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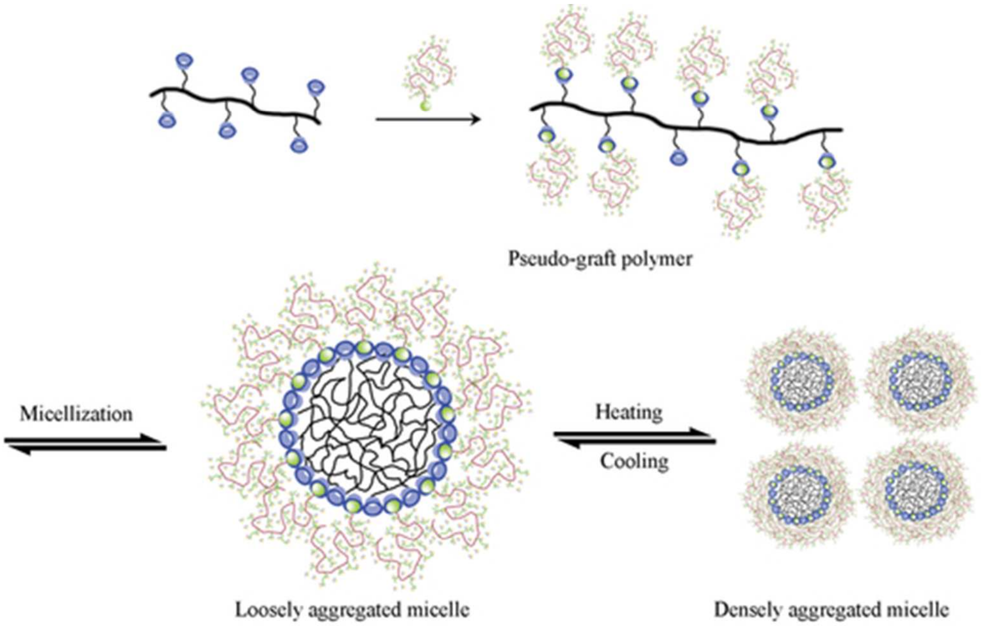

Thermoresponsive and self-assembly behaviors of pseudo-graft polymer based on adamantyl-terminated poly (oligo (ethylene glycol) methacrylate and homopolymer with cyclodextrin pendants.
44x28mm (300 x 300 DPI)

