Polymer 51 (2010) 2556–2564

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Novel supramolecular system of amphiphilic hyperbranched polymer with β-cyclodextrin and hyperbranched topography cavities: Synthesis and selective encapsulation

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ARTICLE INFO

Article history:
Received 15 December 2009
Received in revised form
1 March 2010
Accepted 2 April 2010
Available online 14 April 2010

Keywords:
Amphiphilic hyperbranched polymer
β-Cyclodextrin
Selective encapsulation

ABSTRACT

Novel supramolecular system of amphiphilic hyperbranched polymer with hyperbranched poly(β-cyclodextrin) core was designed and synthesized to accomplish a so-called selective encapsulation, where two types of guest molecules can be encapsulated into two types of molecular cavities from β-cyclodextrin (β-CD) and topography structure of hyperbranched polymer, respectively. The double molecular recognition behaviors from β-CD and hyperbranched cavities drive one guest to go into the former, the other guest to the latter. This selective encapsulation was further confirmed *via* the release profiles and sequences of Levofloxacin lactate (LL) and Phenolphthalein (PP). LL presents a sustained release period followed by an almost non-release stage, while PP releases on a quite slow rate at first, subsequently on the linearly increasing rate. At the early stage, the release of LL dominates in comparison with PP, and then the release rate of PP increases to play a determinate role in the release system. It can be attributed to the existence of two guests in the different molecular cavities with the different microenvironments. The observed selective encapsulation of supramolecular system is a new phenomenon, which is helpful to extend the application of CD-based hyperbranched polymers in supramolecular science and complex drug delivery system.

1. Introduction

Encapsulation of functional species is one of the most attractive areas in contemporary supramolecular host-guest chemistry and shows great potential in drug delivery and cosmetics [1,2], medical diagnostics [3], and materials science [4,5]. Materials used in encapsulation include liposomes [6], chitosan [7], carbon- or silicate-based hollow spheres [8], functional polymers [9–20], and host molecules [21,22], such as crown ethers, cryptates, calixarenes, and cyclodextrins etc. Among these materials, functional polymers show superior encapsulation-release properties due to their special macromolecule structures and unique physicochemical characteristics in solution. For example, polymeric hydrogel [11], microcapsules [12], and nanoparticles (NP) [13] possess high encapsulation efficiency and controlled release abilities for proteins or drugs. Amphiphilic linear polymers self-assemble into micelles in aqueous media to achieve encapsulation and controlled release of hydrophobic drugs [9,10]. Recently, dendritic polymers and their amphiphilic derivatives with the core–shell architecture have been applied in encapsulation and release of hydrophobic compounds, water-soluble dyes or metal colloids [14–20].

However, up to now, almost all the encapsulation of functional polymers reported were focused on the single-guest, which means that only one kind of guest molecules is encapsulated into the cavities at once [9–16,18,19]. With the development of host-guest chemistry, the demand for the host with the selective multi-guest encapsulation ability is greatly increasing. In addition, the establishment of double- or multi-drug delivery system is very important and necessary with the development of controlled and selective delivery [23,24], although the single-drug system has been usually used in this field. Recently, the synergistic encapsulation phenomena of double-guest molecules using amphiphilic hyperbranched polymers were reported by Liu et al. [17] and our group [25,26]. However, there are still few attentions and researches on selective encapsulation and controlled release sequences of functional polymers for double-guest molecules [20].

Our idea is to establish a polymeric supramolecular system possessing two types of molecular cavities, which is able to selectively encapsulate double-guest molecules. In other words, in the selective encapsulation, one guest comes into one cavity and the

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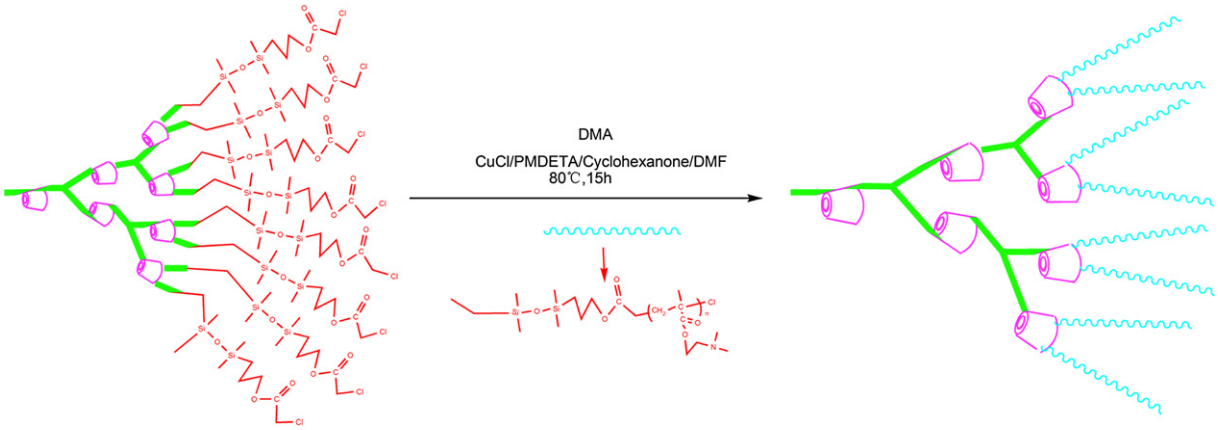
doi:10.1016/j.polymer.2010.04.009



Scheme 1. Schematic representation for preparation of amphiphilic hyperbranched polymers.

other guest goes into the other cavity. And then, one guest releases at first while the other one releases subsequently. Due to the unique molecular structures [27], hyperbranched poly(β-cyclodextrin)s (β-CD) were selected for the construction of this supramolecular system (Scheme S1, Supporting Information). β-CD with a hydrophobic cavity can be employed as a host for a variety of smaller molecular guests *via* noncovalent interactions [28,29]. The topography structure of hyperbranched polymer is also a nature cavity [30]. Therefore, it is possible to encapsulate one guest into β-CD cavity and the other one into hyperbranched cavity when the two types of molecular cavities are combined in one supramolecular structure.

