Encapsulation studies and selective membrane permeability properties of self-assembly hollow nanospheres†

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This paper presents a kind of self-assembled hollow nanosphere for enzyme encapsulation. The enzyme molecules were encapsulated in hollow spheres directly in aqueous solution through the self-assembly of rod-coil Alg-*g*-PEG and α-CDs complexes. These Alg-*g*-PEG/α-CD hollow spheres showed semi-permeability which could prevent big enzyme molecules from leaving while allowing small substrates and products to pass through to maintain the enzyme activity. The permeability properties of Alg-*g*-PEG/α-CD hollow nanospheres were investigated by encapsulating different probes. The results showed that the molar mass cutoff (MMCO) of these hollow nanospheres was between 20 and 40 kDa. The encapsulation capability of Alg-*g*-PEG/α-CD hollow nanospheres was also investigated, the results indicated a maximal values for L-asparaginase encapsulation. Furthermore, the encapsulation behavior for L-asparaginase showed PEG graft density (GD) dependence.

1. Introduction

Encapsulation has been developed as a tool for the transport of cells or proteins into the human organism. The therapeutic potential of encapsulated cells or proteins is promising for treating patients who suffer from tissue loss, neurodegenerative disorders, diabetes, liver failure, and other diseases caused by specific vital cellular dysfunctions.[1] Most biological processes, such as molecular recognition, signal transduction, and molecular transport, occur at native cell surfaces. Thus, a variety of capsules with semi-permeability membrane were extensively investigated as cell membrane models in the past decades.[2–5] To be efficient for biomedical applications, the membrane of capsules must be able to not only protect the cells or proteins from attack by the host immune system but also maintain viable functions by allowing the passage of oxygen, nutrients and substrates as well as the egress of the products. Consequently, it is necessary to measure, and then to control, the permeability of the membrane of capsules. Furthermore, as a nanocarrier, large encapsulation capability is also desirable.

Polymeric hollow nanospheres are attractive functional materials due to their potential for encapsulation of large quantities of guest molecules or large sized guests within the "empty" core domain.[6–8] Hollow nanospheres could be obtained by different approaches, such as layer-by-layer (LBL) self-assembly, core-shell micelles made of block copolymer or non-covalently connected micelles (NCCM),[9–11] however, these methods need physical or chemical procedures to remove the core or the template.[12] Since Jenekhe and Chen reported that rigid-coil copolymers could self-assemble to form hollow nanospheres directly in their selective solvent,[13,14] over the past ten years, this novel approach aroused many researchers' interest in using rigid-coil systems to construct hollow nanospheres.[15–19] However, these self-assembled hollow nanospheres lack biocompatibility and non-biodegradability because the rod-like blocks are π-conjugated long chains formed by the synthesis method.

We have developed a novel approach to construct hollow nanospheres by inclusion alginate-*graft*-poly(ethylene glycol) (Alg-*g*-PEG) and α-cyclodextrin (α-CD), in which the rod-like blocks are formed by self-assembly of α-CD with poly(ethylene glycol) (PEG).[20] Because of having biocompatibility and degradability, Alg-*g*-PEG/α-CD hollow nanospheres were extended to investigate the encapsulation behavior of enzyme. It was found that the Alg-*g*-PEG/α-CD hollow nanospheres not only enable loading of more L-asparaginase due to having cavities, but also show semi-permeability which could prevent the enzyme from leaving to stabilize the enzyme while allow substrates and products to pass through to maintain the enzyme activity. The encapsulated L-asparaginase showed significantly higher stability and maintained most of the activity.[21] In this paper, we expand the study on evaluating the permeability properties of Alg-*g*-PEG/α-CD hollow nanospheres. Vitamin B_2, vitamin B_{12} and four globular shape proteins with different molar masses were used as permeability probes. Furthermore, as a nanocarriers for enzyme encapsulation, the encapsulation capability and the influences of PEG content in Alg-*g*-PEG on the encapsulation efficiency of L-asparaginase were also

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investigated. The aim of this research is to give useful information for applying Alg-*g*-PEG/α-CD hollow nanospheres as cell or proteins carriers in the biomedical field or as model systems to study biological processes at membranes.

2. Experiments

2.1 Materials and instruments

Alginate was purchased from Tianjin Yuanhang Chemicals Co., Ltd., China. α-CD was purchased from TCI Co. Ltd., Tokyo. L-Asparaginase from *E. coli* with absolute activity of 889.34 U/mg (the absolute activity of the enzyme was determined by the literature method)[22] was purchased from Changzhou Bio-Pharma Qianhong Co., China. mPEG ($M_W = 2000$), vitamin B_2, vitamin B_{12}, and Albumin (from chicken egg white) were purchased from Sigma-Aldrich Co., Inc. Albumin bovine fraction and trypsin inhibitor (soybean) were purchased from Amresco Inc. Other reagents were analytical pure and used directly without further purified.

