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Encapsulation Studies and Selective Membrane Permeability Properties of Self-assembly Hollow Nanospheres

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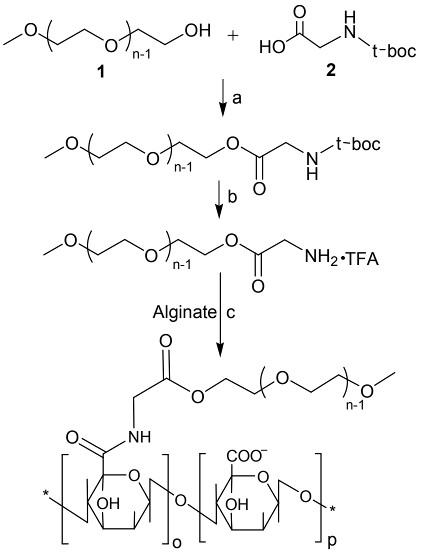
CONTENTS:

1. Synthesis of the PEG-Branched Alginate.



Scheme S1. Synthesis of the PEG-Branched alginate. Reagents and conditions: a) DCC, DMAP, CH_2Cl_2, 0℃; b) CH_2Cl_2/TFA(1:1), 0℃; c) NHS, EDCI, H_2O, 25℃.

Poly(ethylene glycol) methyl ether(M_n=2000) was utilized. Poly(ethylene glycol) methyl ether (**1**, 7.5 mmol，M=2000) was reacted with N-*t*-Boc-glycine (**2**, 8.1 mmol) and DMAP (2.25 mmol) in 75 ml anhydrous CH_2Cl_2. Subsequently, 9 mmol of DCC was added, and the reaction mixture was stirred at 0 °C for 24 h. The dicyclohexylurea (DCU) was filtered, and the filtrate was concentrated in a vacuum at room temperature. The resultant was dissolved in minimal amount of acetone and cooled overnight, and the precipitated DCU was filtered off. For the removal of a *t*-Boc group, PEG-*t*-Boc-amino acid diester (**3**) was dissolved in a mixture of CH_2Cl_2/TFA (1/1, v/v). The reaction mixture was stirred at 0 °C for 3 h and then evaporated to dryness. The deprotected derivative (**4**) was dissolved in a 15 % NaCl solution, and the pH was adjusted to 5. The solution was filtered to remove water insoluble *tert*butyl salts. The filtrate was extracted three times with chloroform; the organic phases were combined and dried over Na_2SO_4. The Na_2SO_4 was filtered, the solvent was evaporated, and the oily residue was dried under vacuum at room temperature.

4: ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 3.26 (s, O$\mathbf{CH_3}$), 3.85 (s, N$\mathbf{CH_2}$CO), 4.25 (m, COO$\mathbf{CH_2}$$CH_2$), 3.41- 3.66 (m, O$\mathbf{CH_2CH_2}$).

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. Different mass of samples of NHS and EDC were added to a 2 % (w/w) alginate solution to activate the carboxylic acid groups on the polymer backbone. The solution was agitated for 10 h 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, and dried under vacuum at room temperature to obtain a series of Alg-*g*-PEG samples with different DS of PEG moiety (Alg-*g*-PEG 1-5).

Alg-*g*-PEG: ^{1}H NMR (600 MHz, D_2O, ppm): δ 3.32 (s, O$\mathbf{CH_3}$), 3.44-3.75 (m, O$\mathbf{CH_2CH_2}$) 3.91 (s, N$\mathbf{CH_2}$CO), 4.36 (m, COO$\mathbf{CH_2}$$CH_2$), 4.01, 4.28 (**CH**-OH of alginate).