However, it should be noticed that hyperbranched poly(β-CD)s are hydrophobic polymers and hard to encapsulate guest molecules in aqueous solution. In addition, as for hyperbranched poly(β-CD) structure reported by us [27], there are no smart characteristics and controlled release abilities in response to some external simultaneous change of temperature or pH value. Fortunately, amphiphilic hyperbranched polymers with the core–shell architecture can give a solution [17,19,22,25,26]. Therefore, poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMA) segments possessing the water-solubility and environmental sensitivity are incorporated onto the peripheries of hyperbranched poly(β-cyclodextrin)s (β-CD)s for the construction of amphiphilic hyperbranched polymer. Based on the considerations above, herein the synthesis of amphiphilic hyperbranched polymers with a hyperbranched poly(β-CD) core and a PDMA shell are reported, and the selective encapsulation as well as controlled release behaviors of these polymers using Levofloxacin lactate (LL) and Phenolphthalein (PP) as double-guest molecules are investigated in details.

2. Experimental

2.1. Materials

HBP-(B_y + AB_x) (M_n = 64700, PDI = 1.74, degrees of branching = 0.83) and HBP-AB_2 (M_n = 36700, PDI = 1.89, degrees of branching = 0.40) were synthesized according to our previous work [27]. HBP-*g*-PDMA based on a hyperbranched polycarbosilane core and a PDMA shell (M_n = 2400, PDI = 1.77, degrees of branching = 0.62) was prepared according to our previous work [25]. Chloroplatinic acid (H_2PtCl_6) (39%, platinum) was provided by Shaanxi Kaida Chemical (Xi'an City, Shaanxi Province, China). 1,1,3,3-Tetrmethyldisiloxane (more than 99.0% purity) was obtained from Zhejiang Sanmen Qianhong (Sanmen City, Zhejiang Province, China). Allyl chloroacetate (more than 98.0% purity) was provided by EHST Ltd. (Shanghai City, China). *N*,*N*-dimethyl amino ethyl methacrylate (DMA) was purchased from ACROS Chemical Industries (USA) and purified by distillation under the reduced pressure. *N*,*N*,*N*′,*N*″,*N*″-pentamethyl diehylenetriamine (PMDETA) was supplied by Yutian Chemical Ltd. (Liyang City, China) and used as received without further purification. PP was purchased from Xi'an Chemical Reagents Plant (Xi'an City, China). LL was purchased from Xi'an University of Architecture & Technology (Xi'an City, Shaanxi Province, China). DMF used as the eluent for SEC/MALLS (HPLC grade) was received from Dima Tech (USA) Other reagents were all purchased from Tianjin Kermel Chemical Reagents Development Center (Tianjin City, China) and dried with 4 Å grade molecular sieve before use.

2.2. Synthesis of hyperbranched poly(β-CD) macroinitiators

The detailed synthetic procedures and the characterization data of hyperbranched poly(β-CD) macroinitiators carrying alkyl chloride groups can be found in the Supporting Information.

2.3. Synthesis of HBP-(B_y + AB_x)-*g*-PDMA or HBP-AB_2-*g*-PDMA

In a flask equipped with a magnetic stirring, hyperbranched poly(β-CD) macroinitiator [HBP-(B_y + AB_x)-Cl or HBP-AB_2-Cl] (0.05 g), CuCl (0.05 g, 0.5 mmol) and PMDETA (0.173 g, 1.0 mmol) were first dissolved in a mixed solvent of DMF (3.7 g, 0.05 mol) and cyclohexanone (1.5 g, 0.015 mol), and then, added DMA under the vigorous stirring. The mixed solution was bubbled with nitrogen

Table 1
Polymerization results of HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA.

Sample index	Macroinitiators[a] (g)	DMA (g)	Con[b] (%)	M_n[c]	M_w[c]	MWD[c]	DP[d]
HBP-AB_2-*g*-PDMA-2_g	0.05	2.0	57.5	75,500	79,300	1.10	19
HBP-AB_2-*g*-PDMA-4_g	0.05	4.0	68.8	80,200	88,700	1.20	21
HBP-AB_2-*g*-PDMA-6_g	0.05	6.0	67.7	119,000	138,100	1.23	42
HBP-(B_y + AB_x)-*g*-PDMA	0.05	6.0	68.4	213,000	256,600	1.21	36

[a] HBP-AB_2-Cl or HBP-(B_y + AB_x)-Cl.
[b] Monomer conversion, determined by gravimetric method.
[c] Molecular weight and molecular weight distributions, determined by SEC/MALLS.
[d] Average degree of polymerization per arm, determined by our previous method [25].

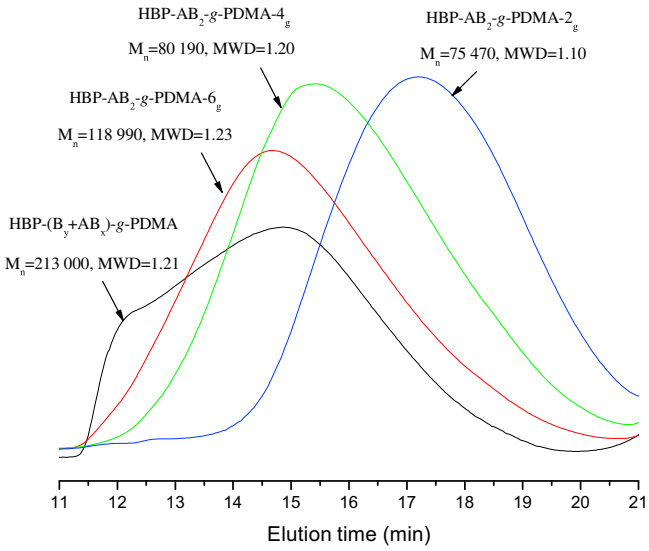


Fig. 1. SEC elution curves of HBP-(B_y + AB_x)-*g*-PDMA and a series of HBP-AB_2-*g*-PDMA.

gas for 45 min, and sealed under vacuum. The polymerization was conducted at 80 °C for 15 h, and then, the mixture was dialyzed in a dialysis bag (molecular weight cut off: 8000–10,000) against distilled water for 5 d. It was refreshed at an interval of 5 h. The dialyzed product [HBP-(B_y + AB_x)-*g*-PDMA or HBP-AB_2-*g*-PDMA] was lyophilized and kept in glassware under vacuum for further characterization.

