}. Physical mixture is similar to the peaks of pure β-CD with a broad band at 3412 cm^{-1}. But it shifts to a lower frequency at 3394 cm^{-1}. As is well known, the association of OH groups makes the stretching peak red shift to a lower frequency.[52] Thus, these above spectrum changes confirm the combination mode of β-CD-phenylalanine system, which clearly reflects the complexation between host-guest molecules and the intermolecular hydrogen bonding between β-CDs.

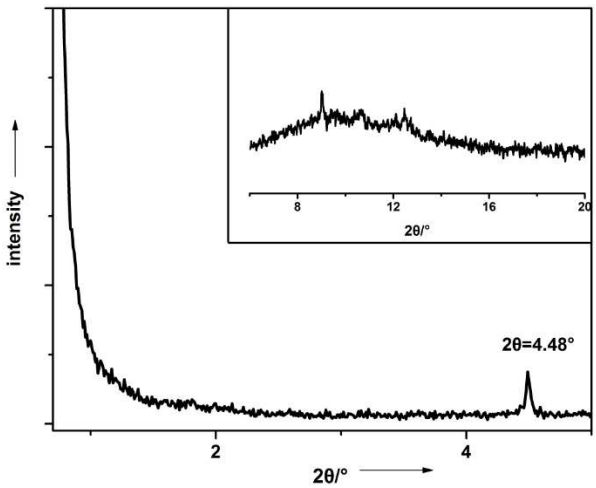


Fig. 8 Powder XRD pattern of L-phenylalanine/β-CD sample.

To further investigate the change in structure, powder XRD, as a powerful method in analyzing species in supramolecular materials, had been utilized to elucidate the molecular packing

mode in the membrane. To preserve the membrane structures, casting film sample was prepared by β-CD/*L*-phenylalanine solution dried on glass. Usually, the low symmetry of β-CD structure leads to a great number of reflections in its XRD patterns.[53] However, most intense peaks weaken significantly in this condition compared with that of β-CD itself in the wide-angle region. In the small-angle region, the emerging sharp reflection peak at 2θ=4.48° corresponds to 19.2Å of the d value according to the Bragg equation (Fig.8). This data is consistent with the membrane thickness detected from AFM results.

Based on the above results, a supposed assembly mechanism was then speculated. As we know, β-CDs contain a great amount of hydroxyl groups and the hydrogen bonds among them should not be ignored. The related packing motif of β-CD complexes with guests is frequently reported.[54, 55] Among most structures, the β-CD molecules are arranged head-to-head with hydrogen bonds of their O2-H/O3-H sides. Through this kind of connected interaction, β-CDs can form basket-like dimers in which guest molecules are accommodated in. The dimer building units can form more complicate self-assemblies such as channel type structures, or arranged in layers which are displaced laterally in high concentration solution or in solid crystal states. However, low concentration of this investigation system is employed and layer structure tends to disperse in the dilute solution, keep independent relatively and then scroll up along two in-plane axes to form vesicle. The membrane thickness obtained from XRD and AFM closely matches an estimated double height of cyclodextrins (17.4 Å), which means that the basket-like dimers accommodated with guests are likely to be the basis composition of the vesicle membrane (Fig. 10a). The membrane thickness is a little larger than twice of the height of CD because even though dimers of β-CDs can form by the hydrogen bonding interaction, the non-covalent hydrogen bond is not strong enough so that they will divide the two β-CD monomer unites with a certain distance between them.

Atomic-based MD simulations can play a powerful role in indicating the properties at a microscopic level and are considered as complements to experiments. Molecular dynamic simulation was performed to prove the possibility of the mechanism model mentioned above. The preassembled state manually instead of starting from a random initial configuration was used, as has been done in most other simulation systems. The model was firstly energy optimized, and then executed 500ps dynamics simulation at 298K. After a relatively long time to achieve the calculated result was shown in Fig. 9.

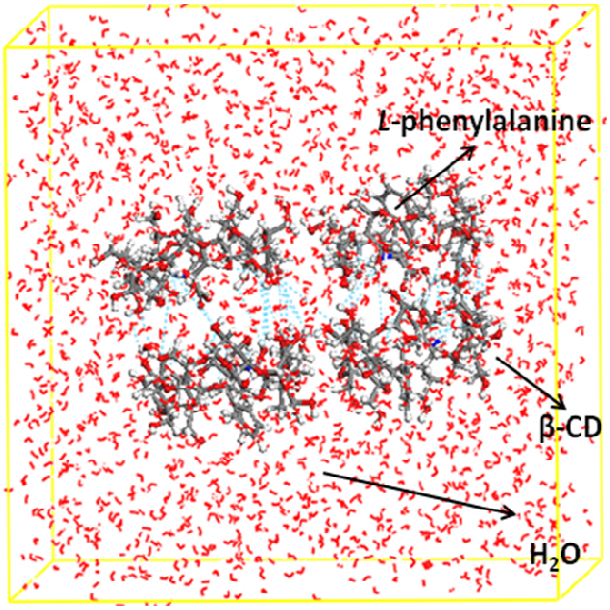


Fig.9 Results of the molecule dynamic simulation of L-phenylalanine/β-CD in the aqueous solution.