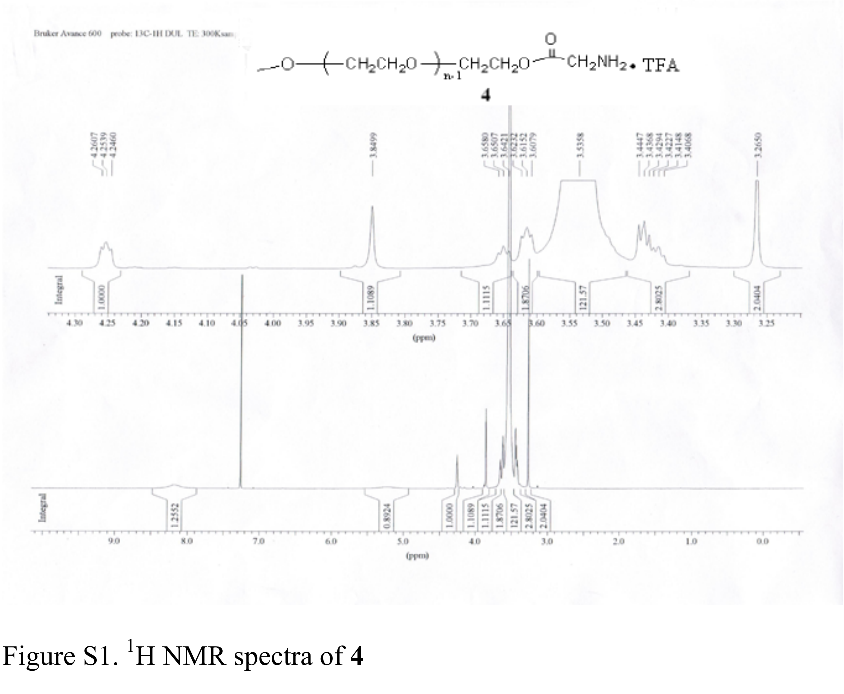


Figure S1. ^{1}H NMR spectra of **4**

2. Structural characteristics of these Alg-*g*-PEG conjugates.

Structural characteristics of these Alg-*g*-PEG conjugates were investigated using ^{1}H NMR and thermogravimetric analyzer (TGA).

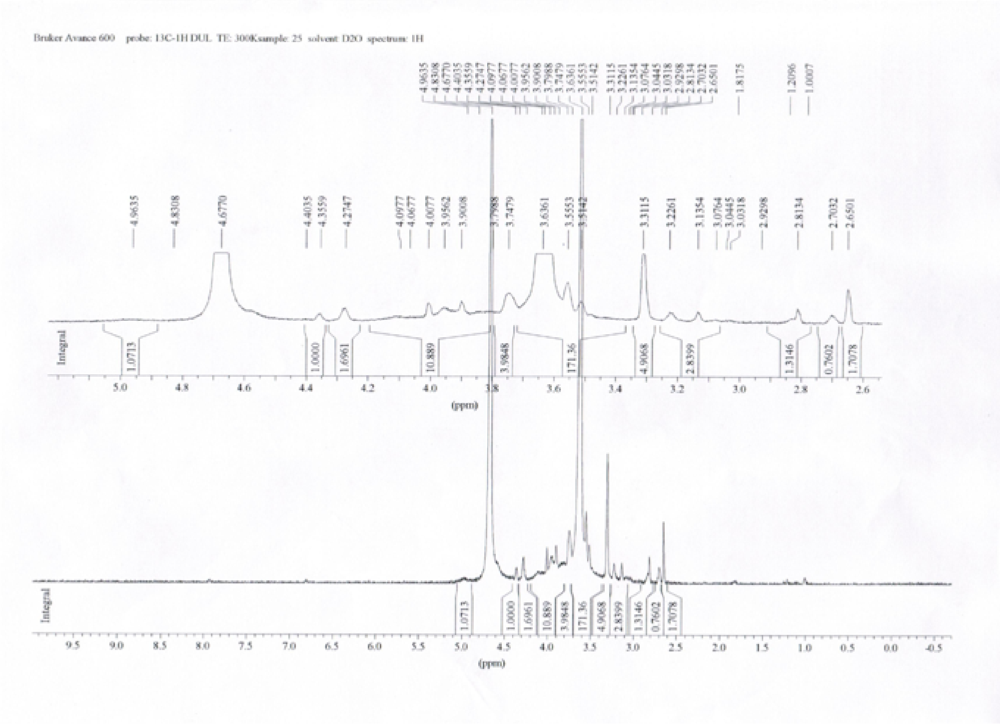


Figure S2. ^{1}H NMR spectra of Alg-*g*-PEG 1.

