

# Vesicles prepared by $\beta$ -cyclodextrins Inclusion complexes based on switching supramolecular interaction models induced by mixed solvents

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**Abstract** Vesicles, based on two-head inclusion model (2:1) and one-head inclusion model (1:1) of carboxymethyl- $\beta$ -cyclodextrin (CM- $\beta$ -CD) with *N,N'*-bis (ferrocenylmethylene) diaminoethane (BFD) were prepared in water as well as in mixed solvents (water/methanol, water/ethanol, water/isopropanol, water/*tert*-butanol and water/glycerol). Morphologies and sizes of the aggregates in water and mixed solvents were observed by TEM and confirmed by DLS. A gradual change was shown with increasing proportion of the organic component in the mixed solvents. The polarities and steric hindrances of the mixed solvents would play key roles in the morphological changes of aggregates. Moreover, two possible formation mechanisms in different solvents were proposed based on UV, CV and  $^1\text{H-NMR}$  data. The work in this paper may extend the applications of vesicles in biosimulation, drug-delivery and smart materials.

**Keywords** Vesicles ·  $\beta$ -cyclodextrin · Inclusion complexes · Switching superomolecular interaction models · Mixed solvent

## Introduction

Cyclodextrins (CDs) are cyclic molecules which conventionally consist of six ( $\alpha$ -), seven ( $\beta$ -) and eight ( $\gamma$ -) glucose units linked by  $\alpha$ -1-4 glycosidic linkages. They have hydrophilic outer surfaces and hydrophobic inner cavities [1], and can form inclusion complexes by binding small guest molecules based on supramolecular interaction [2]. A number of novel supramolecular assemblies have been designed, such as supramolecular gels [3], supramolecular polymers [4, 5], rotaxanes [6], nano-spheres [7], and vesicles based on CDs inclusion complexes. The vesicles prepared by CDs inclusion complexes have gained much attention for their important significances in understanding natural bio-membranes [8, 9] developing new bio-techniques and smart materials [10].

The vesicles prepared from CDs can be divided into two types: one is based on the amphiphilic CDs, or the mixtures of CDs with amphiphilic guest molecules. The amphiphilic CDs could be obtained by covalently modifying native CDs with hydrophobic functions [11]. McNicholas et al. reported that amphiphilic  $\beta$ -CDs synthesized with hexylthio, dodecylthio, and hexadecylthio chains at the 6-positions could self-assemble in water to form vesicles [12]. About the vesicles based on the mixtures of CDs with amphiphilic guest molecules, Jiang et al. reported one vesicle system by adding  $\beta$ -CD to mixed cationic/anionic surfactants [13]. Jing et al. reported the other vesicle system which prepared by the mixture of native  $\beta$ -CD, 1-naphthylammonium chloride and amphiphilic sodium bis(2-ethyl-1-hexyl) sulfosuccinate [14]. The other type is based on the supramolecular amphiphiles. The inclusion complex of CDs with non-amphiphilic linear guests in one-head inclusion model could play as the conventional surfactant with a hydrophilic head and a

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hydrophobic tail. Recently, a redox-responsive vesicle system was prepared based on one-head inclusion model (1:1) of native  $\beta$ -CD with a ferrocenyl guest molecule in our experiment [15].

Here, we report a new vesicle system prepared based on supramolecular interactions between modified  $\beta$ -CDs (carboxymethyl- $\beta$ -cyclodextrin, CM- $\beta$ -CD) and ferrocenyl derivatives (*N,N'*-bis(ferrocenylmethylene) diaminohexane, BFD) with stoichiometries varying from 2:1 (the two-head inclusion model) to 1:1 (the one-head inclusion model) in water, mixtures of water and alcohols, as well as organic solvents. The dramatic changing indicates that the inclusion models of CM- $\beta$ -CD with BFD could switch between one-head and two-head models. Both CM- $\beta$ -CD and BFD could not individually self-assemble into vesicles in solutions. The interesting phenomena may imply that both modified group of  $\beta$ -CD and organic solvents would induce the changing of inclusion models, even leading to the effect on their self-assemblies.

Morphologies and sizes of the vesicles were characterized by TEM, DLS and UV, which would vary gradually. The vesicles would be destroyed with increasing of the alkyl chain of the alcohols, as well as the volume ratio of alcohols/water in the mixed solvents. The polarities and steric hindrances of organic solvents were deemed as main effects on the morphological variations of the aggregates. The possible mechanism of the vesicle formation was proposed based on the experimental data. It is of great value to study the vesicles in mixed solvents since many drugs are insoluble in water and many reactions should be carried out in non-aqueous systems. The study might extend the application of vesicles in bio-simulation, drug-delivery [16, 17], smart materials and synthesis.