2.4. Characterizations and measurements

The ^{1}H NMR and ^{13}C NMR spectra were conducted on a Bruker Avance 300 spectrometer (Bruker BioSpin, Switzerland) operating at 300 MHz (^{1}H) in DMSO-d_6 or DMF-d_6. The molecular structure parameters of hyperbranched polymers were determined on DAWN EOS size exclusion chromatography/multiangle laser light scattering (SEC/MALLS) instrument equipped with viscometer (Wyatt Technology, USA), HPLC grade DMF containing LiCl (0.01 mol/L) (at 40 °C) was used as eluent at a flow rate of 0.5 mL/min. The chromatographic system consisted of a Waters 515 pump, differential refractometer (Optilab rEX) and one column, MZ 10^3 Å 300 × 8.0 mm. MALLS detector (DAWN EOS), quasi-elastic light scattering (QELS), and differential viscosity meter (ViscoStar) were placed between the SEC and the refractive index detector. The molecular weight (M_w) and molecular weight distribution (MWD) were determined by SEC/DAWN EOS/Optilab rEX/QELS model. ASTRA software (Version 5.1.3.0) was utilized for acquisition and analysis of data. UV–vis spectroscopy measurement was preformed on Shimadzu UV-2550 model spectroscopy (Shimadzu, Japan). Fluorescence spectrophotometer measurement was preformed on Hitachi F-4600 model spectrophotometer at room temperature (Hitachi, Japan). All solutions were maintained for more than 12 h to ensure the binding equilibrium and then stirred prior to measurement. All measurements were performed on air-equilibrated solutions at 25 °C. The emission spectra were recorded from 308 to 600 nm with an excitation wavelength of 288 nm. The spectra were recorded with a scan rate at 1200 nm/min.

2.5. Investigation on double-guest encapsulations

Double-guest encapsulations of HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA were measured by a UV–vis, using LL (5×10^{-5} mol/L) and PP (5×10^{-5} mol/L) as guest molecules in a buffer solution with ionic strength equal to 0.1 mol/L and pH = 11. Typically, a 4 mL solution containing LL and PP was mixed with an equal volume, and then polymer was added into the mixture solution. All solutions were kept at 25 °C for 12 h prior to measurements using UV–vis spectroscopy.

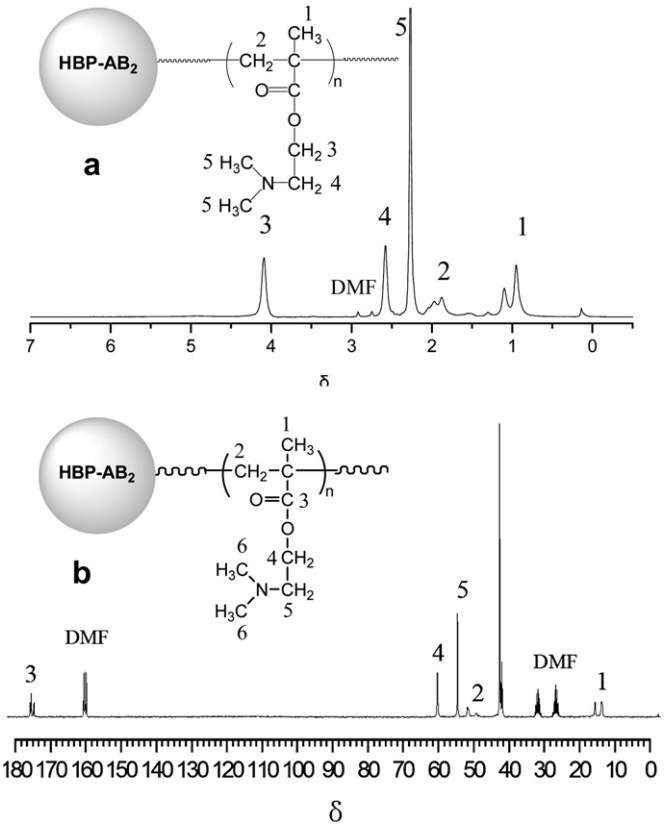


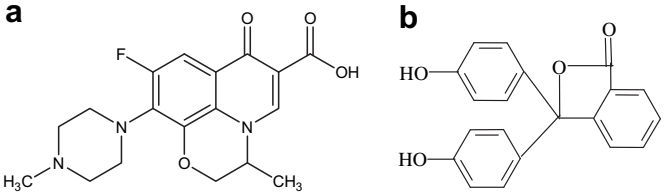
Fig. 2. ^{1}H NMR (a) and ^{13}C NMR (b) spectra of HBP-AB_2-*g*-PDMA.

2.6. Investigation on single-guest encapsulations

Single-guest encapsulations of β-CD and HBP-*g*-PDMA was measured by a UV–vis and fluorescence spectrophotometer, using LL (5×10^{-5} mol/L) as guest molecule in a buffer solution with ionic strength equal to 0.1 mol/L and pH = 11. Typically, β-CD or HBP-*g*-PDMA was gradually added into the solution of LL. All solutions were kept at 25 °C for 12 h prior to measurements using UV–vis and fluorescence spectrophotometer.

2.7. Investigation on molecular recognition behaviors

Molecular recognition behavior of HBP-*g*-PDMA was measured by a UV–vis, using LL (5×10^{-5} mol/L) and PP (5×10^{-5} mol/L) as



Scheme 2. Chemical structures of LL (a) and PP (b).

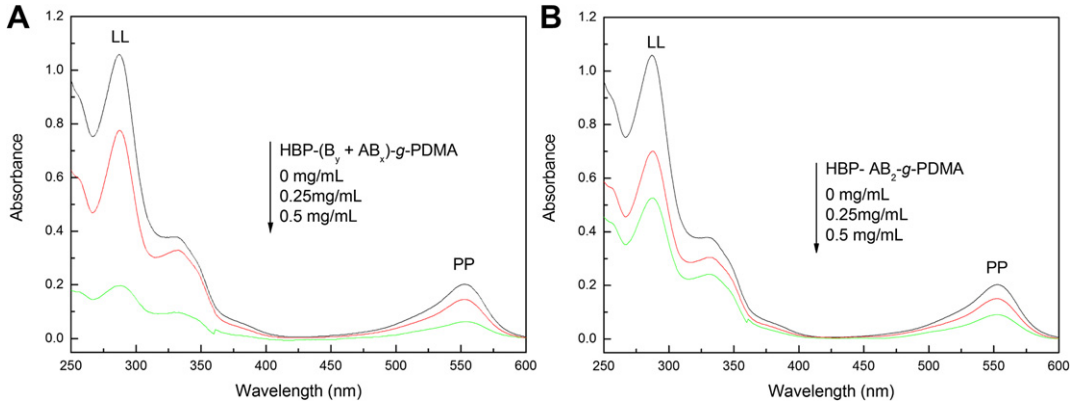


Fig. 3. UV spectra of PP + LL solution in the presence of HBP-(B_y + AB_x)-*g*-PDMA (A) and HBP-AB_2-*g*-PDMA (B) [PP (5×10^{-5} mol/L): 2 mL, LL (5×10^{-5} mol/L): 2 mL].

guest molecules in a buffer solution with ionic strength equal to 0.1 mol/L and pH = 11. When PP was encapsulated in the HBP-*g*-PDMA with the manner of single-dye encapsulation (polymer concentration is 1.0 mg/mL), the solution of LL was gradually added into the bottle by several times with given volumes each time. All solutions were kept at 25 °C prior to measurements using UV–vis.