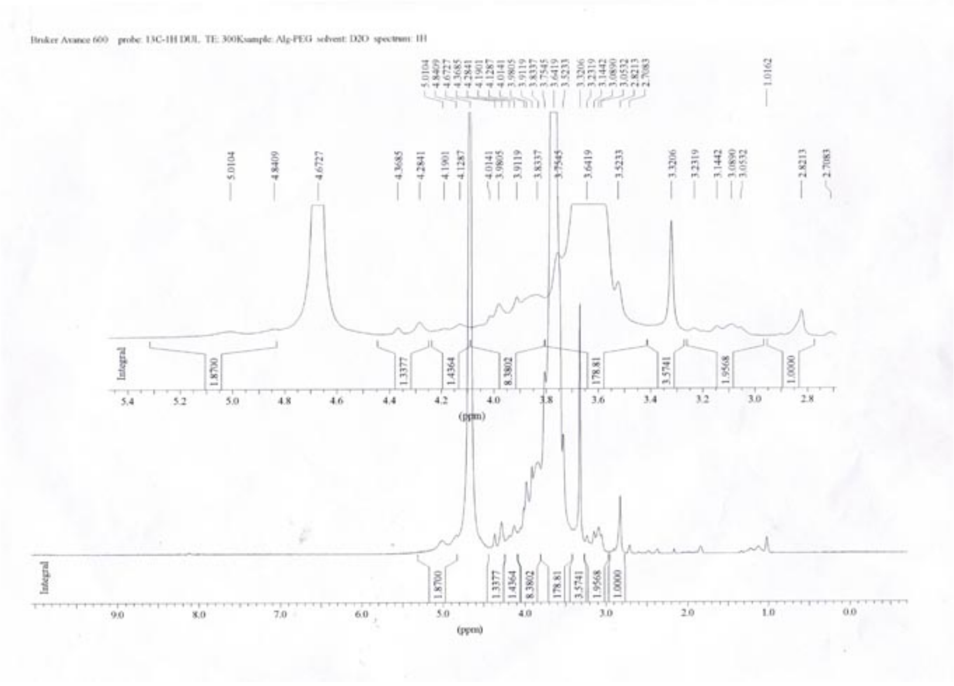


Figure S3. ^{1}H NMR spectra of Alg-*g*-PEG 2.