## Experimental sections

### Materials and methods

Ferrocene, 1,6-diaminohexane, sodium borohydride, chloroacetic acid and other chemicals were all commercially available from Country Medicine Reagent Co. Ltd, P. R. China. Methanol, ethanol, isopropanol and tert-butanol were of chromatographic purity and other organic reagents were analytical purity and used as received without further purification. The  $\beta$ -CD was purchased from Guangdong Yunan Chemical Reagent Co. Ltd, P. R. China, then recrystallized twice from distilled water and dried in vacuum for 12 h. Silica gel, 02HHJ, was purchased from Qingdao Tenghai Fine Silica gel Chem., P. R. China. Water was triply distilled. BFD was synthesized according to the literature [15].

The sonication was performed on KO116 ultrasonic cleaners, Kushan ultrasonic apparatus Co. Ltd, P. R. China; TEM was performed on JEM-100CX II transmission electron microscope operated at an acceleration voltage of 100 kV; DLS was performed on a Wyatt QELS Technology DAWN HELEOS instrument using a 12-angle replaced detector ( $99^\circ$ ) and a 50 mW solid-state laser (658.0 nm); CV was performed in a conventional three-electrode cell with a model CH1650 electrochemical workstation, using a glassy carbon electrode (GCE) as the working electrode with a platinum plate as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode; UV spectra was recorded on TU-1800pc UV-Vis Spectrophotometer at 298 K;  $^1\text{H-NMR}$  spectra, for the inclusion system of CM- $\beta$ -CD with BFD, were recorded on API Bruker Avance 300 M NMR at 298 K;  $^1\text{H-NMR}$  spectra, for the BFD, was recorded on API Bruker Avance 400 M NMR at 298 K;  $^{13}\text{C-NMR}$  spectra were recorded at 75 MHz on API Bruker Avance 300 M NMR at 298 K. TLC analysis were performed on glass plates precoated with silica gel F254 obtained from Qingdao Haiyang Chem., P. R. China.

### Preparation of vesicles

Two equimolar stock solutions ( $10^{-4}$  mol  $\text{L}^{-1}$ , 50 mL), one of CM- $\beta$ -CD and the other of BFD, were prepared with triply distilled water and mixed solvents. All sample solutions for investigation were freshly prepared by diluting the stock solution. The sample solutions were mixtures of CM- $\beta$ -CD and BFD with a molar ratio of 2:1, which were sonicated for 15 min at 300 K before detections. Mixed solvents were prepared with the volume ratio of water/alcohols 2:1, 1:1 and 1:2, respectively, and the alcohols were methanol, ethanol, isopropanol and tert-butanol.

### Transmission electron microscope

A drop of the sample solution was placed onto a Transmission electron microscope (TEM) copper grid covered by a polymer support film. After 30 s, excess solution was blotted away using a strip of filter paper. The samples were stained by phosphotungstic acid ( $\text{H}_3\text{PO}_4 \cdot 12\text{WO}_4$ ) by depositing a drop of 0.2 wt%  $\text{H}_3\text{PO}_4 \cdot 12\text{WO}_4$  aqueous solution onto the surface of the sample loaded grid. After 30 s, the solution was blotted with a filter paper.

### Dynamic light scattering (DLS) measurements

DLS measurements provide the data of the hydrodynamic radius ( $R_h$ ) of the aggregates. In this study, the CONTIN method was used to obtain the mean hydrodynamic

diameter with Astra software. All solutions were filtered with a 450 nm Millipore filter into a clean scintillation vial. All DLS measurements were performed at room temperature and the DLS measurements have been corrected for a change in dielectric constant from water to water/alcohols mixtures.

#### Cyclic voltammetry measurements

Cyclic voltammetry (CV) was performed that, prior to each experiment, the glassy carbon electrode was polished with  $\alpha$ -alumina power, then rinsed thoroughly with triply distilled water and sonicated in a 1:1 mixture of nitric acid and water by volume, and then washed again with triply distilled water. The solutions were deoxygenated by  $N_2$  bubbling for 10 min prior to the experiments, and a blanket of  $N_2$  were maintained during the experiments.

#### Stoichiometry determination by UV

The complex stoichiometry of CM- $\beta$ -CD with BFD was determined using Job's continuous variation method. A set of working solutions were obtained by mixing  $V_g$  mL of the stock BFD solution with  $(V_t - V_g)$  mL of the stock CM- $\beta$ -CD solution, where  $V_t$  is a fixed total volume and  $V_g$  is a variable value (from 0 to 10 mL,  $0 \leq V_g \leq V_t$ ). To determine the constant of complex stability of CM- $\beta$ -CD and BFD, the concentration of BFD ( $2 \times 10^{-5}$  mol L $^{-1}$ ) was kept constant with the concentration of CM- $\beta$ -CD ranging from  $2 \times 10^{-4}$  to  $3.2 \times 10^{-3}$  mol L $^{-1}$ .