2.8. Drugs loading

LL, PP and polymer [HBP-*g*-PDMA, HBP-(B_y + AB_x)-*g*-PDMA or HBP-AB_2-*g*-PDMA] were dissolved in appropriate THF under vigorous stirring for 12 h. The weight ratio of LL, or PP to polymer is 2.5:2.5:100. The mixture was subjected to centrifugation for the removal of two free drugs, and then, the solution containing drugs-loaded polymer and the solid consisting of two free drugs were separated. For one thing, THF in the solution was volatilized at 40 °C in a vacuum oven for 4 d, and then sample slices with 0.2 mm in thickness consisting of two model drugs and polymer were prepared.

2.9. Investigation on controlled release behaviors

The sample slices were sealed separately using a dialysis bag with 4 cm in length. The dialysis bag was used because these polymers have a significantly higher molecular weight, so that they would stay inside the dialysis bag (molecular weight cut off: 3500), whereas the drug with small molecular weight would readily diffuse out. The bags were immersed into 40.0 ml of a buffer solution with pH = 10.0 and 0.1 mol/L ionic strength at 37 °C. In a certain time interval, 5.0 ml of buffer solution was withdrawn and replaced with 5.0 ml of fresh one. The LL released was analyzed using 288 nm as characteristics bands, respectively. The PP released was 553 nm. All solutions withdrawn were kept at 37 °C for 48 h prior to measurements. All release measurements were carried out in triplicate for each sample, and an average value was adopted. The cumulative release was calculated by using Eq. (1) as follows.

$$\text{Cumulative release}(\%) = \frac{100 \times (40.0C_n + 5.0\sum C_{n-1})}{W_0} \qquad (1)$$

Where W_0 (mg) is weight of drug in the polymer; C_n (mg/ml) is the concentration of LL or PP in buffer solution, which was withdrawed for n times, C_{n-1} (mg/ml) is the concentration of LL or PP in buffer solution, which was withdrawed for $n-1$ times.

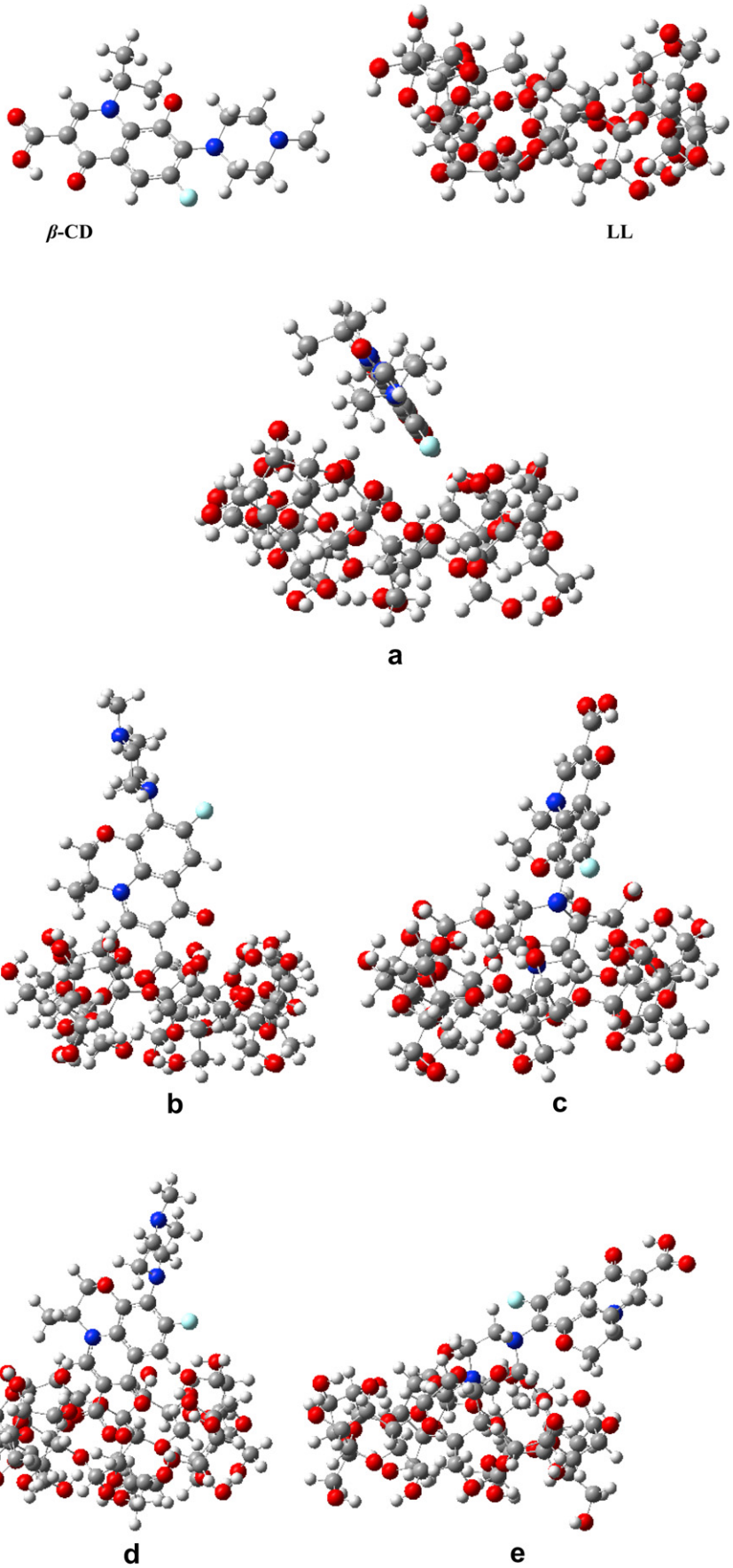
3. Results and discussion

3.1. Synthesis of amphiphilic hyperbranched polymers

To investigate the selective encapsulation behaviors in aqueous solution of HBP-(B_y + AB_x) and HBP-AB_2, PDMA segments were incorporated onto the peripheries of these polymers to obtain amphiphilic hyperbranched polymers HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA. PDMA is often used to prepare amphiphilic hyperbranched polymers due to the perfect water-solubility and environmental sensitivity that can control the release rate in drug delivery system [31–33]. It should be pointed out that HBP-AB_2 possesses certain water-solubility in comparison with HBP-(B_y + AB_x) [27]. However, HBP-AB_2 was still considered as a related hydrophobic core when the PDMA segments possessing the better water-solubility were grafted on its peripheries. Thus HBP-AB_2-*g*-PDMA presents amphiphilic characteristic.