^{1}H NMR were measured on a Bruker 600 MHz spectrometer. The chemical shifts of ^{1}H NMR are expressed in parts per million downfield relative to the internal tetramethylsilane ($\delta = 0$ ppm) or chloroform ($\delta = 7.26$ ppm). The crystalline changes in the hollow nanospheres were confirmed by X-ray diffraction measurements, which were performed by using Cu-Kα irradiation with PHILP X'Pert MPD (20 kV; 35 mA; 2°/min). The centrifugations were taken on TGL-20M, Saite Centrifuge Co., Shanghai, China. The absorbance value of the solution was recorded on APL-UV-2000 spectrophotometer, Shanghai, China. The dynamic light scattering (DLS) measurements were carried out on Brookhaven BI-200SM equipment at a wavelength of 532 nm and a scattering angle of 90°. The transmission electron microscopy (TEM) were measured by Jeol JEM-100CX, and observation was done at an accelerating voltage of 80 KV. Atomic force microscope (AFM) were measured by Nanoscope Multimode, Vecco Instruments, USA.

2.2 Synthesis of the Alg-*g*-PEG

The Alg-*g*-PEG was synthesized by following our previous work and literature procedure.[21,23] In this case, for synthesis of Alg-*g*-PEG, a 2% (w/w) sodium alginate solution was prepared in a buffer solution of 0.1 M MES and 0.5 M NaCl, and the pH was adjusted to 6. The molar ratio of EDC : NHS : COO^- was 1 : 0.5 : 1, a series of different moles of NHS and EDC were added to a 2% (w/w) alginate solution to activate the carboxy groups on the polymer backbone. The solution was agitated for 10 h at room temperature to obtain a homogeneous solution followed by the addition of PEG-amine. The solution was dialysed by bag filter (MWCO: 8000–14000) at 37 °C for 36 h, the solvent was evaporated, washed with acetone 6 times and dried under vacuum at room temperature.

Alg-*g*-PEG: ^{1}H NMR (600 MHz, D_2O, ppm): δ 3.33 (s, $\mathbf{OCH_3}$), 3.53–3.76 (m, $\mathbf{OCH_2CH_2}$) 3.91 (s, $\mathbf{NCH_2}CO$), 4.37 (m, $COO\mathbf{CH_2}CH_2$), 3.85, 4.00, 4.06, 4.29 (**CH**–OH of alginate).

2.3 Preparation of the hollow nanospheres

2 mL Alg-*g*-PEG solution were added dropwise into 6 mL α-CD [6% (w/v)] solution and then stirred for 3 h at room temperature. After the solution turned to muddy and slightly blue, the hollow nanospheres were collected by centrifugation at 16,000 rpm and lyophilized for the further utilization.

2.4 The morphology of Alg-*g*-PEG/α-CD hollow nanospheres

The lyophilized Alg-*g*-PEG/α-CD powder was dispersed by deionized water to obtain 3‰ and 0.1‰ (w/v) Alg-*g*-PEG/α-CD hollow nanosphere solutions, the samples for TEM investigation were prepared by applying a drop of the hollow nanosphere solution (3‰) onto a copper screen and drying at room temperature, the observation was done at an accelerating voltage of 80 kV. The samples for AFM investigation were prepared by applying a drop of the hollow nanospheres solution (0.1‰) onto freshly cleaved mica and dried at room temperature in desiccator.

2.5 The permeability of Alg-*g*-PEG/α-CD hollow nanospheres

Vitamin B_2, vitamin B_{12}, trypsin inhibitor (soybean), Albumin (from chicken egg white), Albumin bovine fraction and L-asparaginase were used as permeability probes to investigate the permeability of Alg-*g*-PEG/α-CD hollow nanospheres.

2.5.1 The encapsulation behavior for vitamin B_2. 1 mL Alg-*g*-PEG solution [0.25% (w/v)] (with 0.04–0.12 mg vitamin B_2) were added dropwise into 3 mL α-CD (6%) solutions and stirred for 3 h at room temperature. The solution turned to muddy obviously and the hollow nanospheres with encapsulated vitamin B_2 were collected by centrifugation at 16,000 rpm. The supernatants of the centrifugation were collected. The absorbance value of the supernatants solution at 440 nm was recorded on a spectrophotometer. The absolute mass of the vitamin B_2 in the supernatants were calculated with the standard curve plotted by the $\Delta A_{440\ nm}$ of the standard *versus* the vitamin B_2 concentration.