#### Synthesis of 2-O-CM- $\beta$ -CD

23.4 g chloroacetic acid was dissolved in 20 mL water, and then injected into 150 mL aqueous solution of 16.2 g  $\beta$ -CD with 17.6 g sodium hydroxide. The reaction was kept at 90 °C for 3 h. Then the pH of the solution was adjusted to 7 with 5% HCl. The solution was concentrated to about 20 mL and then poured into 60 mL methanol to obtain precipitate. The precipitate was further purified by silica gel chromatography (eluent, isopropanol:ammonia (25%):water = 5:2:2, by volume) to give white powder product. Data of CM- $\beta$ -CD:  $R_f = 0.28$  ( $R_f = 0.42$  for  $\beta$ -CD; developer, isopropanol:ammonia (25%):water = 5:2:2, by volume).  $^1\text{H-NMR}$  (400 MHz,  $\text{D}_2\text{O}$ , 300 K, TMS,  $\delta$  ppm): 4.97–5.18 (d, 2.32 H, H-1, H-1'), 4.08–3.21 (m, 18.84 H, H-2, H-3, H-4, H-5, H-6,  $-\text{CH}_2\text{CO}-$ ).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{D}_2\text{O}$ , 300 K, TMS,  $\delta$  ppm): 177.95, 177.67 (C=O), 101.00 (C-1), 99.63, 99.27, 99.06 (C-1'), 80.82, 80.34 (C-4), 73.19 (C-3), 72.85 (C-2), 71.86 (C-5), 70.53, 70.38, 70.30 ( $\text{CH}_2$ ), 61.37 (C-6). ESI-MS: calculated  $m/z$  1309, found  $m/z$  1326 ( $\text{M} + \text{NH}_4^+$ ).

## Results and discussion

### The formation and the solvent effect on the vesicles

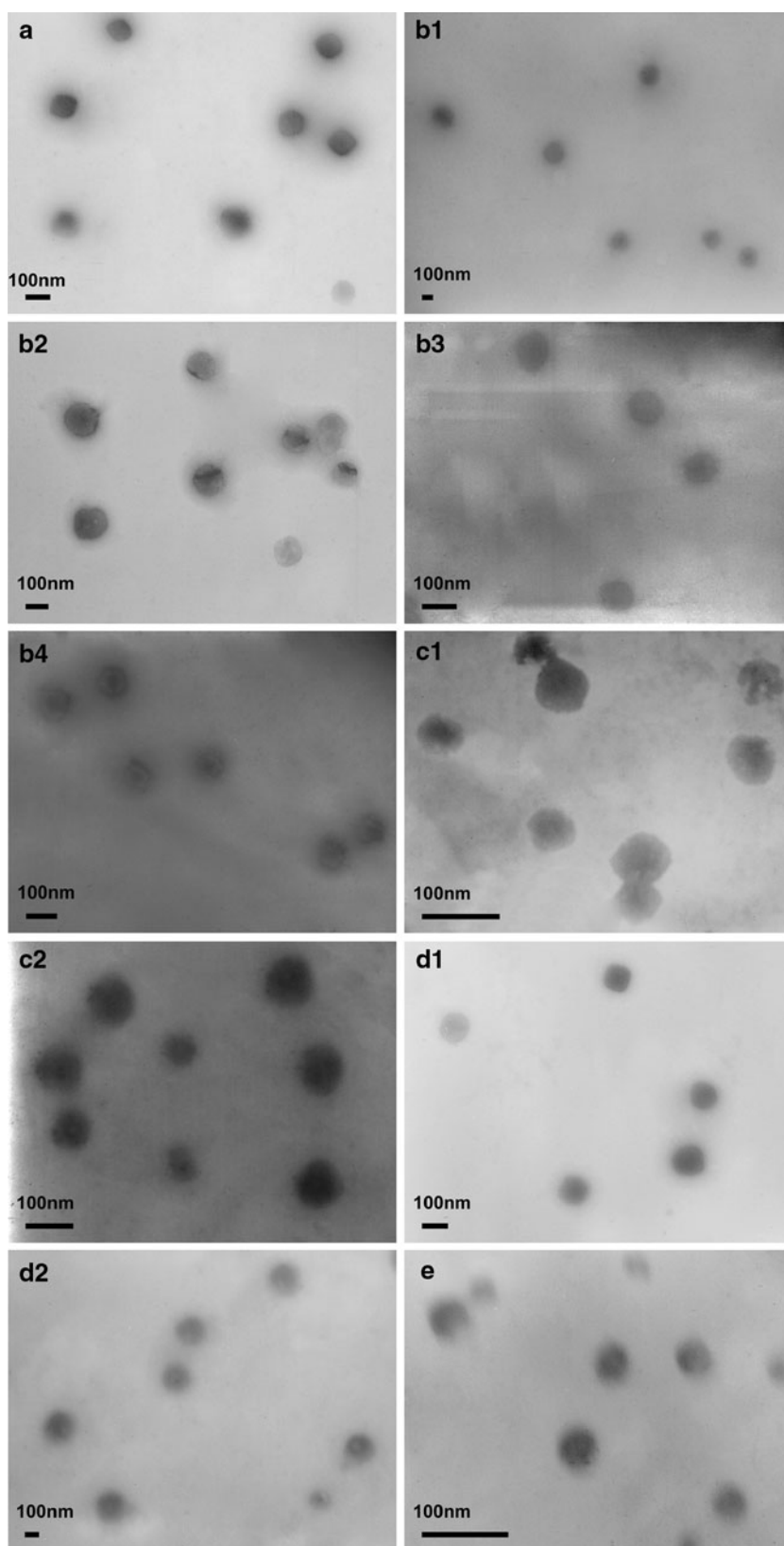
TEM observations were employed to investigate the aggregates of the inclusion complex of CM- $\beta$ -CD and BFD with molar ratio of 2:1. Spherical vesicular structures with diameters of about 60 nm were observed in water, as shown in Fig. 1a. However, no vesicles were observed by TEM in solution of BFD or CM- $\beta$ -CD alone. Also, only can micro-aggregates be observed in this concentration scale. The related reasons have been discussed in our previous work [15].