The synthetic routes of HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA are shown in Scheme 1. Hyperbranched poly(β-CD) macroinitiators carrying alkyl chloride groups including HBP-(B_y + AB_x)-Cl and HBP-AB_2-Cl were synthesized firstly from 1,1,3,3-tetrmethyldisiloxane and allyl chloroacetate (Scheme S2). ^{1}H NMR and ^{13}C NMR spectra are shown in Fig. S1–S4. Initiator site number in HBP-(B_y + AB_x)-Cl and HBP-AB_2-Cl surfaces calculated by our previous method [27] is about 25 and 12, respectively. Then PDMA segments were introduced into the terminal groups of macroinitiators by a robust controlled "living" radical polymerization method, i.e. atoms transfer radical polymerization (ATRP) [34–36] (Scheme 1). The initiation efficiencies of HBP-(B_y + AB_x)-Cl and HBP-AB_2-Cl as macroinitiators are 88.5% and 91.5%, respectively, which can be calculated by the initiator site number and the data listed in Table 1. From the polymerization and SEC/MALLS results listed in Table 1 and Fig. 1, the graft length of PDMA increases with the increased feed ratio of monomer to macroinitiator when the same hyperbranched poly(β-CD) core is employed. Compared with HBP-AB_2-*g*-PDMA-2 and HBP-AB_2-*g*-PDMA-4, HBP-AB_2-*g*-PDMA-6 shows the highest M_n and average degree of polymerization (DP) values in per arm, i.e. the M_n of 119,000 and the DP of 42.

NMR spectra for HBP-AB_2-*g*-PDMA are shown in Fig. 2. As can been seen from Fig. 2a, the chemical shifts at δ 4.09 (–O–C***H***$_2$–CH_2–), δ 2.58 (–O–CH_2–C***H***$_2$–), δ 2.27 ($(CH_3)_2$–N–), δ 1.88–2.04 (–CH_2–), and δ 0.95–1.10 (–CH_3) are attributed to the protons originated from PDMA segments. The same conclusion can be validated from ^{13}C NMR spectrum with the carbons at δ 174.74–175.80 (–COO–), δ 55.56, 61.21 (–O–$(CH_2)_2$–), δ 50.25, 52.78 (–CH_2–), δ 43.04–43.72 ($(CH_3)_2$–N–), and δ 15.07, 16.85 (–CH_3) (Fig. 2b). NMR spectra results indicate that the



Scheme 3. Schematic representation for the different interaction types of β-CD with LL (a–e).

Table 2
Calculation results of Gaussian 03 software.

Sample	E,[a] a.u.	ZPE,[b] kJ/mol	ΔZPE,[c] kJ/mol	ΔE,[d] kJ/mol	$\Delta E'$,[e] kJ/mol
β-CD	−0.26268173	959.7844	–	–	–
LL	−2.28646782	3128.1087	–	–	–
β-CD–LL (a)	−2.58192229	4069.8316	18.1	−86.0	−67.9
β-CD–LL (b)	−2.58964664	4070.5238	17.4	−106.0	−88.6
β-CD–LL (c)	−2.58736943	4077.8227	10.1	−100.3	−90.2
β-CD–LL (d)	−2.59544624	4078.7578	9.1	−121.5	−112.4
β-CD–LL (e)	−2.58833280	4070.7139	17.2	−102.8	−85.6

[a] Total energies.
[b] Zero-point energies.
[c] Zero-point energies correction.
[d] Interaction energy.
[e] Interaction energy corrected by zero-point energies.

polymerization reaction is completed and HBP-AB_2-*g*-PDMA has been successfully synthesized. It should be noticed that both of the protons and carbons from the core layer are overlapped because of the introducing of PDMA segments (Fig. 2). The similar NMR spectra of HBP-(B_y + AB_x)-*g*-PDMA are shown in Fig. S5.

3.2. Double-guest selective encapsulation behaviors

Unique amphiphilic hyperbranched macromolecular structures of HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA with two types of molecular cavities from hyperbranched and β-CD have been constructed. In this section, we try to establish a double-guest encapsulation system using LL and PP as guest molecules (Scheme 2). We hope that one guest is encapsulated into hyperbranched cavity, and the other one is encapsulated into β-CD cavity. Based on this consideration, LL was selected as the double model molecules due to its special molecular structure. From the viewpoint of the molecular size [37,38], LL may not be suitable to be encapsulated into β-CD cavity but into hyperbranched cavity due to its tri-ring structure (Scheme 2a). However, LL has the different UV absorption peaks at 229 nm, 288 nm and 332 nm resulting in the overlap with the peaks of many guest molecules (Fig. S6). On the other hand, the main absorption peak of PP is at around 550 nm, and is often used to conduct inclusion complex with CDs as a probe molecule. Therefore, it is suitable for PP as the partner of LL in the double-guest encapsulation process. From Fig. 3, with an increasing of the polymer concentration, the obviously decreased peak intensities of LL and PP solution indicate that both of HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA can conduct encapsulation with guest molecules. It is in accordance with the results in reference [17], where double-guest molecules can be simultaneously encapsulate into amphiphilic hyperbranched polymers.