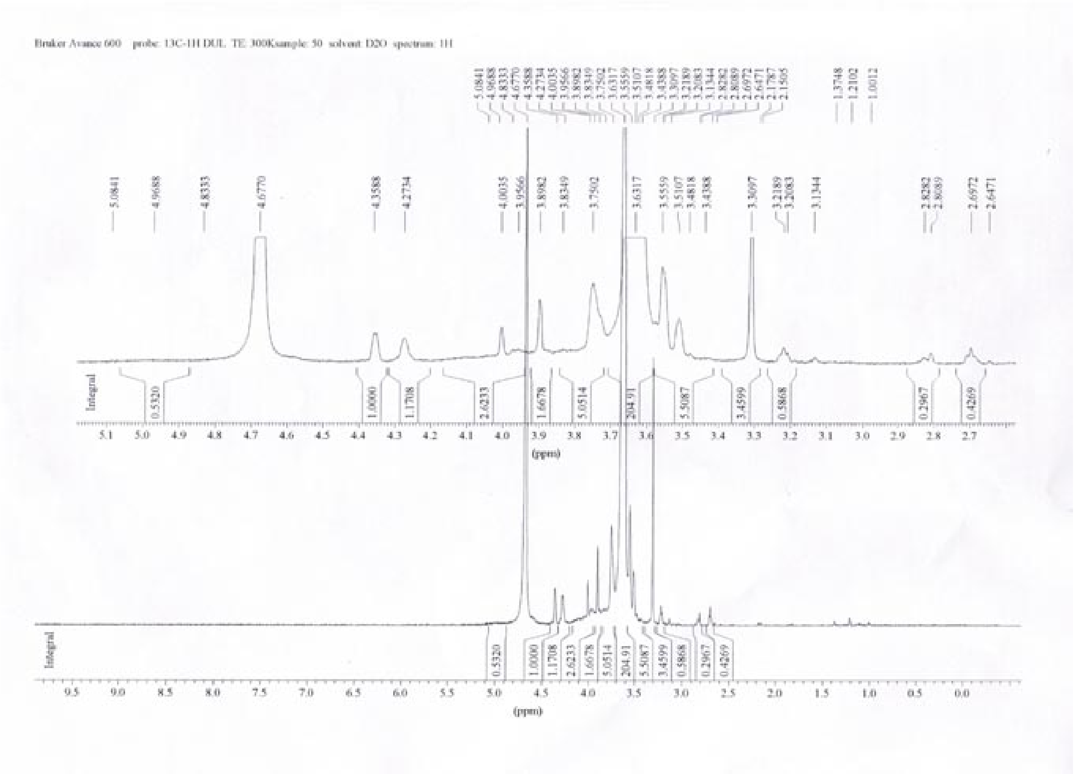


Figure S4. ^{1}H NMR spectra of Alg-*g*-PEG 3**.**

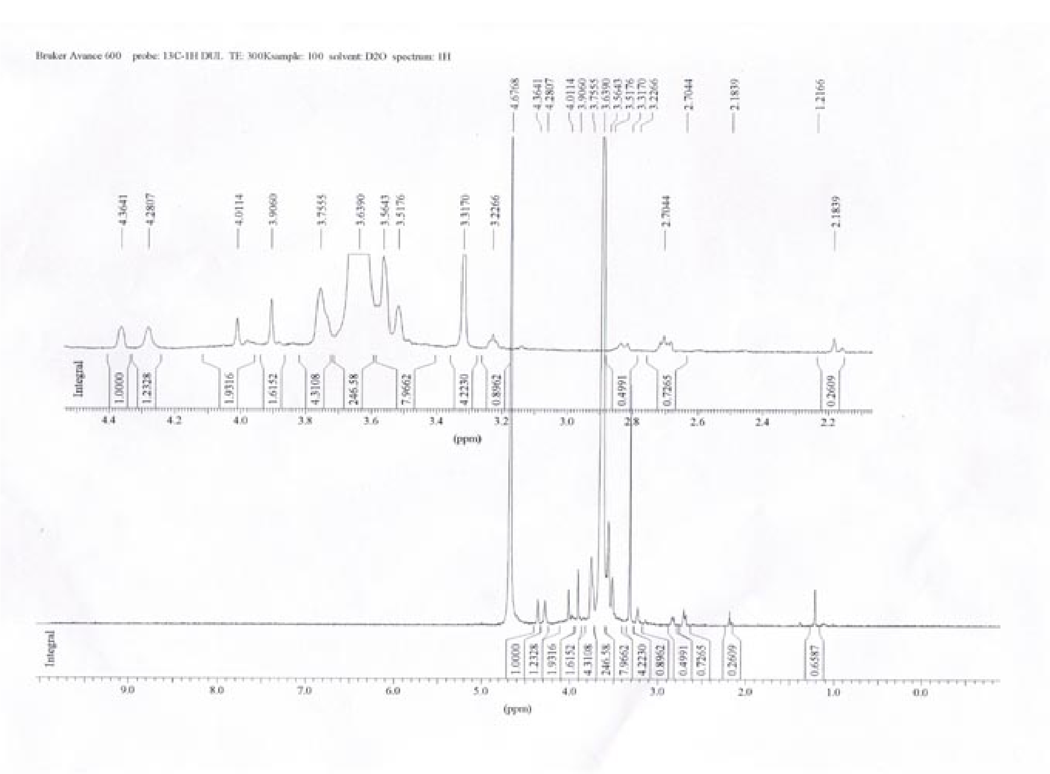


Figure S5. ^{1}H NMR spectra of Alg-*g*-PEG 6**.**

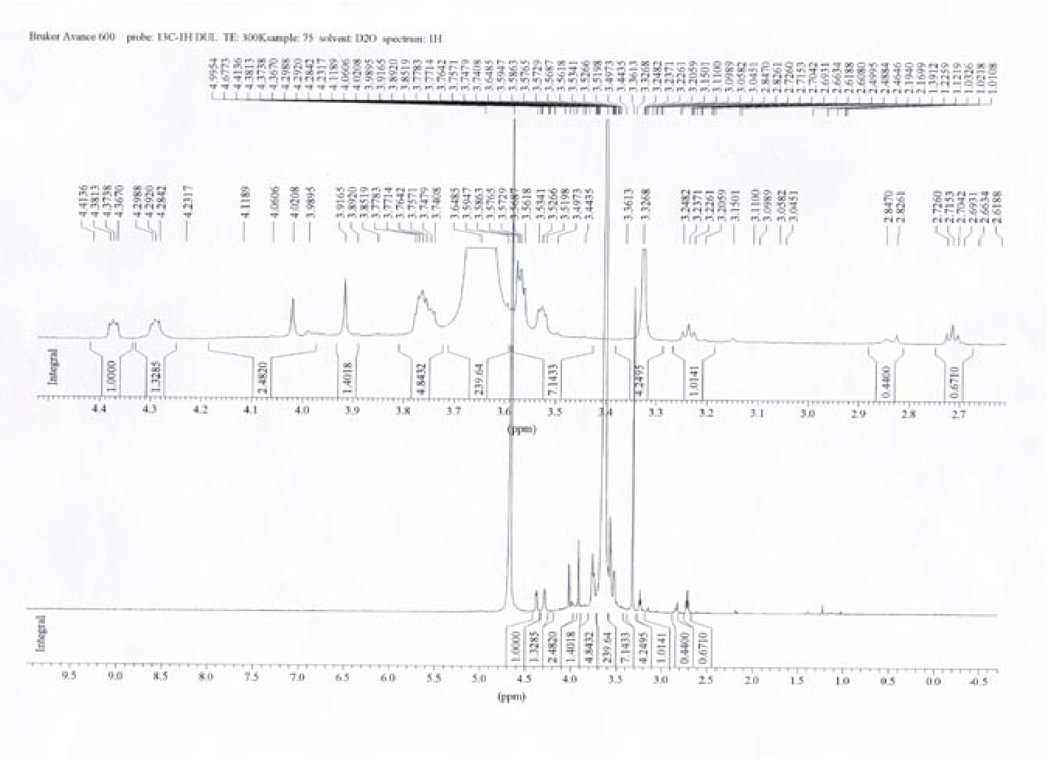


Figure S6. ^{1}H NMR spectra of Alg-*g*-PEG 5**.**

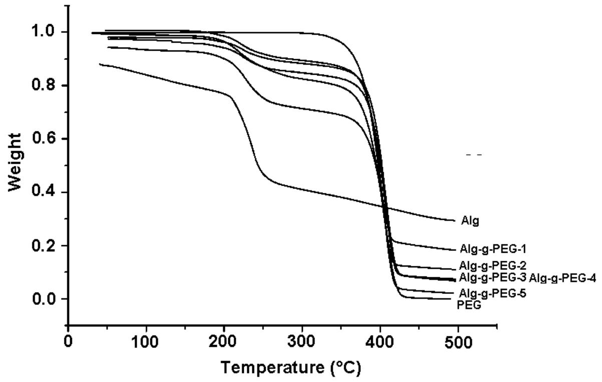


Figure S7. TGA thermograms of Alg, Alg-*g*-PEG copolymers and PEG.

3. Determination of degree of PEG substitution (DS) in Alg-*g*-PEG conjugates.

As the decomposition temperature interval of the CKGM component and the PEG component is significant, the degree of PEG substitution (DS) can be calculated using the following Eqs. (1) and (2); where 2000 is the average molecular weight of PEG, and 198 is the molar mass of a Alg unit.

$$\text{Percentage of Alg component in Alg-}g\text{-PEG (\%)} = \frac{\text{Alg component weitght loss+sample residue}}{\text{1-water weight loss}} \times 100 \quad (1)$$