The hollow nanospheres which contain vitamin B_2 prepared above were kept in 1 mL deionized water at 4 °C for 48 h. Then, the mixture was separated by centrifugation at 16,000 rpm. The clear supernatant was collected and the mass of vitamin B_2 it contained was determined.

2.5.2 The encapsulation behavior for vitamin B_{12}. 1 mL Alg-*g*-PEG solution [0.25% (w/v)] (with 0.08–0.24 mg vitamin B_{12}) were added dropwise into 3 mL α-CD (6%) solutions, stirred for 3 h, the solution turned to muddy obviously, the hollow nanospheres with encapsulated vitamin B_{12} were collected by centrifugation at 16,000 rpm. The supernatants of the centrifugation were collected. The absorbance value of the supernatants solution at 361 nm was recorded on a spectrophotometer. The absolute mass of the vitamin B_{12} in the supernatants were calculated with the standard curve plotted by the $\Delta A_{361\ nm}$ of the standard *versus* the vitamin B_{12} concentration.

The hollow nanospheres which contain vitamin B_{12} prepared above were kept in 1 mL deionized water at 4 °C for 48 h. Then, the mixture was separated by centrifugation at 16,000 rpm. The

clear supernatant was collected and the mass of vitamin B_{12} it contained was determined.

2.5.3 The encapsulation behavior for trypsin inhibitor (soybean), Albumin (from chicken egg white), Albumin bovine fraction and L-asparaginase. 1 mL Alg-*g*-PEG solution [0.25% (w/v)] (with 1–3 mg proteins) were added dropwise into 3 mL α-CD (6%) solutions, stirred for 3 h, the solution turned to muddy obviously, the hollow nanospheres with encapsulated proteins were collected by centrifugation at 16,000 rpm. The supernatants of the centrifugation were collected. The amount of free proteins in the clear supernatant was measured by spectrophotometry at 595 nm using Bradfold protein assay.[24]

The hollow nanospheres which contain proteins prepared above were kept in 1 mL deionized water at 4 °C for 48 h. Then, the mixture was separated by centrifugation at 16,000 rpm. The clear supernatant was collected and the mass of proteins it contained was determined.

The encapsulation efficiency (EE) was calculated using the eqn (1).

$$\text{EE}\ (\%) = \frac{\text{mass of probes used in formulation} - \text{mass of free probes in supernatant}}{\text{mass of probes used in formulation}} \times 100 \quad (1)$$

The leakage efficiency (LE) from the hollow nanospheres was calculated using eqn (2).

$$\text{LE}\ (\%) = \frac{\text{mass of probes detected in the supernatant}}{\text{mass of probes encapsulated}} \times 100 \quad (2)$$

2.6 The encapsulation capability (EC) for L-asparaginase

1 mL 0.25% Alg-*g*-PEG solution with 0.5–10 mg L-asparaginase were added into 3 mL α-CD (6%) solutions, after the process of hollow nanospheres formation completed, the EE and LE were detected by above mentioned method.

2.7 The influences of PEG graft density (GD) in Alg-*g*-PEG on the encapsulation behavior of L-asparaginase

Alg-*g*-PEGs with different GD of PEG [0.25% (w/v)] (with 1 mg L-asparaginase) were added dropwise into 3 mL α-CD (6%) solutions, after the process of hollow nanospheres formation completed, the EE and LE were detected by above-mentioned method.

3. Results and discussion

3.1 Self-assembly of Alg-*g*-PEG/α-CD hollow nanospheres for enzyme encapsulation

Respectively added the Alg-*g*-PEG solution with or without L-asparaginase dropwise to the α-CD solution in water, both mixed solution became slightly turbid gradually, indicating the formation of some kind of aggregates. The morphology of the aggregates was studied by TEM and AFM. As shown in Fig. 1c, an obvious contrast between the central and outer part of the particles was observed for the aggregates without L-asparaginase, which is a typical of TEM images of hollow nanospheres

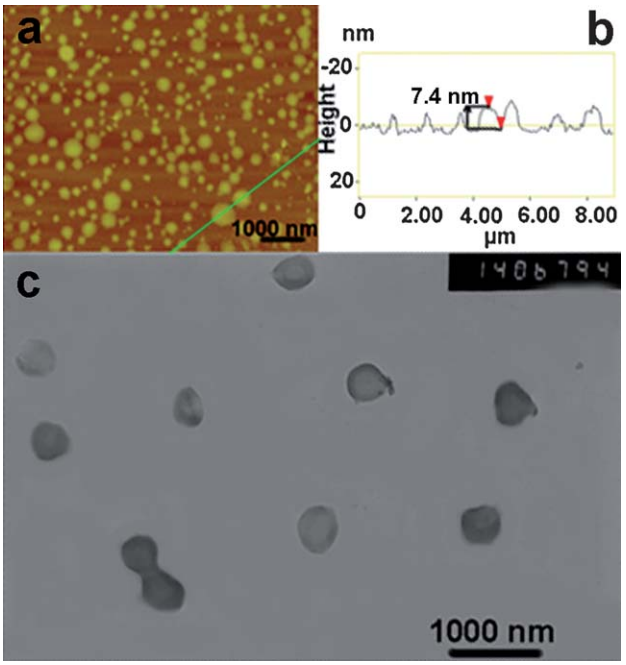

Fig. 1 (a) A height AFM image of Alg-*g*-PEG/α-CD hollow nanospheres. (b) An AFM scan line for the particle-height analysis. (c) A TEM image of Alg-*g*-PEG/α-CD hollow nanospheres.