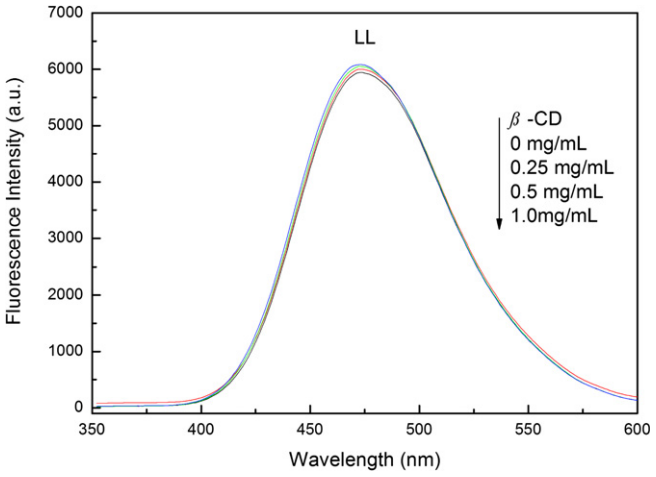


Fig. 5. Fluorescence spectra of LL solution in the presence of β-CD (excitation at 288 nm) [LL (5×10^{-5} mol/L): 4 mL].

LL and PP will be encapsulated into the different cavities, respectively. Firstly, it was confirmed that LL was almost encapsulated only into hyperbranched cavity. From the calculation results of Gaussian 03 software in a semiquantitative manner, the five types of interaction between β-CD and LL (Scheme 3) should be attributed to the intermolecular weak interaction from the view of thermodynamics (Table 2). The results indicate that it is hard for β-CD and LL to form steady inclusion complex. Furthermore, UV–vis spectrum and fluorescence spectroscopy were employed to confirm the above suggestion. From Figs. 4 and 5, no significant change of the peak intensities is observed from UV absorption at 288 nm and fluorescence emission at 474 nm when the β-CD concentration increases in LL buffer solution of pH = 10. In general, these peak intensities should increase or decrease regularly with the increase of β-CD concentration if the inclusion complex is formed [39–42]. It is attributed to the molecular recognition ability of β-CD determined by size/shape match effect between host and guest as well as the cooperative effect of hydrophobic, electrostatic and hydrogen

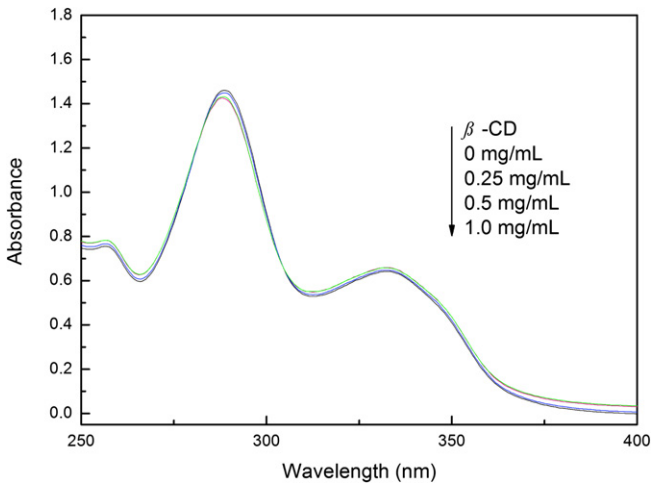


Fig. 4. UV spectra of LL solution in the presence of β-CD [LL (5×10^{-5} mol/L): 4 mL].

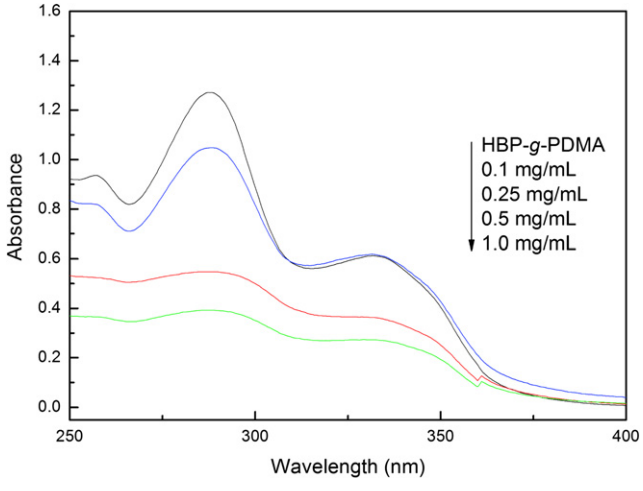


Fig. 6. UV spectra of LL solution in the presence of HBP-*g*-PDMA [LL (5×10^{-5} mol/L): 4 mL].

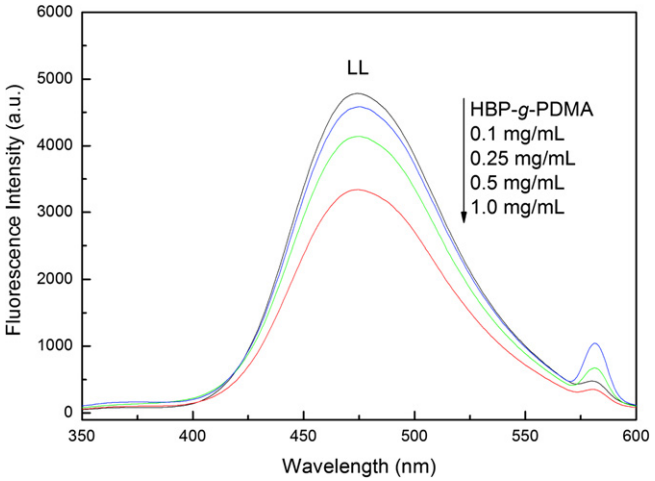


Fig. 7. Fluorescence spectra of LL solution in the presence of HBP-*g*-PDMA (excitation at 288 nm) [LL (5×10^{-5} mol/L): 4 mL].

bonding interactions [43,44]. In this paper, the size/shape match between LL and β-CD cavity should play a main role in determining the inclusion interaction. Anyway, the linkage of the tri-ring structure of LL is too big to fit for the β-CD cavity. In addition, when large amounts of β-CD units were implanted into the hyperbranched structure, the steric hindrance also hindered the formation of the inclusion complex between β-CD and LL. Therefore, these results further confirm that LL almost cannot be encapsulated into β-CD cavity. On the other hand, LL was encapsulated into hyperbranched cavity by using HBP-*g*-PDMA without β-CD units as a host, as shown in Figs. 6 and 7. Here, considering the similar architecture of HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA except β-CD units (Scheme 1 and Scheme S3), HBP-*g*-PDMA was selected as the reference compound to confirm the encapsulation between hyperbranched cavity and LL.