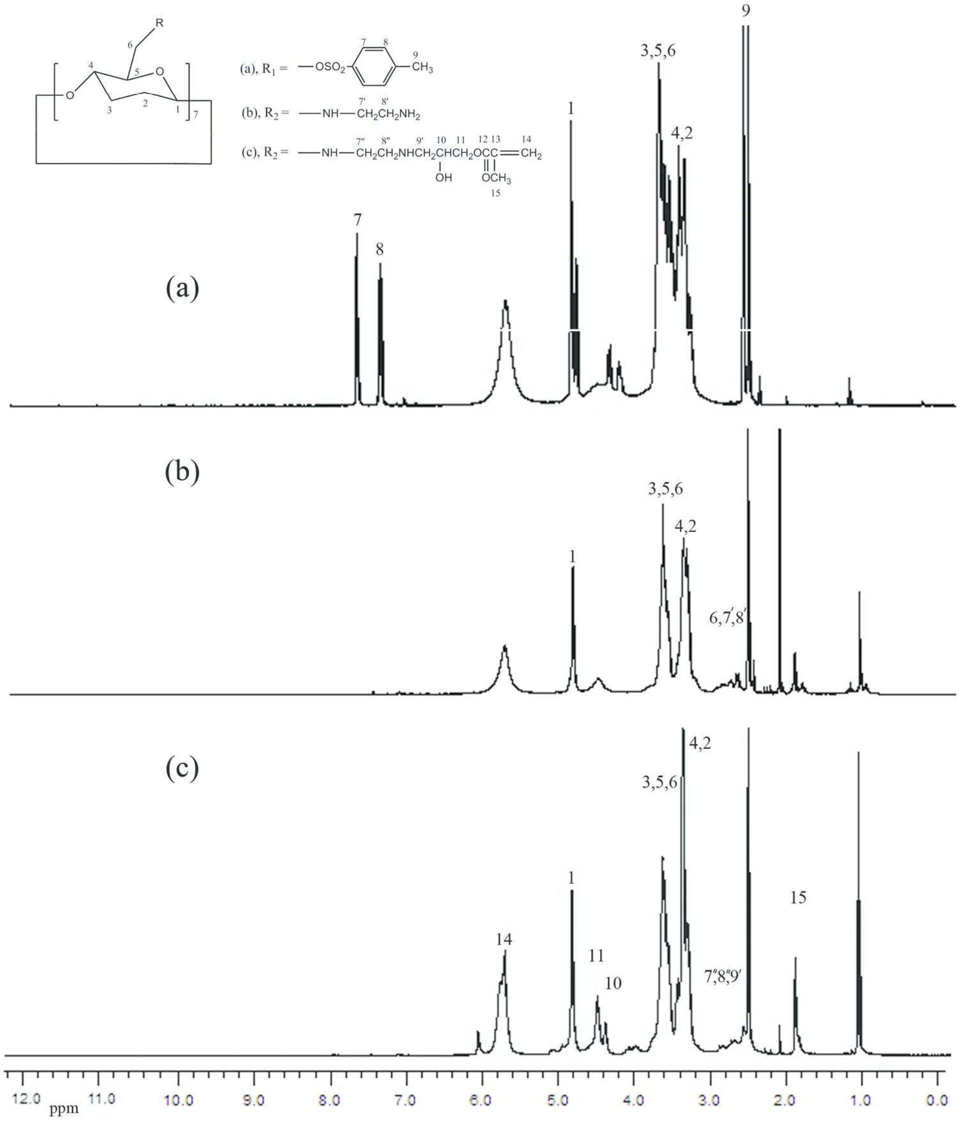

Figure 1. 1H NMR spectra of β-CD based monomers (TCD and ECD) and GCD.
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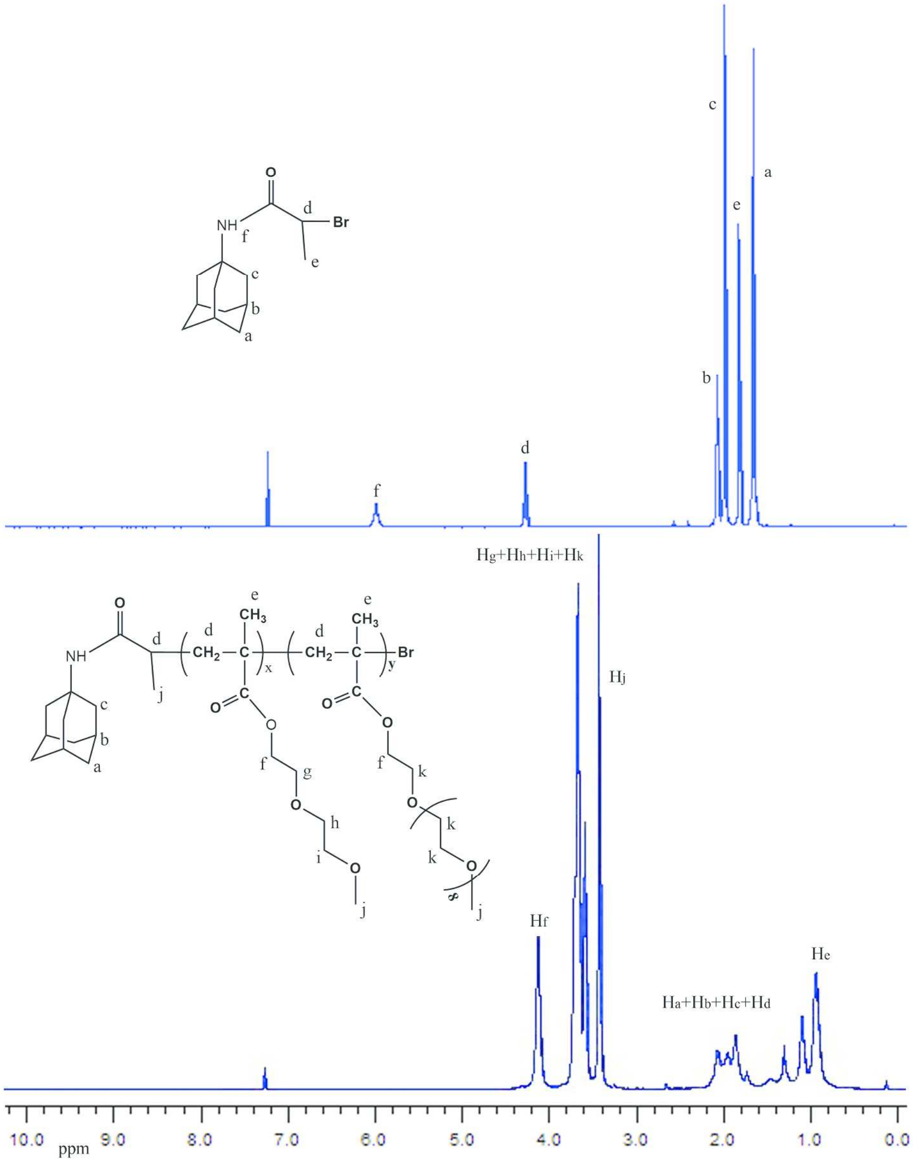


Figure 2. 1H NMR spectra of AdBB and a Ad-terminated P(MEO2MA-co-OEGMA) copolymer (A1) containing 5 mol % of OEGMA per chain recorded in CDCl3.
216x262mm (600 x 600 DPI)

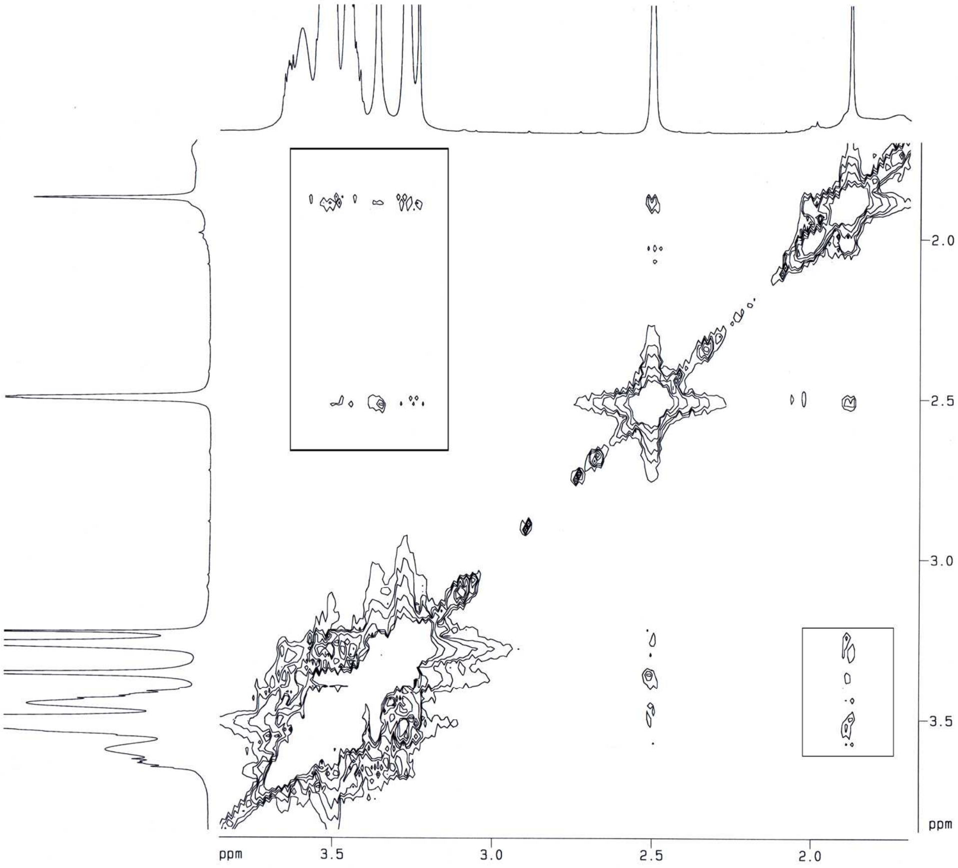


Figure 3. 2D 1H NOESY spectrum of the host-guest mixture of PGCD host and Ad-POEGMA guest in DMSO-d6 at 25 oC.
195x175mm (300 x 300 DPI)