$$\text{Degree of PEG substitution (\%)} = \frac{198\times(1\text{-percentage of Alg component in Alg-}g\text{-PEG}}{2000\times\text{percentage of Alg component in Alg-}g\text{-PEG}}\times 100 \qquad (2)$$

According to the equation (1) and (2) the degree of PEG substitution in Alg-*g*-PEG conjugates were determined to be: Alg-g-PEG-1 12.9 %; Alg-g-PEG-2 20.6 %; Alg-g-PEG-3 32.8 %; Alg-g-PEG-4 40.1 %; Alg-g-PEG-5 49.7 %.

4. Determination of the concentration of α-CD used in the process of forming hollow spheres.

Table S1 The characteristics of hollow nanospheres with different concentrations of α-CD.

Entry	**1**	**2**	**3**	**4**
Concentrations of α-CD	2 %	4 %	6 %	9 %(saturated solution)
Concentrations of Alg-*g*-PEG	0.25 %	0.25 %	0.25 %	0.25 %
Feed ratio of α-CD and graft PEG [a]	1:1	2:1	3:1	4.5:1
Time of hollow spheres start to form [b]	—	—	30 min	10 min
Total time of agitating	3 h	3 h	3 h	3 h
Amount of hollow spheres obtained	—	—	70 mg	76 mg

[a] feed ration is calculated from the PEG graft density (GD) of Alg-*g*-PEG. [b] the time of the solutions turn to turbid.