reported for different kinds of hollow particles.[15–19,25] In the AFM micrograph (Fig. 1a), the aggregates without L-asparaginase also showed round outline shapes and their diameters were similar to those observed by TEM (400–500 nm). It should be noted that the height of the aggregates was around 7 nm (Fig. 1b), two order of magnitude smaller than the average diameter of horizontal direction. As reported for AFM obsevation, such remarkable size difference between the horizotal and vertical directions of spheres was always attributed to hollow structure.[26] Clearly, what we observed here are collapsed hollow nanospheres, like two-layered cakes. The introduction of L-asparaginase into the system have no effect on the self-assembly procedure. However, the TEM image (Fig. 2c) showed that no bright domains can be found within the spheres with entrapped L-asparaginase. In contrast, the cores of spheres showed a little darker than the outlines of spheres. This suggests that the presences of enzyme molecules have no effect of the self-assembly procedure and the hollow spheres are filled with enzyme molecules. AFM micrograph showed that the height of Alg-*g*-PEG/α-CD nanospheres with L-asparaginase was around 38 nm (Fig. 2b), which was about 5 times the height of hollow spheres without enzyme.

Such significant change further demonstrated that the enzyme was encapsulated in the hollow spheres. After the nanospheres collapsed, the enzyme was sandwiched between two layered membranes and contributed to the increase in the height.

X-Ray diffraction (XRD) results showed that the inclusion formation between the Alg-*g*-PEG and α-CD molecules. As shown in Fig. 3, the pattern of Alg-*g*-PEG/α-CD particles was similar to that of the PEG-α-CD inclusion complex ($2\theta = 20°$), which has been reported to have a rod channel structure,[27–29] while the homoPEG crystalline peaks ($2\theta = 19.2°$ and 23.3°) and

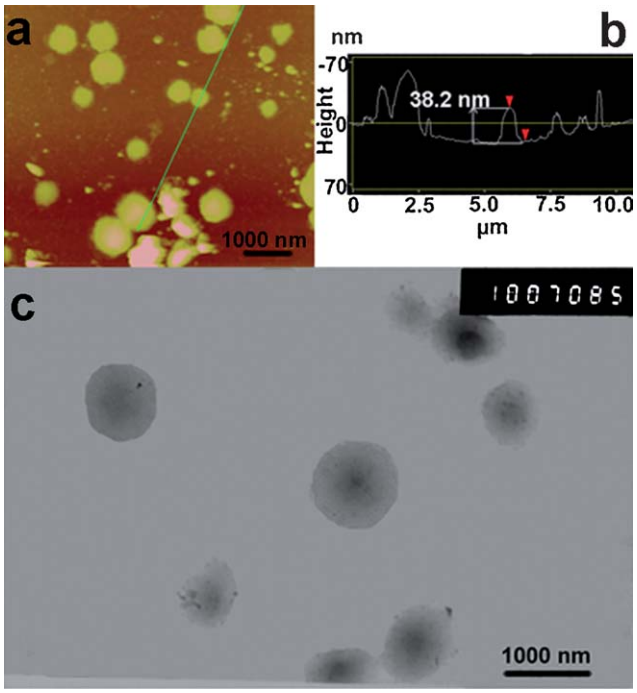

Fig. 2 (a) A height AFM image of Alg-*g*-PEG/α-CD hollow nano spheres entrapped with enzyme. (b) An AFM scan line for the particle-height analysis. (c) A TEM image of Alg-*g*-PEG/α-CD hollow nanospheres entrapped with enzyme.