In order to further study the effects of alcohols on the vesicles, four systems (water/methanol, water/ethanol, water/isopropyl alcohol and water/tert-butanol) were employed, and the volume ratio of water and alcohols were changed from 2:1 to pure alcohols. The morphologies of the aggregates were investigated by TEM observations, as shown in Fig. 1b–e. The sizes and the size distributions of the vesicles were measured by DLS, as listed in Table 1, and shown in Fig. 2. It is worth to noting that the sizes of the vesicles measured by DLS are much larger than that observed by TEM (Table 1). This may be due to the shrinking of the vesicle samples dried in TEM observation which is different from that the samples in DLS measurement. Based on the TEM and DLS measurements, the morphologies of the aggregates were found to vary greatly. The vesicles would be destroyed with decreasing of the volume ratio of water/organic in the mixed solvents.

In the system of water/methanol, vesicles with an average diameter about 100 nm could be observed in all the mixture of the solvents, even in pure methanol. In the system of water/ethanol, vesicles could be formed when the volume ratio of water/ethanol was more than 1:1, while particles could be observed when that was less than 1:2, and irregular aggregates could be observed in pure ethanol. The phenomenon in the system of water/isopropanol was similar to that in water/ethanol. In the system of water/tert-butanol, vesicular hollow spheres could be obtained when the volume ratio of water/tert-butanol got to 2:1, and amorphous particles could be observed when that was about 1:1 while no aggregates could be observed when that was less than 1:2, including pure tert-butanol.

It is obvious that the vesicles can be destroyed easily not only with decreasing the ratio of water/alcohols, but also with the longer hydrophobic alkyl chain of the alcohol. Meanwhile, the vesicles prepared in the mixed solvents and pure alcohols are less stable than those in water. When the volume ratio of water/alcohols is less than 2:1, the vesicles could stay no more than 4 days. However, the vesicles prepared in water could stay more than 1 week. Generally, the higher concentration of alcohols results in the weaker

**Fig. 1** TEM images of the vesicles of CM- $\beta$ -CD with BFD (molar ratio, 2:1) in different solvents using phosphotungstic acid as the negative staining agent (*scale bars* = 100 nm). **a** water; **b1** water/methanol (2:1, v:v); **b2** water/methanol (1:1, v:v); **b3** water/methanol (1:2, v:v); **b4** methanol; (**c1**, v:v) water/ethanol (2:1, v:v); **c2** water/ethanol (1:1, v:v); **d1** water/isopropanol (2:1, v:v); **d2** water/isopropanol (1:1, v:v); **e** water/tert-butanol (2:1, v:v)



**Table 1** Morphologies and sizes of vesicles in different solvents

| Item                         | Aggregate morphology    | TEM (nm) | DLS (Rh, nm) |
|------------------------------|-------------------------|----------|--------------|
| Water/methanol, 2:1 (v:v)    | Vesicle                 | 200      | 150          |
| Water/methanol, 1:1 (v:v)    | Vesicle                 | 150      | 100          |
| Water/methanol, 1:2 (v:v)    | Vesicle                 | 100      | 80           |
| Methanol                     | Vesicle                 | 100      | 75           |
| Water/ethanol, 2:1(v:v)      | Vesicle                 | 60       | 50           |
| Water/ethanol, 1:1(v:v)      | Vesicle                 | 80       | 70           |
| Water/ethanol, 1:2(v:v)      | Particle, no vesicle    | 100      | –            |
| Ethanol                      | Irregular particles     | –        | –            |
| Water/isopropanol, 2:1(v:v)  | Vesicle                 | 100      | 80           |
| Water/isopropanol, 1:1(v:v)  | Vesicle                 | 200      | 150          |
| Water/isopropanol, 1:2(v:v)  | Particle, no vesicle    | 60       | –            |
| Isopropanol                  | No vesicle, no particle | –        | –            |
| Water/tert-butanol, 2:1(v:v) | Vesicle                 | 40       | 50           |
| Water/tert-butanol, 1:1(v:v) | Particle, no vesicle    | 60       | –            |
| Water/tert-butanol, 1:2(v:v) | No vesicle, no particle | –        | –            |
| Tert-butanol                 | No vesicle, no particle | –        | –            |

polarity of the solvents, and this would decrease the supramolecular interaction between CM- $\beta$ -CD and BFD.