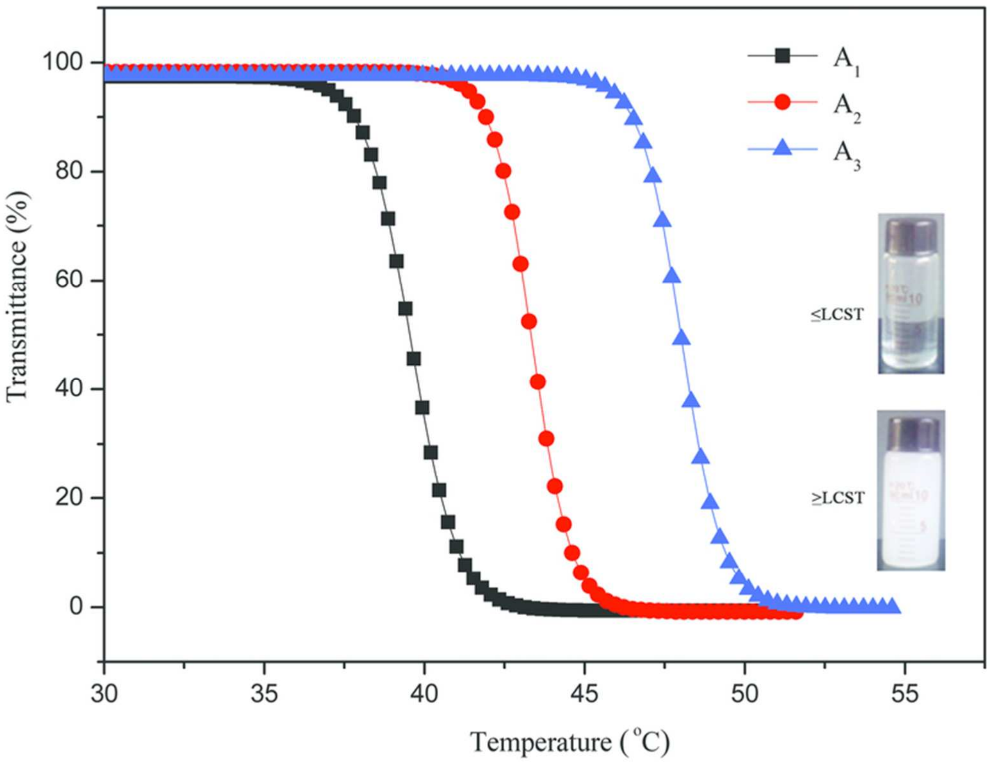


Figure 4. Plots of transmittance as a function of temperature measured for aqueous solutions (3 mg mL-1) of Ad-POEGMAs (A1, A2 and A3).
69x53mm (300 x 300 DPI)

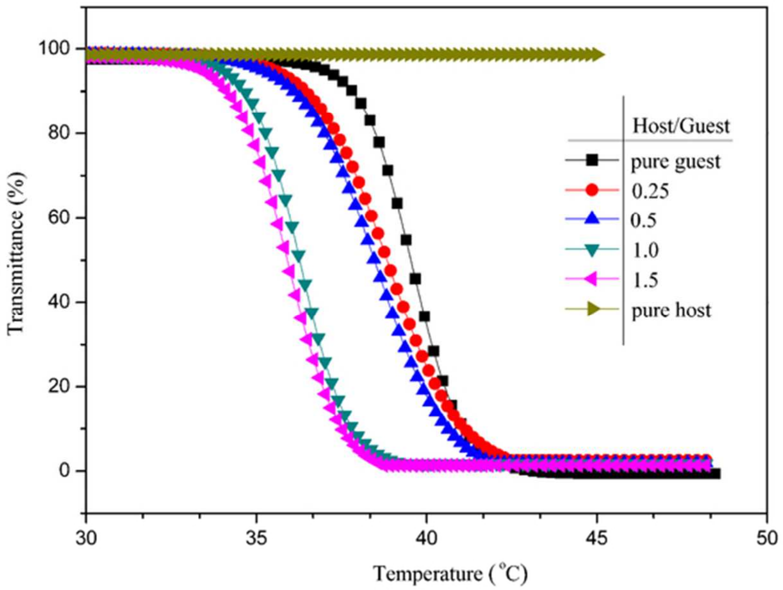


Figure 5. Turbidity variations of aqueous solutions of Ad-POEGMAs (A1) (guest) as a function of temperature upon the addition of PGCD (host). Cguest = 3 mg mL-1 (pure guest); nPGCD/AD-POEGMA= 0.25, 0.5, 1, 1.5 separately.
63x44mm (300 x 300 DPI)

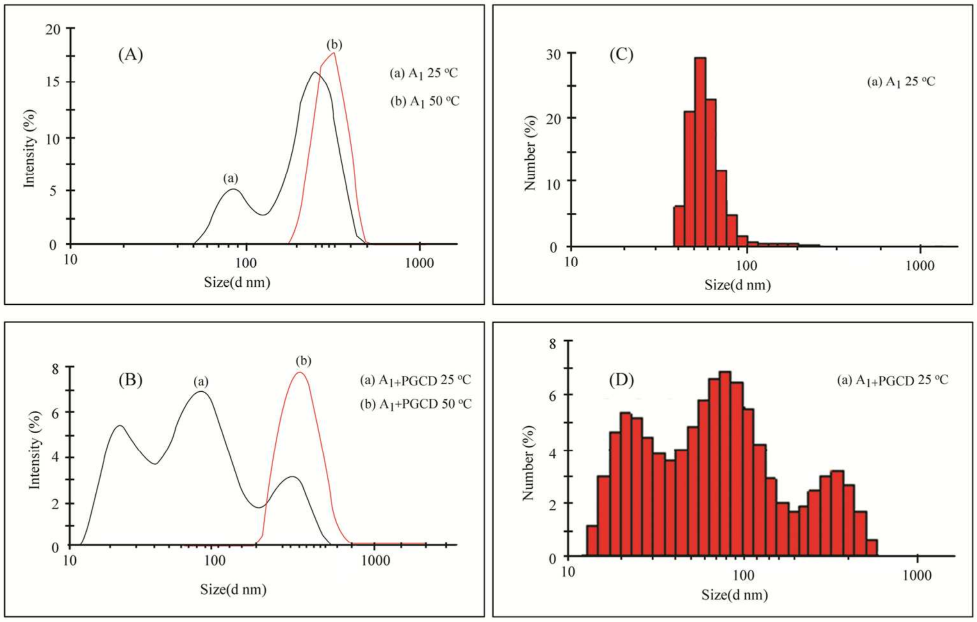


Figure 6. Size distributions of the aggregates formed by A1 (A and C), and A1+PGCD (B and D) in aqueous solution (3 mg mL-1) at 25 and 50 oC, separately.
150x97mm (300 x 300 DPI)