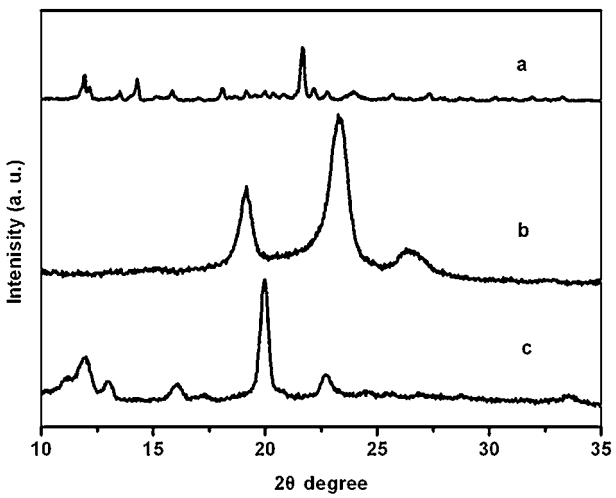

Fig. 3 X-Ray diffraction patterns of (a) α-CD (b) Alg-*g*-PEG (c) Alg-*g*-PEG/α-CD particles.

α-CD crystalline peak ($2\theta = 21.5°$) were absent. This implies that α-CD rings are stacked along the graft PEG chain axis to form a channel-type crystalline structure. As a result, the Alg-*g*-PEG/α-CD complexes have both a rod block (PEG-α-CD inclusion) and a coil block (protonated Alg backbone). Jenekhe and Chen[13,14] and Jiang and coworkers[15–19] have reported that the rod-like block in the rod-coil system preferred crowded parallel packing and resulted in the formation of hollow spheres for necessary of the efficient space-filling packing. With the formation of insoluble PEG-α-CD inclusion blocks, water became a selective solvent for the Alg-*g*-PEG/α-CD, so hollow spheres were formed. The expected structure of such an aggregate in aqueous solution is an inner PEG-α-CD inclusion block surrounded by the protonated coil-like Alg shell.

The Alg-*g*-PEG/α-CD spheres with L-asparaginase was separated by centrifugation. The encapsulation efficiency, the leakage efficiency and the activities of the encapsulated L-asparaginase were calculated.[21] As shown in Table 1, the encapsulation efficiency was increased with the concentrations of Alg-*g*-PEG increasing. This maybe due to the fact that the amount of hollow spheres increased as the concentration of polymer increased. But when the concentration of Alg-*g*-PEG was above 0.375%, the encapsulation efficiency almost remain stable. It is showed that the leakage efficiency is low (below 10%) and the activity of encapsulated enzyme is high (above 77%) for all of samples. These results indicate that: the membrane of Alg-*g*-PEG/α-CD hollow spheres is semi-permeable for L-asparaginase, which prevent the enzyme from diffusion into the surrounding solution while allowing substrates and products to pass through. Furthermore, It should be noticed that when the concentration of α-CD was above 6%, the formation, structure and compact degree of the hollow spheres as well as the encapsulation and leakage efficiency of enzyme would not be affected by the concentration of α-CD anymore (Table S1, Fig. S8 and S9†). The schematic illustration of the process of enzyme encapsulation was shown in Fig. 4.

3.2 The permeability of Alg-*g*-PEG/α-CD hollow nanospheres

As an extended work for investigating the encapsulation behavior of Alg-*g*-PEG/α-CD hollow nanospheres, it is necessary to measure the permeability of the membrane of those hollow nanospheres. During our experiments, it was found that the high

Table 1 The encapsulation efficiency, leakage efficiency and relative activity of encapsulated enzyme of nanospheres

Entry	Concentrations of Alg-*g*-PEG (%)	Encapsulation efficiency (%)	Leakage efficiency (%)	Relative activity of encapsulated enzyme (%)
1	0.125	45.4	6.3	87.2
2	0.187	49.7	7.3	77.7
3	0.250	72.4	1.3	85.5
4	0.375	81.9	5.4	110.7
5	0.500	80.1	1.3	109.7

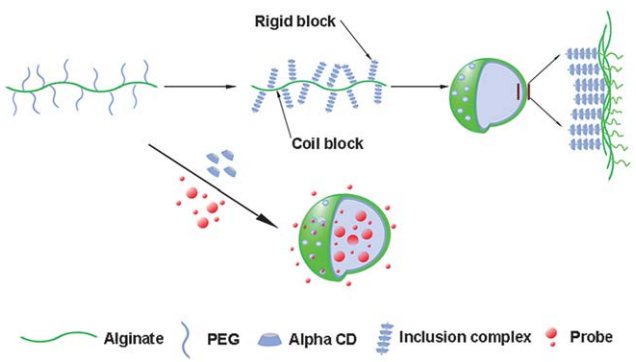

Fig. 4 A schematic illustration of the formation of the hollow nanospheres and the membrane permeability for probe molecules.

enzyme encapsulation efficiency always accompanied low enzyme leakage efficiency. On the contrary, the low enzyme encapsulation efficiency always accompanied high enzyme leakage efficiency. We have confirmed that the enzyme was encapsulated into the hollow nanospheres during the process of hollow nanospheres formation, therefore, if the membrane of the hollow nanospheres allows the enzyme to pass through freely, the high enzyme encapsulation efficiency and low enzyme leakage efficiency would not be obtained (Fig. 4). Thus, the encapsulation and leakage efficiency of the probe molecules could reflect the permeability of the membrane directly.