Secondly, PP will be encapsulated into β-CD cavity but not into hyperbranched cavity. It has been proved that PP can be simultaneously encapsulated into β-CD and hyperbranched cavities according to our previous work [25–27]. However, due to the existence of molecular recognition, PP encapsulated in these cavities can be displaced by other guest molecules, such as methyl orange [26,27]. In this paper, the similar phenomenon is still observed in Fig. 8. It presents that the original peak intensity of PP in the presence of HBP-*g*-PDMA without β-CD units increases after the addition of LL solution. It means that PP encapsulated in hyperbranched cavity is displaced by LL and comes into the bulk buffer solution again. Wan et al. [18–20] reported that the core of amphiphilic hyperbranched can highly selectively encapsulate a guest from a mixture deriving from the molecular recognition property independent of the sequence of guest addition. Therefore, PP has lost the ability to take up the hyperbranched cavity due to molecular recognition property, but there is no opportunity for PP encapsulated in β-CD cavity to be displaced by LL based on the discussion of the above section.

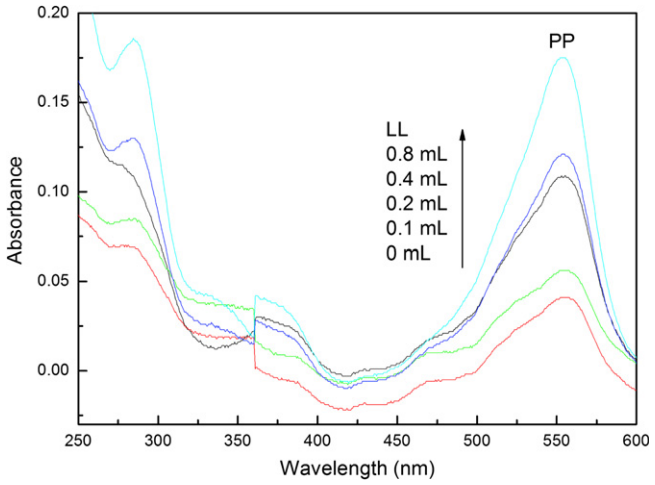


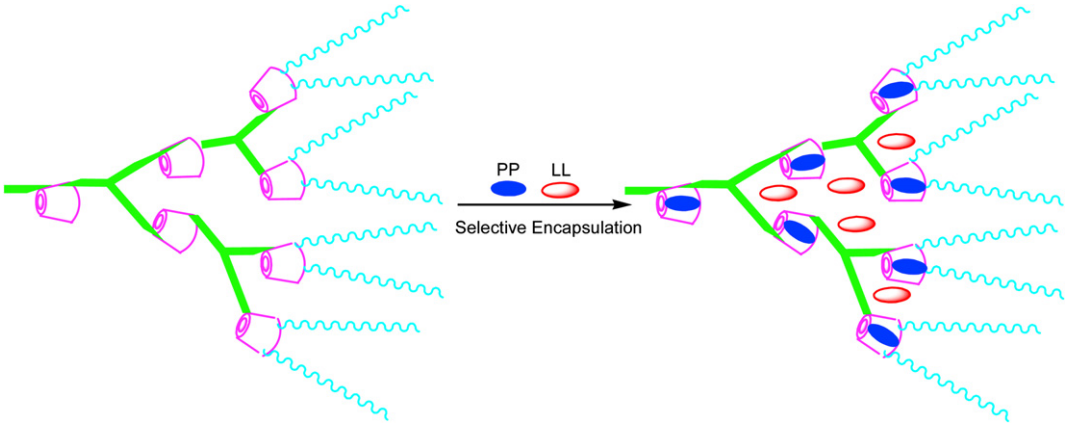
Fig. 8. UV spectra of PP + HBP-*g*-PDMA solution in the presence of different concentration LL solution [PP (5×10^{-5} mol/L): 4 mL, LL (5×10^{-5} mol/L): 0–0.8 mL, HBP-*g*-PDMA: 1.0 mg/mL].

In summary, the amphiphilic nature of HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA provides an opportunity for double-guest molecules to be encapsulated, where hyperbranched poly(β-CD)s act as a hydrophobic core layer, and water-soluble PDMA segments serve as a hydrophilic shell layer. Furthermore, the double molecular recognition behaviors from β-CD and hyperbranched cavities drive PP to go into the former, LL to the latter. In this case, the selective encapsulation between amphiphilic hyperbranched poly(β-CD) and double-guest molecules was successfully fulfilled. The encapsulation mechanism of HBP-(B_y + AB_x)-*g*-PDMA or HBP-AB_2-*g*-PDMA with double-guests is suggested in Scheme 4. On the other hand, the selective encapsulation of amphiphilic hyperbranched poly(β-CD) with LL and PP can be further confirmed from the view of controlled release in next section.

3.3. Controlled double-drug release behaviors

To test controlled release behaviors of amphiphilic hyperbranched polymers with the core–shell structure, a double-drug release system using LL and PP as double model drugs was established. LL is a hydrophobic anti-bacterial drug [45]. PP can be used as a purgative medicine [46]. Fig. 9 shows the release profiles of LL and PP from HBP-(B_y + AB_x)-*g*-PDMA and a series of HBP-AB_2-*g*-PDMA with the different arm lengths at 37 °C. No significant burst release was observed from either LL-loaded or PP-loaded samples. It indicates that the drug molecules do not locate within the hydrophilic PDMA shell layer and only stays in the core layer due to the relatively slow release rate [47,48]. Furthermore, the LL-loaded samples present a sustained release period (up to 14 h) followed by an almost non-release stage as shown in Fig. 9A. In contrast, the releases of PP from samples show the totally different phenomenon (Fig. 9B). PP releases on a quite slow rate within 14 h, subsequently the release rate increases linearly. Obviously, the different release behaviors between LL and PP further suggest that the double-drug molecules exist in the different cavities under different microenvironments. The sustained release of LL from samples is attributed to the hydrophobic–hydrophobic interactions between the drug molecules and the hydrophobic hyperbranched core layer [47,49]. It means that LL should stay in hyperbranched cavity. For PP, the release behavior is complicated, but it is believable that PP exists in β-CD cavity because there is no other place for them to stay in this supramolecular system based on the above discussion. Therefore, selective encapsulation of amphiphilic hyperbranched poly(β-CD) with LL and PP was proved again from the viewpoint of controlled release.

Due to the existence of selective encapsulation, the release sequences of LL and PP from samples are different from their cumulative release amounts in Fig. 9. The cumulative release amounts of LL and PP from HBP-(B_y + AB_x)-*g*-PDMA were 11.9% and 5.2% within the start 14 h, respectively. It suggests that the release of LL is dominant at the early stage due to the different binding abilities of hyperbranched cavity to LL, and β-CD cavity to PP. It can



Scheme 4. Schematic representation for possible encapsulation mechanism of HBP-(B_y + AB_x)-*g*-PDMA or HBP-AB_2-*g*-PDMA with double-guests.