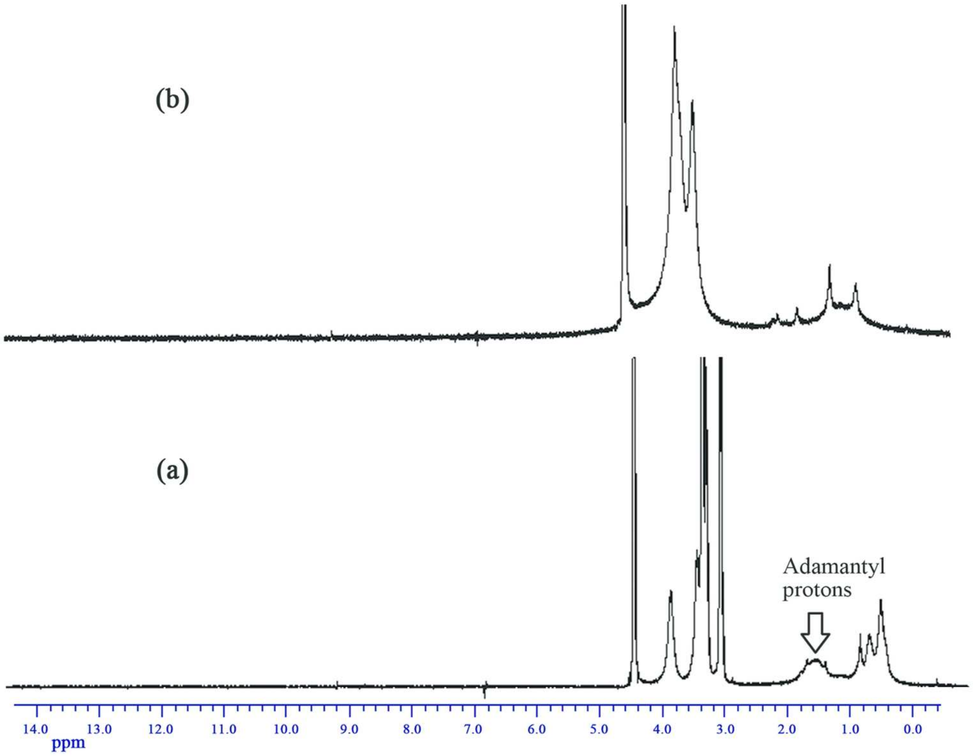


Figure 7. 1H NMR spectra of a copolymer P(MEO2MA-co-OEGMA) containing 5 mol % of OEGMA per chain recorded in D2O at 25 and 50 oC.
60x47mm (600 x 600 DPI)

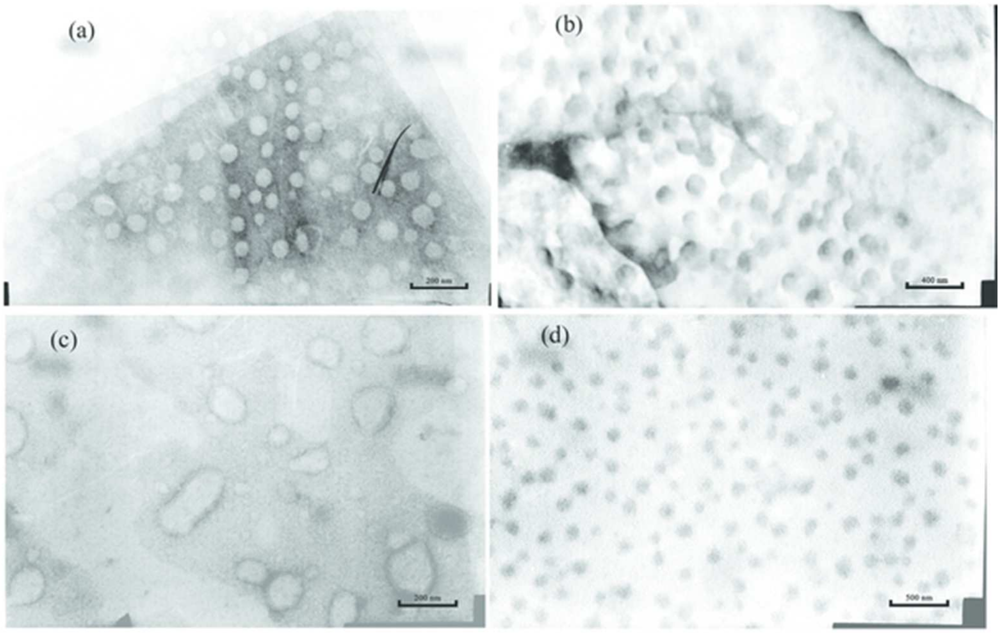
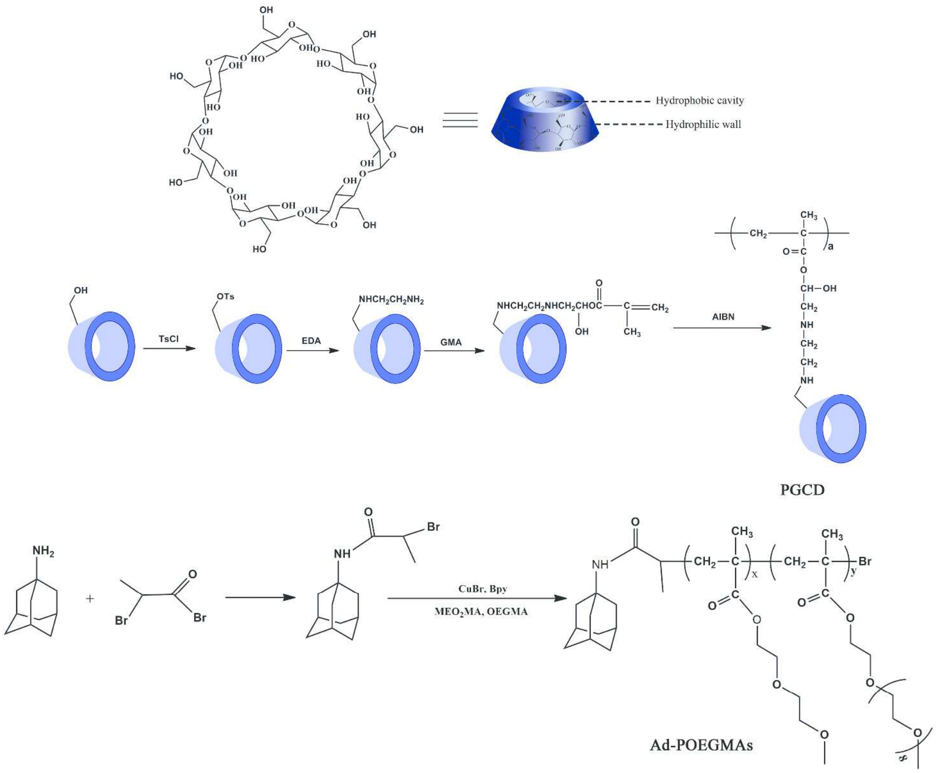
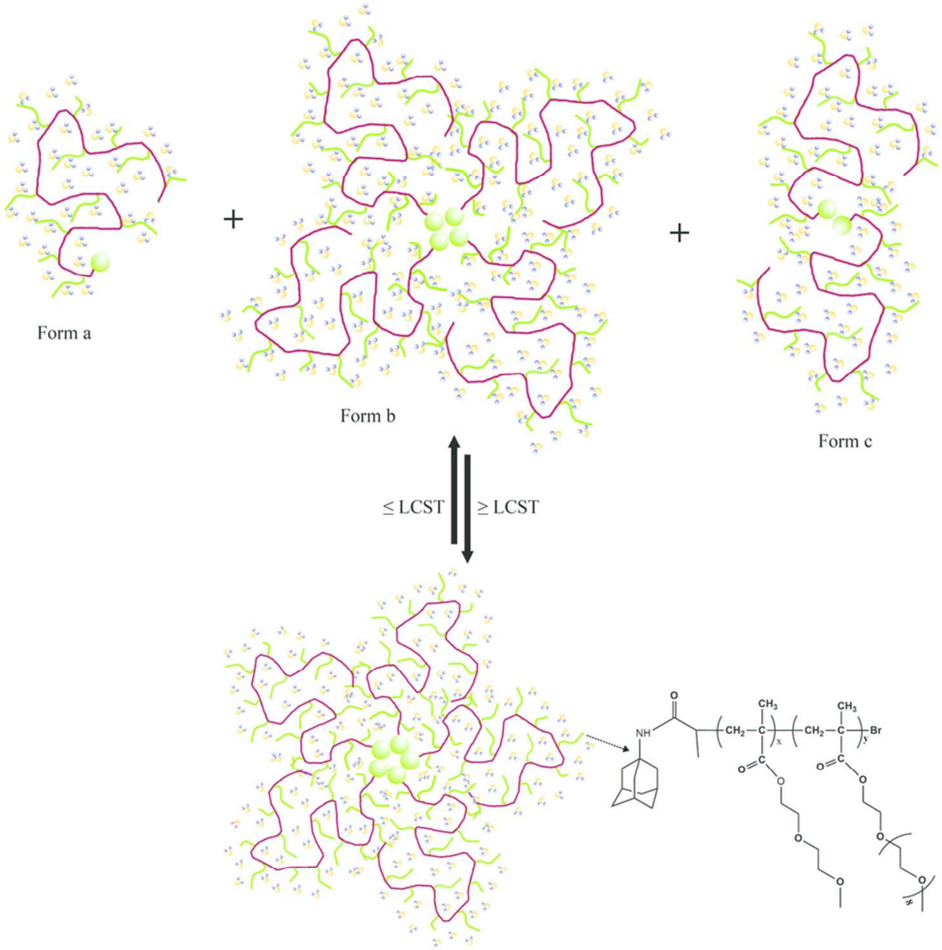