The characteristics of probes utilized for permeability estimation are shown in Table 2, Vitamin B_2 and Vitamin B_{12} are frequently utilized for detecting the permeability of the membrane of capsules, [30,31] other probes are proteins or enzyme which have similar isoelectric point (pI) and structure. Proteins can be classified as complex ampholytes, they are generally charged and can electrostatically interact with charged components of the capsule.Thus, in addition to steric hindrance, electrostatic interactions can also influence permeability measurements with proteins. All the used proteins have a globular shape. Their radius of gyration varies from 2.3 to 4.4 nm (Table 2). All proteins possess an isoelectric point (defined as the pH at which the net charge averaged over all the protein is zero) in the range between 4.5 and 4.7 (Table 2). At the physiological pH used for the permeability experiments (pH 7) the proteins are in an anionic form (Table 2). Following the recommendation of Stewart and Swaisgood,[33] the physiological pH should be above the pI of protein permeants to minimize ionic adsorption to the negatively charged polymers such as alginate. Thus, influence of charge density and shape carried by the protein will be negligible.

As shown in Fig. 5, after 3 h encapsulation, the encapsulation efficiencies of the probes which have a molar mass below 21.5 kDa (vitamin B_2, vitamin B_{12} and trypsin inhibitor (soybean)) are below 20%. However, the encapsulated probes totally permeate the medium after 48 h of diffusion in deionized water. This suggests that the membranes of the hollow nanospheres were permeable to the molecules with molar mass of 21.5 kDa and lower. A small amount of the probes' (vitamin B_2, vitamin B_{12} and trypsin inhibitor (soybean)) encapsulation efficiency may be ascribed to the slight interaction between probes and one or several components of the capsular membrane.

When the molar mass of probes are above 44.3 kDa, (albumin (from chicken egg), albumin bovine fraction and L-asparaginase), their encapsulation efficiency are above 50%, and their leakage efficiency are all below 5% after 48 h of diffusion in deionized water. These results suggest that the membranes of the hollow nanospheres were not permeable to the molecules with a molecular weight above 40 kDa. Moreover, it also indicated those proteins do not absorb at the surface of the hollow nanospheres. The different encapsulation efficiency may be due to the structural discrepancy of those proteins. In summary, the molar mass cutoff (MMCO) of the Alg-*g*-PEG/α-CD hollow nanospheres determined by the encapsulation and leakage behavior of the probes can be approximately estimated as between 20 and 40 kDa.

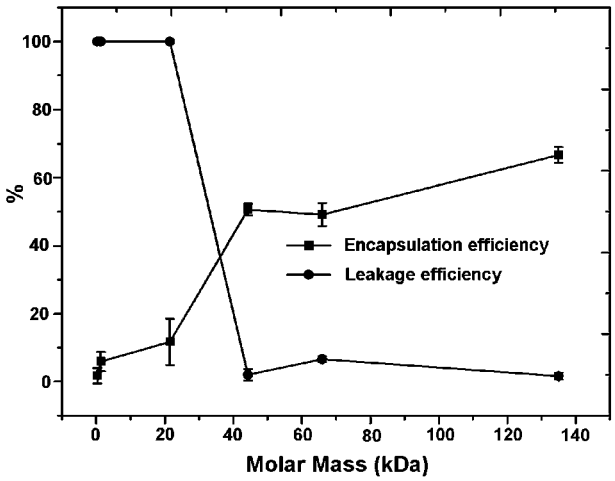

Fig. 5 The encapsulation and leakage efficiency of different probes.

$$R_g = 0.051\ MM^{0.378}\ \text{for globular proteins}\ ^{32} \quad (3)$$

MMCO, as an indicator of permeability, is a relative measure related to the permeate size in solution, and is dependent on the chemistry of the macromolecules and the solvent quality. Therefore, one should not expect the MMCOs to be numerically equivalent to the real membrane permeability of capsule if using protein based probes. However, as each protein we used has a globular shape, and their radius of gyration were estimated using eqn (3) varies from 2.3 to 4.4 nm (Table 2). Therefore, the MMCO results of the Alg-*g*-PEG/α-CD hollow nanospheres determined by the encapsulation and leakage behavior of the probes can reflect the pore size of the membrane of the hollow nanospheres directly. According to the MMCO results, the pore size (radius) of the membrane of the hollow spheres can be approximately estimated as between 2 and 3 nm. This suggests that the molecules which have radiuses of gyration below 2 nm can pass through the membranes of Alg-*g*-PEG/α-CD hollow nanospheres freely.