In addition, another examination of pyrene/β-CD system was carried out aimed at further confirming our assumptions. It has been reported that on the basis of molecular size, pyrene (8.2 Å wide and 10.4 Å long) is too bulky and the internal diameter of β-CD severely limits the total entrance of pyrene into the cavity. Totally, approximately half of the pyrene actually enters the β-CD while the other half (outside the cavity) is equally amenable to complex formation with another β-CD. Therefore, as shown in Fig. 10b, a head to head "sandwich type" inclusion mode with 2:1 stoichiometry is considered.[56] The inclusion mode is similar to the hypothesis we mentioned above and the existence of vesicles of β-CD/ pyrene system was demonstrated by TEM measurement

(Fig.10c). This result is consistent with our vision about the mechanism of the membrane structure.

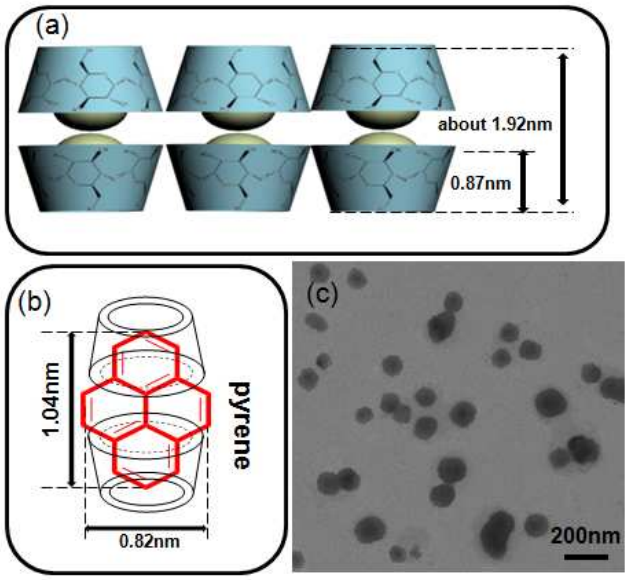


Fig. 10 (a) Illustration scheme of the proposed vesicle-lamellar structure; (b) Chemical model for the inclusion of pyrene in β-CD; (c) TEM images of the micromorphology of vesicular samples formed by pyrene/β-CD solution.

3.3 Construction of multi- stimuli responsive system

Stimuli-responsiveness is a crucial feature in designing and fabricating new supramolecular materials because it enables these substances to be functionalized for practical applications in the fields of controllable drug transport and gene delivery. A great many of research groups have attempted to create these systems in recent years. Although there were plenty of previous responsive reports occurred using pH, light, redox or enzyme as stimulus,[57] complicated designs and syntheses aimed at introducing specific functional groups are indispensable so far and it is still difficult to create multi-functional soft materials with simpler methods. Generally speaking, supramolecular materials are more convenient in realizing responsiveness to conventional materials as the presence of the reversible non-covalent bonds, and among which inclusion complexation has been extensively investigated. Considering that vesicles formation can realize with a wide range of guest molecules in this research, and complexation interaction plays a key role in the formation of vesicles for no aggregates were observed in the aqueous solutions of neither CDs nor guests alone. It is expect that the disassembly of vesicles can be artificially controlled inducing by dissociation of the inclusion complex. Therefore, different functional guests that can alter binding behaviors with β-CD by stimulus are employed to carry out the corresponding responsive vesicles (Fig. 11). We presume that β-CD can be treated as a kind of versatile instrument which provides a new avenue to the construction of novel smart nanomaterials.