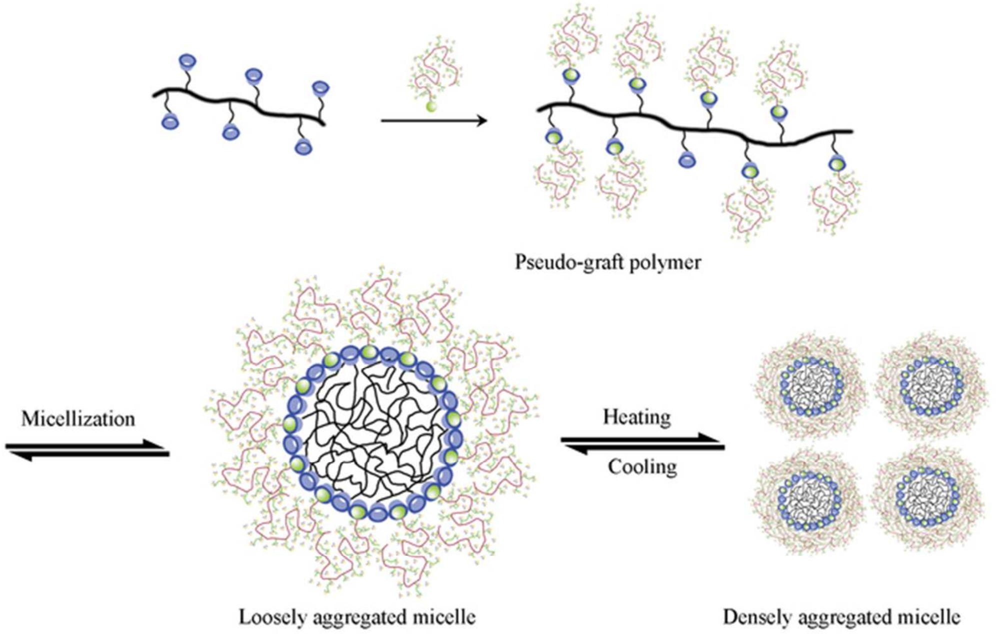
Figure 8. Typical TEM images obtained by aqueous solutions of A1 (a and b) and A1+PGCD (c and d) (3 mg mL-1) at 25 and 50 oC.
51x35mm (300 x 300 DPI)



Scheme 1. Synthetic routes employed for the preparation of CD based monomers and PGCD, and ATRP initiator AdBB and Ad-POEGMAs.
173x137mm (600 x 600 DPI)



Scheme 2. Schematic illustration of the dynamic self-aggregation and self-disaggregation mechanism of Ad-POEGMAs in water during heating and cooling.
88x87mm (300 x 300 DPI)



Scheme 3. The procedures for inclusion complexation, the thermally-induced dehydration and subsequent self-assembly of the pseudo-graft polymer based on Ad-POEGMAs and PGCD.
58x38mm (300 x 300 DPI)

# Pseudo-graft polymer based on adamantyl-terminated poly (oligo (ethylene glycol) methacrylate and homopolymer with cyclodextrin as pendant: its thermoresponsivity through polymeric self-assembly and host-guest inclusion complexation

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## ABSTRACT

Series of well-defined adamantyl-terminated thermally-responsive copolymers (Ad-POEGMAs) were synthesized by atom transfer radical polymerization (ATRP), in which 2-(2-methoxyethoxy) ethyl methacrylate ($MEO_2MA$) and oligo (ethylene glycol) methacrylate (OEGMA) served as the thermosensitive building blocks. Meanwhile, cyclodextrins (CDs) as bulky pendent grafted polymer (PGCD) was synthesized by homopolymerization of aminoethyl methacrylate β-cyclodextrin (GCD). The thermal-responsive behaviors were investigated by combination of $^1$H NMR, UV-vis spectroscopy, dynamic light scattering (DLS), and transmission electron microscopy (TEM). In comparison to other thermal-responsive copolymers based on POEGMAs, Ad-POEGMAs exhibited unusual thermally induced aggregation process. The Ad group assembled and POEGMA chains associated to produce stable water soluble nano aggregates, followed by a rearrangement process at the second thermal transition. Moreover, it was found that supramolecular pseudo-graft noncovalently connected polymer via inclusion complexation in aqueous solution was formed. This pseudo-graft polymer underwent a reversible temperature-induced transition from solution to micelle under suitable conditions. The cyclodextrin (CD) moiety attached to the main chain played two roles. As supramolecular host moieties, CDs formed inclusion complexes with guest-ended polymers, leading to graft-like polymers. As bulky hydrophilic moieties, CDs stabilized the micelles induced by the coil-to-globule transition of POEGMA segments.