Table 2 The characteristics of probes utilized for permeability estimation

Probe	Molar mass (kDa)	Isoelectric point	Inoic Form at pH 7	Structure	Radius of gyration/nm[a]
Vitamin B_2	0.4	—	—	—	—
Vitamin B_{12}	1.4	—	—	—	—
Trypsin inhibitor, soybean	21.5	4.5	Negative	Globular shape	2.26
Albumin (from chicken egg white)	44.3	4.7	Negative	Globular shape	2.92
Albumin bovine fraction	68.0	4.7	Negative	Globular shape	3.42
L-Asparaginase	130–140	4.7	Negative	Globular shape	4.43

[a] Protein dimensions were estimated using eqn (3).

3.3 The maximum encapsulation capacity (EC) for L-asparaginase

As a nanocontainer for enzyme encapsulation, it is necessary to measure the maximum encapsulation capacity for enzyme. Taking the L-asparaginase as a model enzyme and fixing Alg-*g*-PEG/α-CD at a fixed concentration (0.25%/6%) and solution volume (4 mL), the influence of the amount of enzyme on the encapsulation capacity of Alg-*g*-PEG/α-CD hollow nanospheres was investigated.

As shown in Fig. 6, the encapsulation efficiency of L-asparaginase in Alg-*g*-PEG/α-CD hollow nanospheres hovered at high levels (70%) when the amount of L-asparaginase ranged from 1.5 to 6 mg. These results imply that the encapsulation capacity increased with the amount of enzyme increasing. However, the encapsulation efficiency gradually decreased when the mass of L-asparaginase is above 6 mg, which implies the encapsulation capacity for L-asparaginase may arrive at a maximum value. Furthermore, it is shown that the leakage efficiency is low for all samples (below 10%). When the data of encapsulation efficiency were converted to the mass of enzyme encapsulated in the hollow nanospheres, as shown in Fig. 7, the encapsulation capacity for L-asparaginase increased with the amount of enzyme increasing nearly in a linear correlation when the mass of enzyme is below 6 mg. But when the mass of enzyme is above 6 mg, the encapsulation capacity almost keeps stable which confirmed the conclusion obtained from encapsulation efficiency results. In summary, fixing the Alg-*g*-PEG/α-CD concentration and solution volume, the encapsulation capacity of Alg-*g*-PEG/α-CD increased as the amount of enzyme increases. The maximum value of the encapsulation capacity was approximately 4 mg per 4 mL Alg-*g*-PEG/α-CD (0.25%/6%) solution.

3.4 The influences of PEG graft density (GD) in Alg-*g*-PEG on the encapsulation behavior of L-asparaginase

A series of Alg-*g*-PEG with different PEG GDs were synthesized (Scheme S1†) to investigate the influences of GD on the encapsulation behavior of L-asparaginase. Structural characteristics of these Alg-*g*-PEG conjugates were investigated using ^{1}H NMR (Fig. S2–S6†) and thermogravimetric analysis (TGA), and the GDs were calculated from the TGA data (Fig. S7†). As shown in Fig. 8, it can be seen that more grafts favor the formation of small aggregates. We have demonstrated that rod-like blocks are formed by self-assembly of α-CD with PEG. The trend of the particle size varying with the graft density of rod-like blocks is quite consistent with that reported for hollow spheres made from the hydrogen-bonding "graft copolymers".[15–18] When the concentration of Alg-*g*-PEGs and α-CD is at a fixed value, the formation of small aggregates means the number of hollow nanospheres is increased. As discussed in our previous work,[21] the increase in the amount of hollow spheres may result in the encapsulation efficiency increasing. As shown in Fig. 9, the encapsulation efficiency increased with the increase in PEG GD in Alg-*g*-PEG. Additionally, the leakage efficiency is low for all the samples (below 10%), implying that the particle size has no influence on the leakage of enzyme.

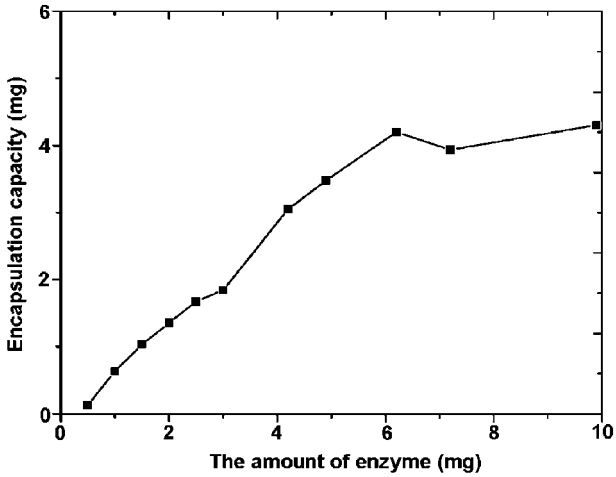

Fig. 7 The encapsulation capacity of hollow spheres with increasing mass of L-asparaginase used in the formation of hollow nanospheres.