When a certain concentration of Alg-*g*-PEG aqueous solution mixed with different concentrations of α-CD aqueous solution, they dissolve perfectly, but after a certain time the samples **3** (30 min) and **4** (10 min) became more and more turbid, indicating the formation of large aggregates (**Table S1**). After agitating 3 h, the aggregates were clollected by centrifugation and lyophilized. It was found that the yield of sample **3** and sample **4** were nearly equivalent, which suggested that the formation of the aggregates (hollow spheres in this study) would not be affected by the concentration of α-CD when the concentration of α-CD above 6 %. However, after agitating for 3 h, the aqueous solutions of samples **1** and **2** still kept transparent, suggesting that no large aggregates were formed in samples **1** and **2**.

In this work, the formation of rod-like PEG-α-CD inclusion block is a key factor to construct hollow spheres. It was reported that the formation of PEG-α-CD inclusion including five steps[1]: (1) diffusion of the two reactants in the solvent; (2) initial threading of graft PEG ends into α-CD cavities; (3) sliding of α-CD over the graft PEG chain; (4) dethreading of α-CD from the graft PEG chain; (5) precipitation of the final hollow spheres. The pseudorotaxane formed between graft PEG and α-CD is a dynamic equilibrium, and the concentration of α-CD is one of key factors effected this equilibrium. When the amount of α-CD in the solutions exceeds a certain value, the step (4) can not be occurred easily, thus the hollow spheres were formed. In this work, when the concentrations of α-CD below 6 % (samples **1**and **2**), the inclusion process between graft PEG and α-CD only occurred from step (1) to step (4), and the step (4) can easily undertake because of the low concentrations of α-CD, thus, after a certain time (3h), the phenomenon of the hollow spheres formation (the solutions turn to turbid) can not be founded. Once the concentration of α-CD was above 6 % in aqueous solution, the hollow spheres were formed quickly.

Because the formation of the hollow spheres would not be affected by the concentration of α-CD when the concentration of α-CD above 6 %, 6 % α-CD was used instead of saturated aqueous solution in order to save the material in this paper.

As shown in **Fig. S8** and **Fig. S9**, in the ^{1}H NMR of the hollow nanospheres obtained from samples **3** and **4**, although signals of α-CD and alginate are mostly overlapped, the signal of anomeric hydrogen in α-CD (δ 5.1 ppm) and the graft PEG (δ 3.7 ppm) are well identifiable.

Therefore, the ratio between the α-CD and graft PEG can be calculated exactly, the results suggest that the number of α-CD complexes in each graft PEG chain of samples **3** (6 % α-CD) and **4** (saturated α-CD) are almost equivalent (6/7.83 and 6/7.73). Those results imply that the structure and compact degree of the hollow nanospheres would not be changed while the concentration of α-CD exceeds 6 %. Therefore, the encapsulation and leakage efficiency of the enzyme would not be affected by the concentrations of α-CD when the concentrations of α-CD between 6 % and saturated aqueous solution.