## Introduction

Polymers respond with a property alteration towards environmental changes are often referred to as "smart" polymeric systems because they exhibit reversible property changes in response to changes in external conditions such as pH, temperature, ionic strength, light irradiation, mechanical force, electric and magnetic fields, and analyte of interest (e.g., ions, bioactive molecules, etc.) or an integration of them.[1-7] In cases where the external trigger is temperature, the polymer is said to exhibit thermo-responsive property. Poly(N-isopropylacrylamide) (PNIPAM) is one of the most popular thermal-responsive polymers, which possesses dramatic and reversible phase transition behavior in aqueous solution and a lower critical solution temperature (LCST).[8-11] The progress in the design and application of PNIPAM based thermo-responsive polymers during the last decades was already covered in a range of review articles.[12,13] Recently, series of methacrylate-based polyethylene glycol(PEG) polymers (POEGMAs) substituted with oligo(ethylene glycol) (OEG) units as side chains were reported to possess similar or even superior thermosensitivity than PNIPAMs, which have attracted a great deal of attention.[14-20] POEGMAs have good phase transition reversibilities and tunable lower critical solution temperatures (LCSTs) in aqueous medium, and the biocompatibility of POEGMAs is also excellent and attractive. Moreover, by the copolymerization of different OEGMA monomers with different side chain lengths and by varying the feed molar ratio of the comonomers, the LCSTs of POEGMAs can be conveniently adjusted. To date, great interests have been evoked for the design of POEGMA based polymers, and these polymers have been widely incorporated into dendrimers, microgels, block or comb-like copolymers, polymeric brushes and gold surfaces which endow these materials with fascinating properties. Furthermore, although studies have indicated that the thermal-responsive mechanism of the POEGMAs can also be regarded as the consequence of the competition between hydrophilic polymer-water interactions and hydrophobic polymer-polymer interactions, the role of POEGMAs in a thermally-induced self-assembly process, especially when POEGMAs serve as thermosensitive components for composite systems composed of different components has still not been sufficiently studied.[21-23]

Recently, compared to traditional chemistry based on covalent bonds, supramolecular chemistry based on non-covalent bonds has become a powerful way to construct supramolecular system in a facile and dynamic way. A variety of subtle non-covalent interactions, such as hydrogen bonding, metal-ligand coordination interaction, electrostatic attraction, host-guest inclusion complexation, etc. have been widely used as the driving forces to construct supramolecular systems.[24-34] Therefore, based on those thoughts, well-defined Ad-POEGMAs was firstly synthesized and applied in order to fine tune the LCSTs of the PEG based thermo-responsive polymers. Moreover, this work focused on self-assembly behavior of composite system between Ad-POEGMAs as guest consisting of POEGMAs as thermal blocks, and PGCD as multi-host with pendent CD cavities via inclusion complexation. The design of this special composite system is based on the following facts and considerations: the main part of Ad-POEGMAs is the thermal sensitive POEGMA segments, so thermal induced self-assembly is possible; moreover, CD species is a bulky group which endows the PGCD with some characters of a comb-like polymer, The hydrophobic interior cavity of β-CD can accommodate a variety of guest molecules. Among them, the β-CD/Ad pair is well-known due to its high association constant, so PGCD may show some peculiar characters in assembly. Finally, the CD cavities as hosts are available for post-polymerization modifications by POEGMA with Ad guest end via inclusion complexation to construct complex supramolecular system. In the current work, this supramolecular system was formed with mixtures of Ad-POEGMAs and PGCD due to the inclusion complexation between terminal CD and Ad moieties. Possessing thermoresponsive POEGMAs, the obtained supramolecular pseudo-graft polymer is expected to exhibit intriguing aggregation properties in aqueous solution.

## Results and Discussion

### Synthesis and structure analysis of β-CD based monomers and PGCD homopolymer

Herein, we report systematically on an efficient and preparatively simple approach for the generation of β-CD based monomers with high purity and excellent yield, which all were confirmed by element analysis, IR, $^1$H NMR, $^{13}$C NMR and MALDI-TOF measurements, the detailed synthesis processes of β-CD based monomers are shown in our previous reports.[35] The synthetic route of TCD, ECD and GCD are shown in Scheme 1 and the $^1$H NMR spectra of TCD, ECD and GCD are shown in Fig. 1. The water soluble PGCD was prepared by homopolymerization, and the SEC polydispersity index (Mw/Mn) of PGCD was low at 1.19.

### Synthesis and structure analysis of AdBB and Ad-terminated P(MEO$_2$MA-co-OEGMA) copolymers (Ad-POEGMAs)

AdBB initiator was synthesized by 2-bromopropiomyl bromide and 1-adamantanamine with high purity and yield. Using AdBB as the initiator, and MEO$_2$MA and OEGMA as comonomers, Ad-terminated polymers, Ad-POEGMAs, were synthesized by atom transfer radical polymerization (ATRP). As reported by Lutz et al., increasing the feed molar ratio of MEO$_2$MA to OEGMA leads to a decrease in the LCST of POEGMAs. Therefore, three Ad-

POEGMAs named $A_1$, $A_2$, and $A_3$ with molar ratios of $MEO_2MA$ to OEGMA being 95:5, 90:10, and 85:15, respectively, were used in this study. The polymerization condition and results are summarized in Table 1. Fig. 2 shows the $^1H$ NMR spectra of **AdBB** and $A_1$. Besides the resonance peaks of the POEGMAs segment in the range of $\delta$= 0.8–4.1 ppm, the characteristic signals of Ad segment, could also be observed at $\delta$= 1.6-2.1 ppm, indicating that Ad-POEGMAs was successfully synthesized, although the signals of Ad moiety were partly overlapped by the relatively signals of the main-chain methylene protons of main chains at $\delta$= 1.3-2.4 ppm.

Table 1. Summary of structural parameters of PGCD and Ad-terminated POEGMA copolymers ($A_{1,}$ $A_2$ and $A_3$).

| Samples | Initiator | $n_{MEO2MA}$:$n_{OEGMA}$ $^c$ | $M_{n,\ SEC}$ $^d$ | $M_{w,\ SEC}$ $^d$ | PDI $^d$ |
|---|---|---|---|---|---|
| PGCD $^a$ | / | / | 11950 | 14220 | 1.19 |
| $A_1$ $^b$ | AdBB | 95:5 | 23163 | 30427 | 1.32 |
| $A_2$ $^b$ | AdBB | 90:10 | 29610 | 39604 | 1.35 |
| $A_3$ $^b$ | AdBB | 85:15 | 34667 | 46535 | 1.34 |

*(a) Synthesized via the homopolymerization of aminoethyl methacrylate β-cyclodextrin (PGCD); (b) Synthesized by the ATRP of $MEO_2MA$ and OEGMA using AdBB as initiator; (c) mole ratio (%) of $MEO_2MA$ and OEGMA in feed; (d) Molecular weights and molecular distributions ($M_w$ /$M_n$, PDI) were determined by SEC using THF as eluent relative to polystyrene standards.*

Fig. 3 presents the 2D $^1H$ NOESY spectrum of the mixture of AD-POEGMA ($A_1$) guest and PGCD host in DMSO-$d_6$, which provided a direct evidence for the formation of the pseudo-graft PGCD/AD-POEGMA polymer. The NOESY cross-peaks between the signals at 3.0-4.0 ppm ascribed to the inner protons C(3)H and C(5)H of β-CD segments and the signals at 1.6-2.1 ppm assigned to the methine protons(Hb) and the methylene protons(Ha and Hc) of adamantyl moieties were clearly observed, indicated that AD moieties were included in the cavity of β-CD, consequently, the Ad-POEGMAs were successfully synthesized by atom transfer radical polymerization.