The supramolecular interaction between CM- $\beta$ -CD and BFD can be shown by the corresponding inclusion constant, and a larger inclusion constant tends to mean that the inclusion compound is more stable with the same stoichiometry.

The apparent inclusion constants of CM- $\beta$ -CD with BFD were determined by UV double-reciprocal method based on the Eq. 1 [18, 19], as listed in Table 2.

$$1/\Delta A = 1/\alpha + 1/\alpha K [\text{CM}\beta\text{CD}]_0^n \quad (1)$$

where  $\Delta A$  is the change of absorbance of BFD in the present of CM- $\beta$ -CD,  $\alpha$  is a constant,  $[\text{CM}\beta\text{CD}]_0$  is the initial concentration of CM- $\beta$ -CD.  $K$  is the apparent constant for the formation of the 1:n inclusion complex, which could be calculated from a plot of  $1/\Delta A$  vs.  $1/[\text{CM}\beta\text{CD}]_0^n$ . As we speculate, the  $K$  decreases with the volume ratio of alcohols increasing in the mixed solvents.

The  $n$  value means the complex stoichiometries of CM- $\beta$ -CD with BFD, which can also be obtained based on the continuous variation method (Job's method) [20, 21]. The Job's plot for the binding of CM- $\beta$ -CD with BFD in water, as shown in Fig. 3, showed a maximum value at a molar fraction of 0.3, corresponding to 1:2 stoichiometry [22–25]. The results were consistent with those obtained by UV double-reciprocal method (Table 2). On the other hand, the stoichiometry changing of CM- $\beta$ -CD with BFD from a molar ratio of 2:1 to 1:1 also showed the weaker of the supramolecular interaction, as shown in Fig. 3.

In the mixed solvents, the polarity of the solvents became smaller and the solvophobic ability [26] of the alkyl chains of BFD became weaker with the addition of

ethanol and other alcohols, which was not beneficial to the formation of the vesicles. When alcohols with longer chains were used, the vesicle formation became much more difficult.

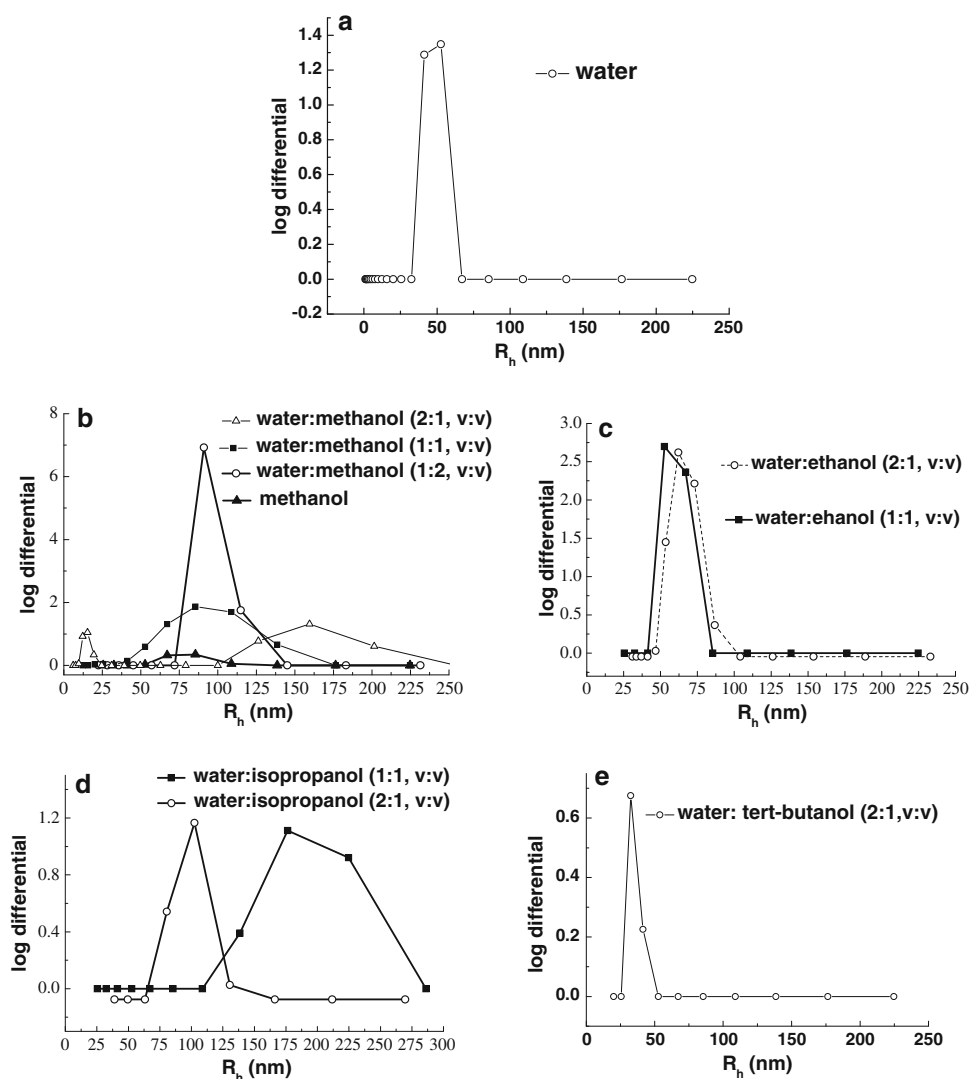
We assume that the two ferrocenyl groups of BFD are included by CM- $\beta$ -CD in water to form a two-head inclusion model like a bola surfactant through host–guest interactions. In the two-head inclusion model, CM- $\beta$ -CD acts as the hydrophilic fraction and the alkyl chain of BFD as the hydrophobic fraction. The two-head inclusion model can aggregate into vesicles when the supramolecular interaction of CM- $\beta$ -CD with BFD is strong enough. With the addition of alcohols in the solvents, the interaction between the two-head inclusion models as well as the inclusion ability of CM- $\beta$ -CD with BFD would become weaker, even the inclusion stoichiometry of CM- $\beta$ -CD with BFD turns to a molar ratio of 1:1 from 2:1.