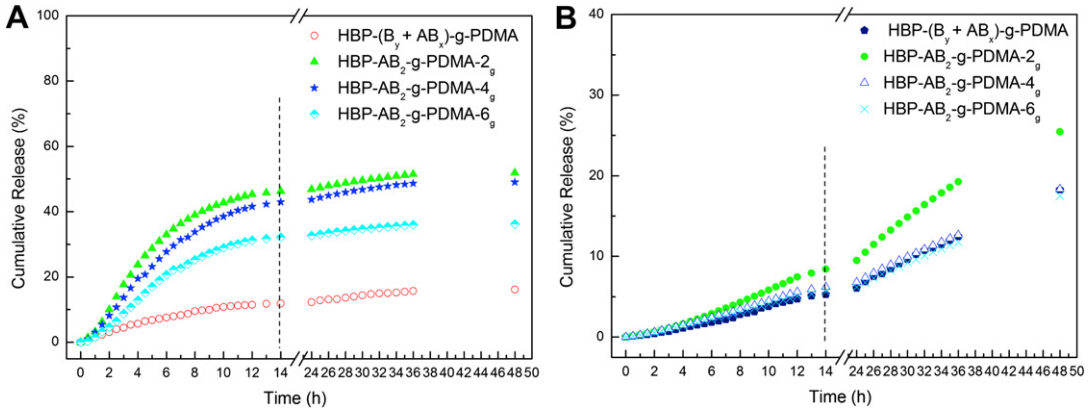
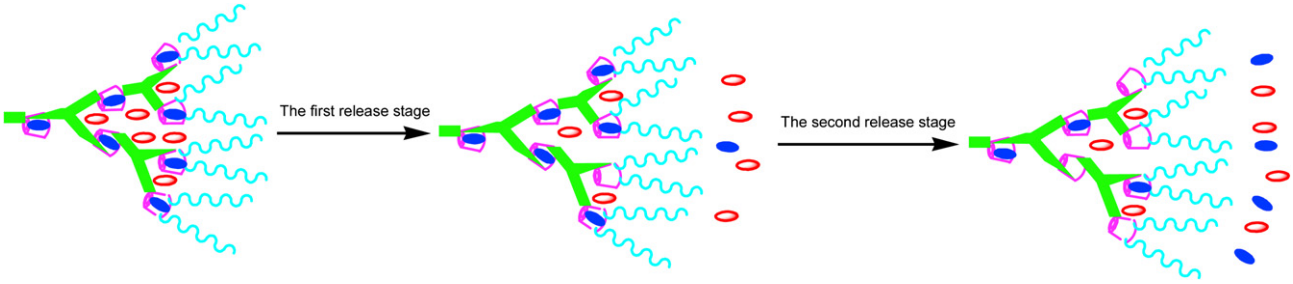


Fig. 9. Release profiles of LL (A) and PP (B) from HBP-(B_y + AB_x)-*g*-PDMA and HBP-AB_2-*g*-PDMA as a function of time at 37 °C.



Scheme 5. Schematic representation for the release processes of LL and PP from samples.

be further confirmed by comparing the cumulative release amounts of LL and PP from a series of HBP-AB_2-*g*-PDMA with the different PDMA chain lengths in Fig. 9. After 14 h, LL will come into a non-release stage resulting from the release equilibrium. In this case, the release rate of PP increases greatly and PP displaces LL to play a determinate role. In addition, the residual amounts of LL and PP still kept the high percentage after 50 h. Thus amphiphilic hyperbranched poly(β-CD)s as polymer carriers possess the ability of the long term release for drugs. The controlled double-drug release mechanism is illustrated in Scheme 5.

The relationships between amphiphilic core–shell structures and double-drug release behaviors were further discussed. The cumulative release amounts of LL and PP from HBP-(B_y + AB_x)-*g*-PDMA are lower than that from HBP-AB_2-*g*-PDMA-6_g as shown in Fig. 9. The reason is that the former has the higher degrees of branching (DB = 0.83 [27]) than that of the latter (DB = 0.40 [27]) in the core layer based on the similar shell layer structure. The hydrophobic hyperbranched poly (β-CD) core possessing high DB value seems to retard the release of drug from polymers. On the other hand, the cumulative release amounts of LL and PP from samples decrease with an increasing of the PDMA chain lengths for HBP-AB_2-*g*-PDMA in the shell layer. It may be attributed to a special macromolecular structure of HBP-AB_2-*g*-PDMA in which the PDMA chains are radially aligned on the three-dimensional core of hyperbranched. It is well known that PDMA has a conformational transition from an expanding shape to a compact coil in accordance with the variation of the surrounding pH value [31,32,50,51]. Accordingly, the release rate of drug can also be changed during the conformational change of the PDMA chains.

Furthermore, PDMA chains are in a compact coil conformation at high pH value (i.e. pH = 10), which retards LL and PP releasing from amphiphilic hyperbranched polymers [52]. Therefore, the release behaviors of drugs from samples can be controlled effectively by regulating the DB values of the core layer or the PDMA chain lengths of the shell layer.

4. Conclusion

A novel supramolecular system of amphiphilic hyperbranched polymer with hyperbranched poly(β-cyclodextrin) core was designed and synthesized to accomplish a so-called selective encapsulation. In this supramolecular system, PP and LL can be encapsulated into two different molecular cavities from β-CD and hyperbranched, respectively. The double molecular recognition behaviors from β-CD and hyperbranched cavities drive PP to go into the former and LL to the latter. This viewpoint can be further confirmed by the release profiles and release sequences of two guest molecules. LL presents a sustained release period followed by an almost non-release stage, while at first PP releases on a quite slow rate and subsequently the release rate increases linearly. The release of LL is dominant at the early stage, and then the release rate of PP increases greatly even to displace LL in release system. The release behaviors of double-guest from samples can be controlled effectively by regulating the DB values of the core layer or the PDMA chain lengths of the shell layer. This discovery will extend the CD-based on hyperbranched polymers applied in supramolecular science and complex drug delivery system.

Acknowledgements

The authors are grateful to the China Postdoctoral Science Foundation funded project (No. 20090461307) and NPU Fundamental Research Foundation (No. JC200920) for financial support.

Supplementary data

Supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.polymer.2010.04.009.

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