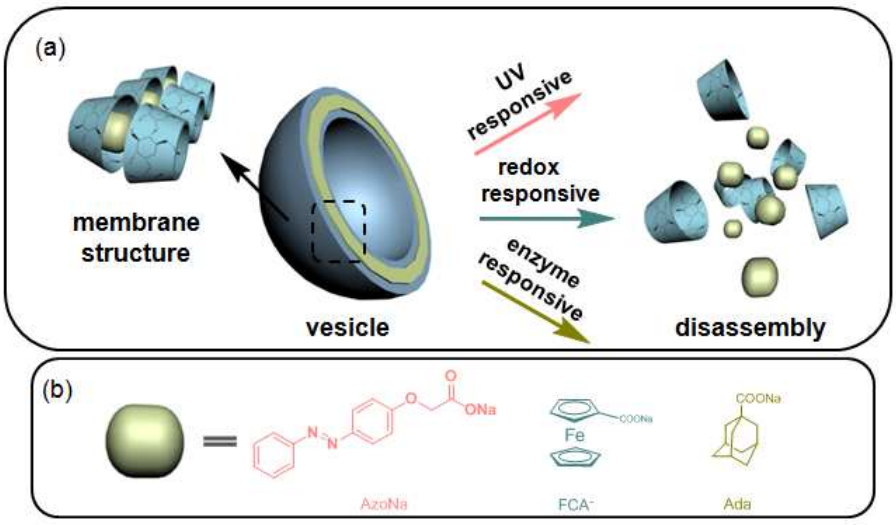


Fig. 11 Schematic representation of assembly and multi-stimuli responsiveness of the vesicles.

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ARTICLE TYPE

Azobenzene (AZO) is a classic guest for reversible inclusion complexation with CDs. *trans*-AZO binds strongly to α- or β-CD, while *cis*-AZO cannot bind or binds very weakly. Many stimuli-responsive self-assemblies were initiated from this knowledge. In this part, a commonly used azobenzene derivative, sodium (4-phenylazo-phenoxy)-acetate (AzoNa) which may undergo *trans/cis* transition triggered by UV irradiation, was synthesized according to the previous literature[58] (see the supporting information). By introducing it into β-CD solution with a molar ratio of 1:1, the inclusion complex of β-CD with the AzoNa self-assembled into vesicles in water (Fig. 12b). After UV light irradiation, the photoisomerization of AZO from *trans* to *cis*-form. The *trans/cis* transition evidenced by UV-vis spectra (Fig. 12a) induced the dissociation of the inclusion complex, which made the vesicles disappeared and irregular aggregates formed (Fig. 10c).

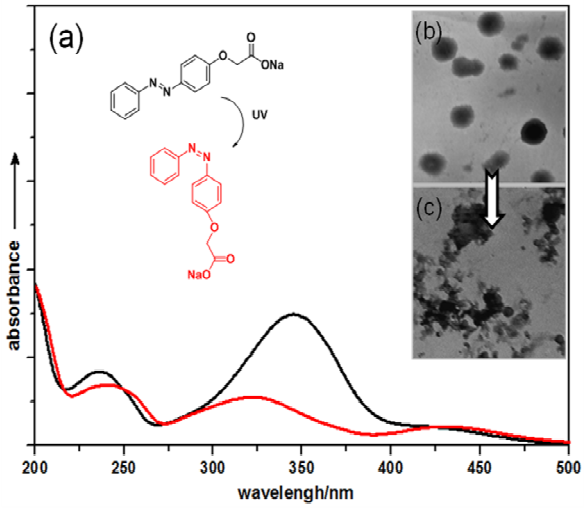

Fig. 12 UV-responsive behavior of the AzoNa/β-CD vesicle system.

The formation of host–guest complexes between ferrocene (Fc) and β-CD in solution has been widely investigated. In general, CD normally binds to neutral or anionic compounds only while some cationic substances are excluded out.[59] So β-CD shows a high affinity for the reduced state of the Fc group due to its hydrophobic nature, whereas the oxidized state of Fc group (Fc^+) exhibit an alienated relationship with β-CD. In this research, we choose ferrocenecarboxylic acid presenting as the anion salt (FCA^-) to achieve the responsiveness target. FCA^--CD complex was prepared with a mole ratio of 1:1 in pH 9.2 aqueous buffer. TEM result indicates the formation of vesicles in solution as we expected (Fig. 13b). Then we investigated the effect of the redox reagents on the FCA^--CD assembly structure. A little excess of NaClO as an oxidant was added into the solution. Cyclic voltammetry measurement reveals the decrease of dramatic half-wave voltage after the addition of NaClO, which demonstrates the disassociation of charged Fc^+ from the β-CD cavity (Fig. 13a), and the disassembly of vesicles at the same time (Fig. 13c).