### Possible formation mechanism of the vesicles

#### The inclusion and conformation

According the above data, we could hold that the supramolecular interaction of CM- $\beta$ -CD with BFD is crucial to the vesicle formation. The interaction of CM- $\beta$ -CD with BFD in water was studied by  $^1\text{H-NMR}$ , which is one of the most powerful tools for realizing supramolecular assemblies in solution [27]. As shown in Fig. 4, the chemical shift of H-3 and H-5 of CM- $\beta$ -CD in combination of CM- $\beta$ -CD with BFD ( $\delta = 3.68\text{--}3.85$ ) have clearly positive shifts compared with those of CM- $\beta$ -CD ( $\delta = 3.62\text{--}3.83$ ). Compared to those of individual BFD ( $\delta = 4.25\text{--}4.31$ ), the

**Fig. 2** DLS size distributions of CM- $\beta$ -CD with BFD (molar ratio, 2:1) in different solvents. **a** water; **b** water/methanol; **c** water/ethanol; **d** water/isopropanol; **e** water/tert-butanol



**Table 2** The apparent inclusion constants between CM- $\beta$ -CD and BFD in different solvents at the maximum absorption

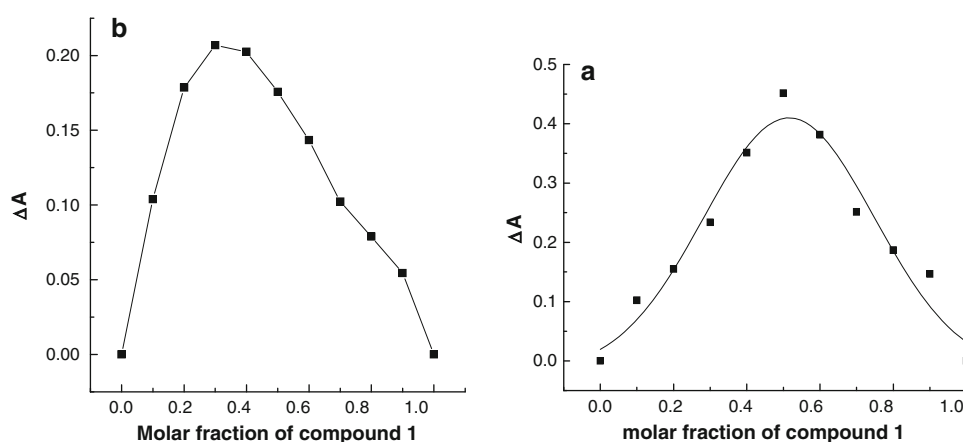
| Solvent composition          | Complex stoichiometry | Apparent inclusion constant (K)                 | R       |
|------------------------------|-----------------------|---|---------|
| Water                        | 2:1                   | $3.29 \times 10^6 \text{ L}^2 \text{ mol}^{-2}$ | 0.99468 |
| Water/methanol, 2:1 (v:v)    | 1:1                   | $4215 \text{ L mol}^{-1}$                       | 0.99792 |
| Water/methanol, 1:1 (v:v)    | 1:1                   | $2565 \text{ L mol}^{-1}$                       | 0.99849 |
| Water/methanol, 1:2 (v:v)    | 1:1                   | $2166 \text{ L mol}^{-1}$                       | 0.99258 |
| Water/ethanol, 2:1(v:v)      | 1:1                   | $2379 \text{ L mol}^{-1}$                       | 0.9972  |
| Water/ethanol, 1:1(v:v)      | 1:1                   | $1867 \text{ L mol}^{-1}$                       | 0.99653 |
| Water/isopropanol, 2:1(v:v)  | 1:1                   | $2210 \text{ L mol}^{-1}$                       | 0.99487 |
| Water/isopropanol, 1:1(v:v)  | 1:1                   | $1715 \text{ L mol}^{-1}$                       | 0.99715 |
| Water/tert-butanol, 2:1(v:v) | 1:1                   | $1878 \text{ L mol}^{-1}$                       | 0.99673 |