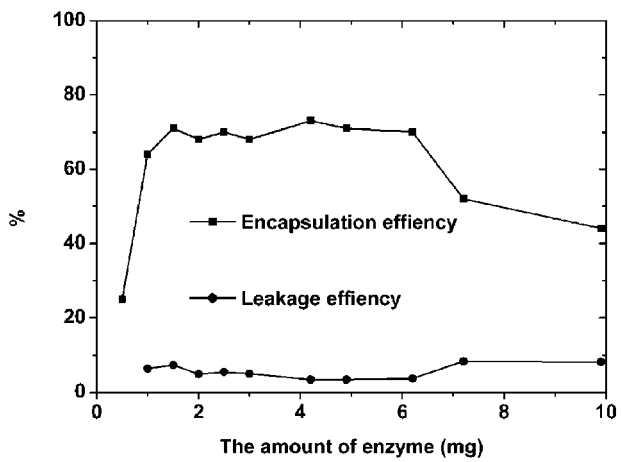

Fig. 6 The relationship between the encapsulation and leakage efficiency of L-asparaginase and the mass of L-asparaginase used in the formation of hollow nanospheres.

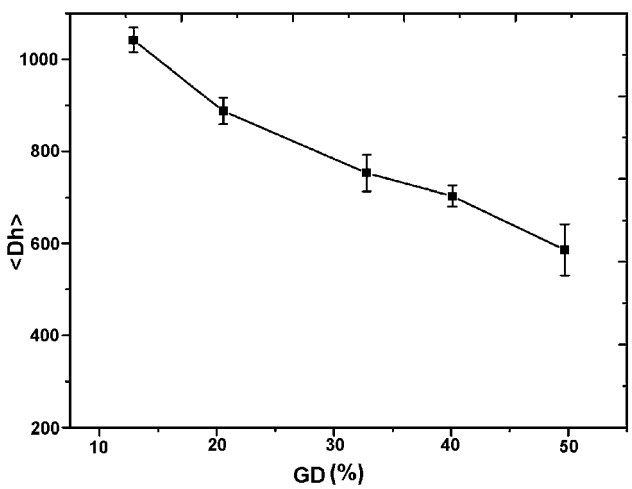

Fig. 8 Dh (hydrodynamic diameter) distribution of Alg-*g*-PEG/α-CD hollow spheres with increasing PEG content in Alg-*g*-PEG.

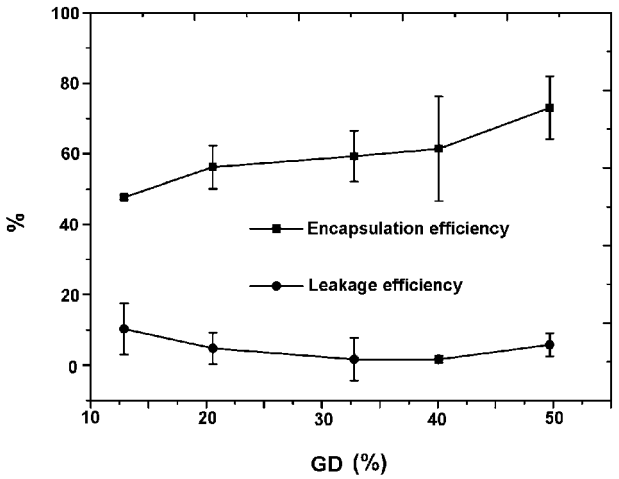

Fig. 9 The encapsulation and leakage efficiency for L-asparaginase with increasing PEG content in Alg-*g*-PEG.

Conclusions

In conclusion, we presented a novel kind of hollow nanospheres for enzyme encapsulation. The strategy for fabrication is based on the supramolecular inclusion between Alg-*g*-PEG and α-CD. The Alg-*g*-PEG/α-CD hollow spheres showed semi-permeability which could prevent the enzyme from leaving while allowing substrates and products to pass through to maintain the enzyme activity. The MMCO of the Alg-*g*-PEG/α-CD membrane was estimated to be between 20 and 40 kDa. The maximum encapsulation capability for enzyme (L-asparaginase as the model enzyme), as an important character for enzyme carrier, was estimated to be approximately 4 mg per 4 mL Alg-*g*-PEG/α-CD (0.25%/6%) solution. Furthermore, high PEG GD resulted in the encapsulation efficiency increasing but had no effect on the leakage efficiency. These promising characteristics create the exciting prospect of applying such hollow nanospheres as drug or cell carriers and controlled release systems.

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