## Thermoresponsive behavior of the Ad-POEGMAs and host-guest inclusion complexation system

Polyethylene glycol (PEG) and its derivatives are well known for its highly hydrophilic or water-soluble nature, and exhibit LCSTs in aqueous solution.[14-19] They are water-soluble and water-insoluble below and above their LCSTs. The mechanism proposed for explaining the thermo-responsiveness of POEGMAs is summarized as follows: the conformationally favored formation of H-bonds between the ether oxygens of poly (ethylene glycol) (PEG) and water hydrogens is one of the key factors responsible for the unusual water solubility of these PEG type polymers.[21,22] Thus, the Ad-POEGMAs were expected to self-assemble into nano-sized aggregates or micelles in their aqueous solutions by altering temperature, moreover, the interesting thermoresponsive behavior was investigated for the host-guest system comprising of polymeric host PGCD with CD as pendant and polymeric guest Ad segment with POEGMAs in aqueous solution. In this study, the thermally-induced self-assembly behaviors were investigated in detail by a combination of UV-vis, DLS, and TEM and $^1$H NMR in $D_2O$.[36]

### (a) Determination of the LCSTs of Ad-POEGMAs

UV-Vis was first used to determine the LCSTs of the Ad-POEGMAs. The results are shown in Fig. 4. A gradual decline in the solution transmittance could be observed with elevated temperature, indicating that the three Ad-POEGMAs were all thermally-responsive. The LCSTs for $A_1$, $A_2$, and $A_3$ were 37.94 $^o$C, 42.07 $^o$C and 46.71 $^o$C, respectively, and increased with increasing content of OEGMA. Studied have already indicated that the LCSTs of the copolymers of 2-(2-methoxyethoxy) ethyl methacrylate ($MEO_2MA$) and ω-methoxy (oligoethyleneoxy) ethyl methacrylate (OEGMA) increased significantly with increasing content of OEGMA. In this study, in contrast to previous reports, the Ad group was firstly introduced to POEGMAs system, but similar results were further confirmed for different POEGMA copolymers with hydrophobic Ad groups. However, the difference for Ad-POEGMAs is that the LCSTs are higher than those POEGMA copolymers without Ad groups.

**(b) Determination of the LCSTs of the supramolecular system of Ad-POEGMAs and PGCD.**

In this study, the interesting thermoresponsive behavior was further investigated for the host-guest system comprising of polymeric guest Ad-POEGMAs and host PGCD in aqueous solution. Both the host and the guest polymers used in this study were all soluble at room temperature. Fig. 5 shows the information on the thermoresponsive behaviors of a series of mixtures of PGCD and Ad-POEGMAs with varied host/guest ratio. It could be seen that individual aqueous pure guest exhibited thermoresponsive profile, but the host polymer exhibited none thermoresponsive profile. When the two components were mixed together, the LCSTs of the host-guest supramolecular systems decreased, finally when the adding amount of polymeric host PGCD increased to some extent, the LCST became almost saturated when the composite system involved a 1:1 complexation between adamantyl moiety and β-CD core. The detailed experimental results for the aqueous mixtures of $A_1$ and PGCD were given below as a typical example.

Fig. 5 shows that the LCST of pure guest ($A_1$) was 37.94 $^{o}$C somewhat higher than body temperature promising for biomedical application, and shifted to a lower temperature upon the addition of the host (PGCD). The LCST significantly changed with a LCST decrease (ΔLCST, referenced to pure guest) of 1.45 $^{o}$C, 1.74 $^{o}$C, 3.13 $^{o}$C and 3.65 $^{o}$C when the host/guest ratio is 0.25, 0.5, 1.0 and 1.5. At last, the LCST became almost unchanged indicating that the composite system involved complexation equilibrium between adamantyl moiety (Ad) and β-CD core. The Ad-terminated POEGMAs could be incorporated to the β-CD core via inclusion complexation to form a pseudo-graft supramolecular polymer. Through forming the pseudo-graft polymer, such networks stabilized not only by single CD/Ad complexes but also by pairs of CD/Ad complexes, the hydrophilic POEGMA arms on guest polymer contributed to increase the water solubility of the supramolecular polymeric system. However when the adding amount of PGCD exceeded the requirement of complexation equilibrium, the excess PGCD reduced the thermal sensitivity of this supramolecular system.

## Size and morphology of the Ad-POEGMAs and host-guest inclusion complexation system

### (a) Size and morphology of the assemblies formed of Ad-POEGMAs

Although researchers have demonstrated that comb-like polymers with multiple PEG side chains generally adopt a compact coil conformation in water, the existence of intermolecular associations resulting in the formation of larger aggregates such as polymer vesicles was also suggested by researchers.[37,38] Moreover, previous reports have already indicated that hydrophilic propionate-terminated poly( $MEO_2MA$-OEGMA) solution which had the coexistence of small particles with a hydrodynamic radius (Rh) less than 10 nm.[21] Thus, in the present case, it is important to study the exact conformation of Ad-POEGMAs in aqueous solutions above and below the LCSTs. Fig. 6 shows the typical size distributions measured by dynamic light scattering (DLS) for an aqueous solution (3 mg $mL^{-1}$) of the Ad-POEGMAs ($A_1$) containing 5 mol % of OEGMA. This solution was optically clear at room temperature but the DLS intensity distribution indicated the coexistence of small particles with a hydrodynamic radius (Rh) of approximately 10-100 nm and larger aggregates having a radius of roughly 350 nm, and the latter were indeed a negligible minority, as evidenced by the number distribution shown. Hence, the size distributions of the Ad-POEGMAs in aqueous solutions presented a wide poly-dispersity with large hydrodynamic radius below the LCSTs, indicating that Ad-POEGMAs not only exist as a random coil conformation in aqueous solution but also with pseudo aggregates. However, as a matter of fact, under lower temperature (<LCSTs), the Ad-POEGMAs were still easily soluble.