hydrogen of the ferrocene moieties ( $H_a\delta = 4.33$ ) in the combination system of CM- $\beta$ -CD with BFD have clearly positive shifts, and the peak intervals among  $H_a$ ,  $H_b$  and  $H_c$  become wider. All these variations in  $^1\text{H-NMR}$  spectra imply that the ferrocene moieties in the combination

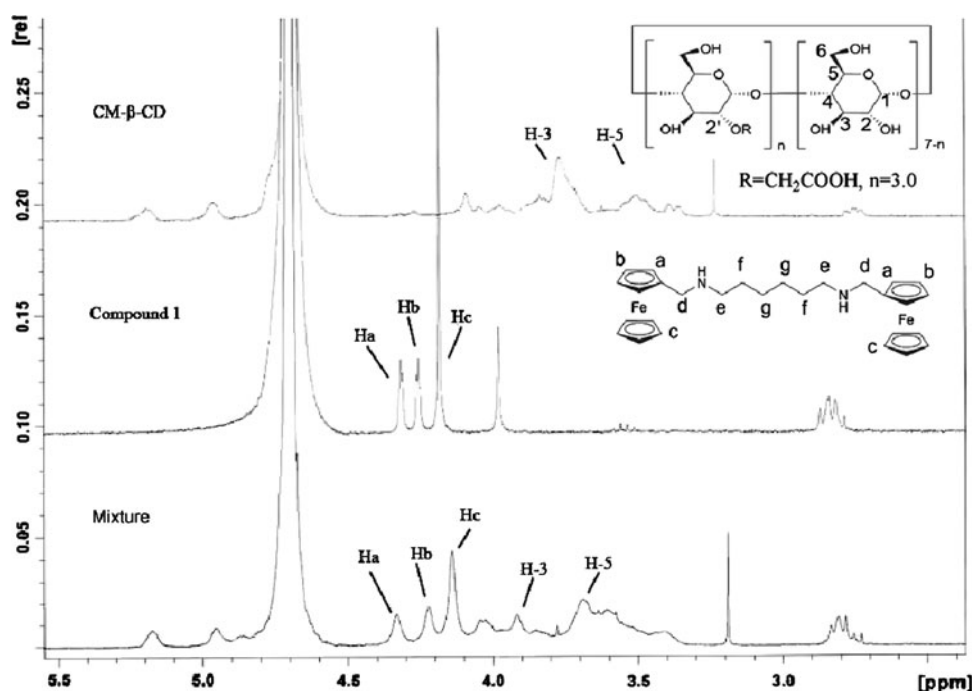
system of CM- $\beta$ -CD with BFD are included in the cavity of CM- $\beta$ -CD.

The conformation of the inclusion complex was also confirmed by the cyclic voltammetry (CV). The typical cyclic voltammograms of the solution of CM- $\beta$ -CD with

**Fig. 3** Job's curve of solution of CM- $\beta$ -CD with BFD by UV in different solvents: **a** water; **b** water/methanol (2:1, v:v)



**Fig. 4**  $^1\text{H-NMR}$  spectra (300 MHz) of the inclusion system of CM- $\beta$ -CD with BFD ( $\text{D}_2\text{O}$ ,  $10^{-4}$  mol  $\text{L}^{-1}$ ) in comparison with the individual compound BFD ( $\text{D}_2\text{O}$ ,  $10^{-4}$  mol  $\text{L}^{-1}$ ) and CM- $\beta$ -CD ( $\text{D}_2\text{O}$ ,  $10^{-4}$  mol  $\text{L}^{-1}$ ) at 295 K ( $\delta = 4.69$  ppm is the reference for  $\text{H}_2\text{O}$ )



BFD are shown in Fig. 5. It is clearly that the exclusive couple of the anodic and cathodic curves caused by  $\text{Fc}/\text{Fc}^+$  and the decrease of the anodic peak current of the solution of CM- $\beta$ -CD with BFD, which suggest the inclusion of both the ferrocene moieties by CM- $\beta$ -CD [28]. The inclusion phenomenon is consistent to that obtained by  $^1\text{H-NMR}$  spectra (Fig. 4).