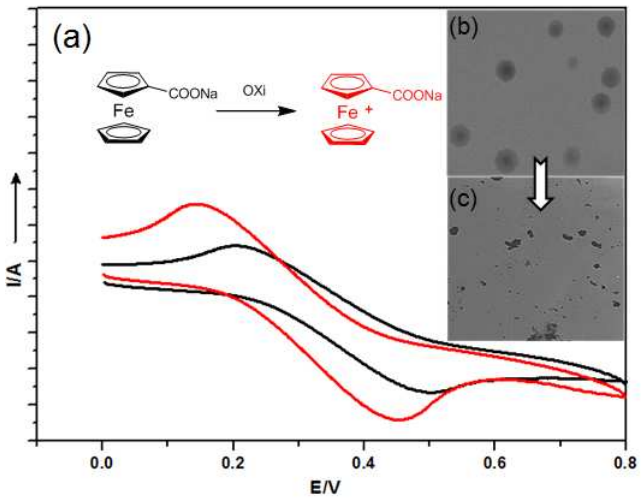

Fig. 13 Redox - responsive behavior of the FCA^-/β-CD vesicle system.

Then we further explored the utility of this general approach to construct aggregates with enzyme responsive character. α-Amylase, as a kind of digestive enzyme, can catalyze the breakdown of starch into sugars by cleave α-1, 4 linkages

between glucose units of starch molecules.[60] Cyclodextrins are prone to degradation by this enzymatic attack. One of the most investigated molecules, adamantanecarboxylic acid (ADA) was employed as it has strong binding constant with β-CD while do not possess responsive ability to amylase. Sodium salt of ADA was present to ensure the solubility in water. ADA/β-CD complex exists in the form of vesicles before enzyme treatment. Then enzymatic reactions were carried out at 310 K for 48 h. Amylase with a gravity of 1.5 g/10 ml was added into the complex system and the solution pH was kept at a range of 6.5–7.5. Such a high dosage of amylase was used to ensure the complete degradation of β-CD. The result indicates that the activity of amylase in degrading β-CD terminates the host-guest interactions and consequently destroys the vesicular structure into lumps (Fig. 14). Moreover, both α-amylase and β-CD are biocompatible so that this system may provide medical applications with some diseases with α-amylase abnormal increase such as acute pancreatitis.[61] In addition, in order to finally verify the vesicular structure and to test the packaging performance of the vesicles, encapsulation experiment of Rhodamine B was carried out. Considering adamantane group has strong binding ability with an association constant around $10^5 M^{-1}$. It is stronger than that of RHB in water, so the addition of relatively small quantity of RHB would not affect the inclusion behavior of β-CD and ADA. As we can see, through a dialysis process, only trace amount of RHB was observed in absence of the ADA/β-CD system after 24h (Fig. 14e). However, Fig.14d reveals that the model molecule RHB added into the ADA/β-CD solution was loaded into the vesicles so that it could still exist in the dialysis tube evaluated through the UV spectroscopy. What still needs to be explained is that the ultimate concentration of RHB is not such high compared with the initial concentration and the RHB encapsulation efficiency is calculated to be 26.4% according to UV-vis absorption spectra. This is because the encapsulation ability is limited by the low concentration of the vesicle solution. This phenomenon indicates the present supramolecular vesicle can encapsulate drug molecules inside and can be used as nanocarrier for drugs.

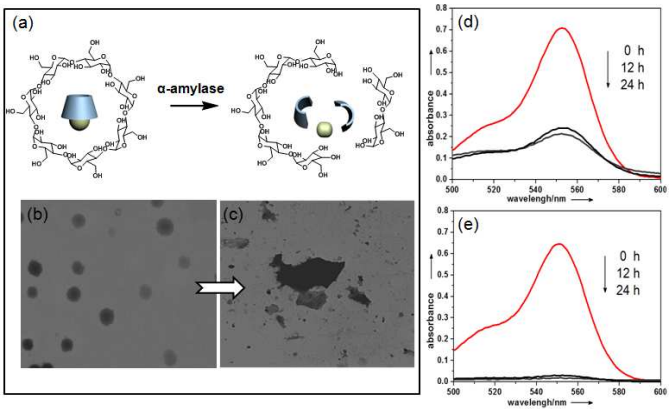

Fig. 14 (a) (b) and (c) Enzyme - responsive behavior of the ADA/β-CD vesicle system using α-amylase as the degradation reagent;(d) and (e) encapsulation of RHB with and without the ADA/β-CD vesicle.

4. Conclusion

In summary, this paper reports a facile approach to directly construct a series of common aromatic molecules into vesicles by β-CD. Morphology and size of these vesicles of supramolecular assemblies were identified and a possible mechanism was proposed. It is believed that the results reported herein offer new insights in constructing CD-based architectures in water. More interestingly, vesicular architectures with diverse stimuli such as light, redox or enzyme could be designed according to different requirements by the addition of different functional guest molecules. It is anticipated that our system would be helpful in constructing CD-based architectures and provide diverse applications in a range of areas.

Notes and references

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