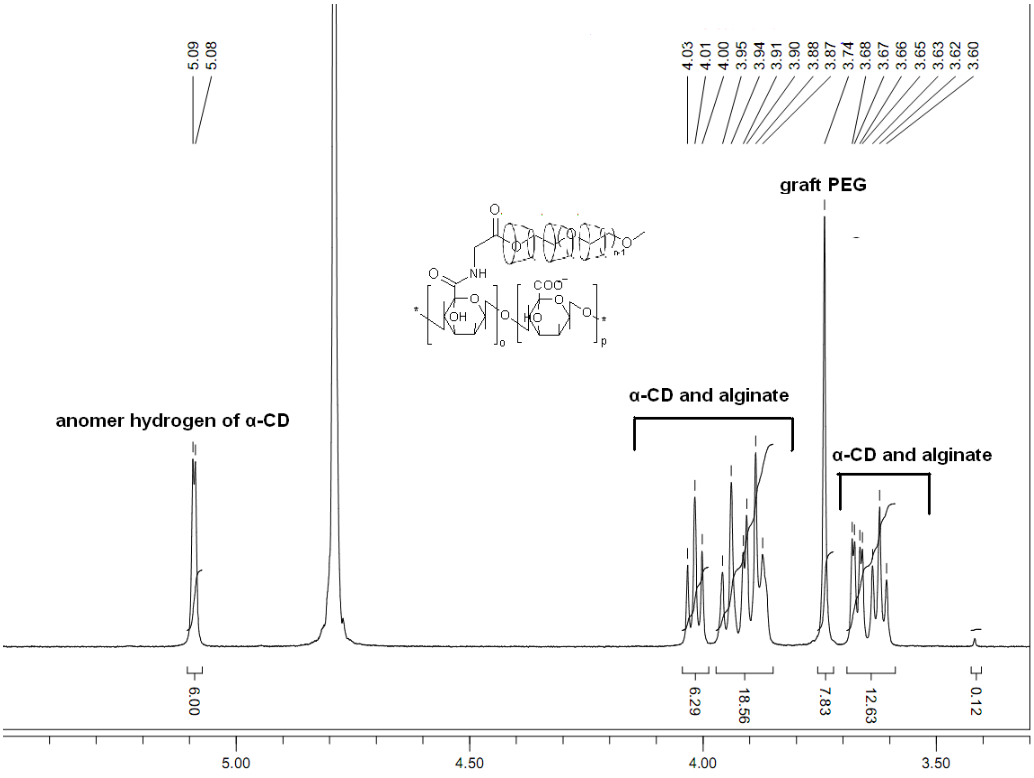


Figure R1. The ^{1}H NMR (D_2O) of the hollow nanospheres obtained from samples **3** (6 % α-CD)

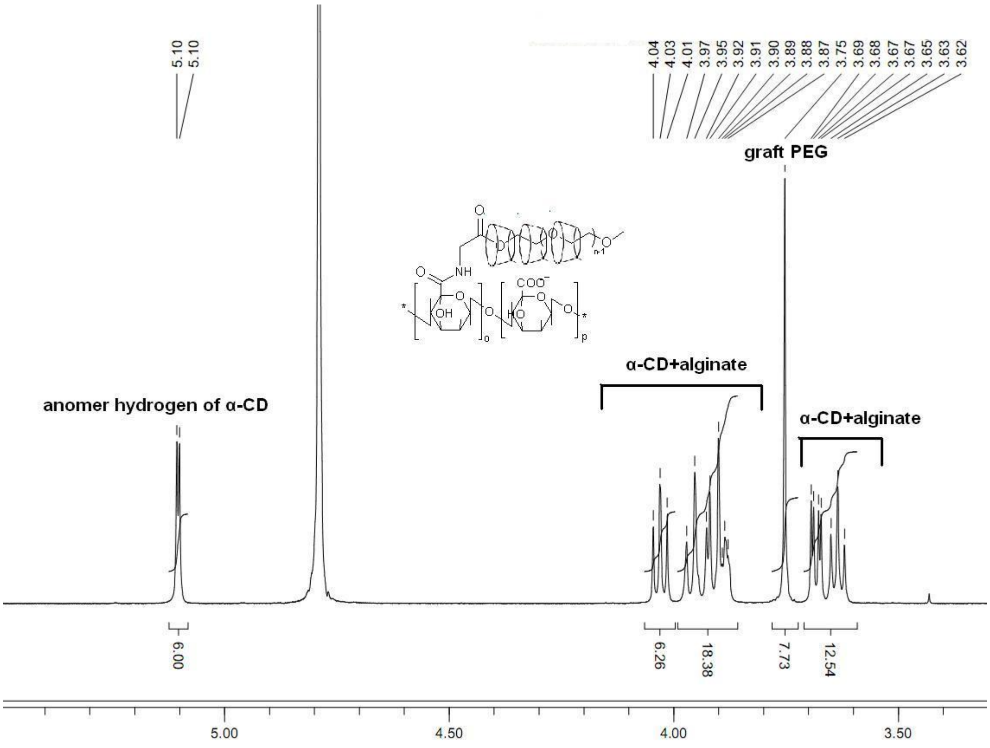


Figure R2. The ^{1}H NMR (D_2O) of the hollow nanospheres obtained from samples **4** (saturated α-CD solutions)

Reference:

1. M. Ceccato, P. L. Nostro and P. Baglioni, *Langmuir* 1997, **13**, 2436-2439.