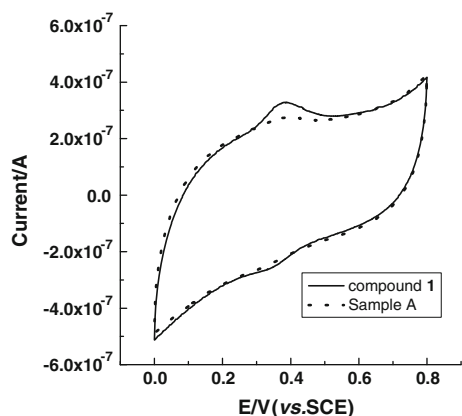
Models for the possible formation mechanism of the vesicles

Based on the data as indicated by  $^1\text{H-NMR}$ , CV and UV, mechanism “A” is assumed for the formation of the vesicles in the inclusion system of CM- $\beta$ -CD with BFD and mechanism “B” is suggested for the transformation of the

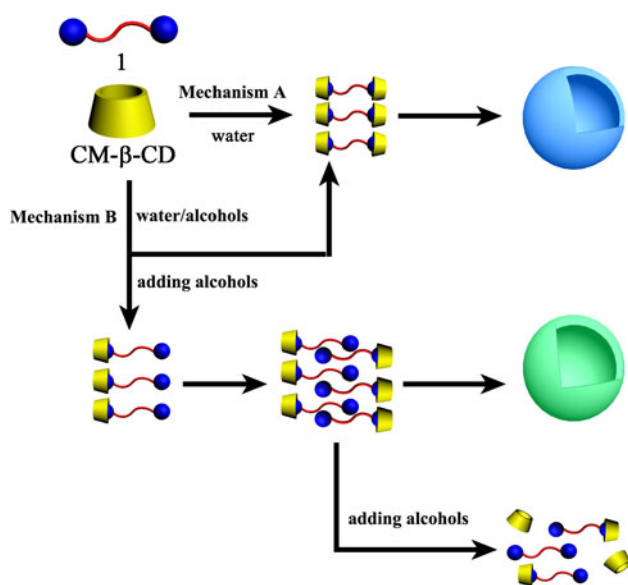
vesicles in the mixed solvents from water, which is illustrated in Fig. 6.

In mechanism “A”, both of the ferrocene moieties are included by the hydrophobic cavities of CM- $\beta$ -CD to form two hydrophilic “heads” like a bola amphiphile through host–guest interactions [29]. The carboxymethyl functions (three per host molecule on average) could form strong hydrogen bonds with nitrogen atoms of compound BFD, which could transform into ionic bonds and enhance the stability of the supramolecular complex for the formation of the vesicles.

In mechanism “B”, with the addition of alcohols, the stability of the inclusion compounds of CM- $\beta$ -CD with BFD would be decreased [30]. And then some of the inclusions would dissociate, even the stoichiometry of CM-



**Fig. 5** Cyclic voltammograms of compound BFD ( $10^{-4}$  mol L $^{-1}$ ) in H $_2$ O with NaCl (0.05 mol L $^{-1}$ ) as the supporting electrolyte, recorded at a glassy carbon electrode (2 mm diameter) at a scan rate of 50 mV s $^{-1}$ , in the absence (straight line) or presence of CM- $\beta$ -CD (dotted line)



**Fig. 6** Possible mechanisms for the formation of the vesicles in water (a); and the transformation of the vesicles in mixed solvents (b)

$\beta$ -CD with BFD would turn to the molar ratio of 1:1 from 2:1. On the other hand, the inclusion compounds of CM- $\beta$ -CD and BFD with a molar ratio of 1:1 could still self-aggregate into vesicles until the alcohols become relatively too much in the solvent, for example, the solution of water/ethanol (1:2, by v:v) or water/tert-butanol (1:1, by v:v) (Table 1).

## Conclusions

The self-assembly vesicles, which are based on the supramolecular complexation of CM- $\beta$ -CD and BFD in two-

head inclusion model as well as one-head inclusion model, were prepared and characterized by TEM and DLS,  $^1$ H-NMR and CV in aqueous solution and mixed solvents. The polarities and steric hindrances of the mixed solvents would play key roles on the morphological changes of the aggregates. The morphologies of the aggregates would vary gradually, even be destroyed with the increasing of alkyl chain of the alcohols, as well as the increasing of the volume ratio of the alcohol/water in mixed solvents. It is notable that the complex of CM- $\beta$ -CD with BFD can form vesicles based on different inclusion compounds in molar ratio of 1:1 or 2:1. The study might be of great value for the applications of vesicles in biosimulation, drug-delivery, smart materials and synthesis.

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