According to previous report, the self-assembly mechanism of random copolymer of P(MEO$_2$MA-co-OEGMA) is a four consecutive conformation changes “hydrated chains-dehydrated chains-loosely aggregated micelles -densely aggregated micelles” during the self-aggregation process.[39,40] Therefore, A reasonable explanation could be summed as follows for Ad-POEGMAs, just like shown in scheme 2, the special hydrophobic Ad groups connect to POEGMAs covered in PEG chains or self assembled partly resulted for different size aggregates in their aqueous solutions. A plausible structure of aggregates should be a structure with the hydrophobic adamantyl (Ad) chains towards the interior while the hydrophilic POEGMAs chains exposed and stretched in water by the hydrophilic-hydrophilic interactions, therefore behaved water-soluble. As the temperature increasing, the partly dehydration of PEG side chains which collapsed first to get close to the hydrophobic backbones or Ad group, and then distorted to expose hydrophilic ether oxygen groups to the outer shell of polymer chains as much as possible. When the temperature rose above the LCSTs, it was found obviously that Ad-POEGMAs could self-assemble into nano-sized aggregates in their aqueous solutions. The uniform aggregates formed with values ranging from 150 nm to 250 nm with a Z-average diameter (DZ=181nm, PDI=0.116) revealing the occurrence of thermally-induced self-assembly of the Ad-POEGMAs. However, the size of this nano-sized aggregates of Ad-POEGMAs was obvious larger than that of previous reports about P(MEO$_2$MA-co-OEGMA) copolymers resulted from the Ad group.

In order to further confirm the self-assembly mechanism, Ad-POEGMAs (A$_1$) was investigated by $^1$H NMR in various deuterated solvents. Fig. 7 compares the spectra measured for the Ad-POEGMAs (A$_1$) in deuterated water (D$_2$O) at 25 $^{o}$C and 50 $^{o}$C. As shown in Fig. 2, in deuterated chloroform, all the protons of $A_1$ lead to sharp and intense signals, which suggested that the macromolecule was uniformly molecularly dissolved. Rather different results were observed in $D_2O$. In $D_2O$ at 25 $^oC$ and 50 $^oC$, the peaks corresponding to the protons of the oligo (ethylene glycol) side chains remained sharp, but the signals of the protons belonging to the Ad group and backbone or located in close proximity of the backbone $\delta$=1.6-2.1 ppm considerably reduced and broadened as compared to those observed in $CDCl_3$. In $D_2O$ at 50 $^oC$, the signals of the protons belonging to the Ad group were disappeared compared to those observed in $D_2O$ at 25 $^oC$. Based on the analysis above, it could be concluded that the nano-assemblies formed by the Ad-POEGMAs were stabilized by the Ad segment aggregated and POEGMAs chains dehydrated and aggregated jointly through hydrophobic-hydrophobic interactions.

**(b) Size and morphology of the assemblies formed of host-guest inclusion complexation system**

DLS is proved to be a sensitive method to trace the formation of noncovalently connected nano-assemblies according to the changes in particle size and scattered light intensity.[41,42] The changes of micelle size with temperature (25 $^oC$ and 50 $^oC$) for the 1:1 host- guest system of Ad-POEGMAs ($A_1$) and PGCD are shown in Fig. 6. It could be seen that the values of nano-assemblies size were with a hydrodynamic radius (Rh) of approximately 10-300 nm, and kept unevenly under lower temperature (<LCST). However, the values increased dramatically to a 200-350 nm with a Z-average diameter (DZ=273nm, PDI=0.121) when the temperature was increased above LCST. This increase could be attributed to a change in the refractive index of the guest molecule as its POEGMAs arms underwent a phase transition from random coil to condensed globule.[15,43,44] This change indicated clearly that the micelles began to form at around LCST, and this result from DLS study was consistent with that following TEM investigation.

The self-assembly mechanism of the host-guest composite system was further confirmed by comparing the TEM images of the polymeric solutions, which were prepared at 25 $^{o}$C and 50 $^{o}$C. Fig. 8 shows the representative TEM micrographs of noncovalently connected micelles formed from Ad-POEGMAs ($A_1$), and 1:1 host-guest mixtures of Ad-POEGMAs ($A_1$) and PGCD at 25, and 50 $^{o}$C, respectively. From TEM micrographs, At 25 $^{o}$C, the Ad-POEGMAs dissolved in water as uneven and loose single- or multi-molecular spherical particles with a diameter ranging from 10 nm to 100 nm, whereas bigger micelles (150-230 nm in diameter) were observed for sample solution prepared and dried at 50 $^{o}$C, showing that the noncovalently connected micelles formed at higher temperature had bigger size. In contrast, spherical uneven and loose nano-assembled vesicles were observed at 25 $^{o}$C for host-guest mixtures of $A_1$ and PGCD, which agreed quite well with the results measured by DLS at the same temperature, demonstrating that nano-sized aggregates indeed formed via the inclusion complexation of the Ad-POEGMAs and PGCD. For the host-guest composite system of $A_1$ and PGCD, dense dark spherical particles could be observed, which was especially clear for the sample prepared and dried at 50 $^{o}$C, this was due to the action of the staining agent, phosphotungstic acid, which stains the hydrophobic segments to a greater extent. However, it was found that the diameters of the micelles from the TEM micrographs were clearly smaller than those from DLS measurements. This could be related to the fact that DLS measures the hydrodynamics diameter of the micelles in an aqueous environment whereas the TEM micrographs show the dehydrated solid state of the micelles.[17]

Furthermore, obvious differences in morphology could be observed by comparing the TEM images of the nano-assemblies. The nano-assemblies constructed from Ad-POEGMAs show relatively light aggregates below the LCST, and dark condensed globule above the LCST, presenting typical micellar characteristic of POEGMAs based polymers.[39,45] For the ones formed from host-guest mixtures of $A_1$ and PGCD, a strong contrast between the light center and the dark thin periphery is seen below the LCST, which is characteristic of a vesicular nano-structure.[46,47] When the temperature rose above the LCST, densely aggregates formed. Hence, from the results of DLS and TEM it was found that nano-assemblies with different morphology could be constructed from Ad-POEGMAs and the supramolecular system of Ad-POEGMAs and PGCD by simply elevating the temperature, with Ad-POEGMAs forming micelle-like nano-structures and the supramolecular system of Ad-POEGMAs and PGCD organized into vesicle-like nano-aggregates. The procedures for inclusion complexation, the thermally-induced dehydration and subsequent self-assembly of the pseudo-graft polymer based on Ad-POEGMAs and PGCD have been illustrated in Scheme 3.

## Conclusions

The thermally-responsive Ad-POEGMAs were synthesized and shown tunable LCSTs behavior in aqueous solution, which could self-assemble into noncovalently connected micelles with the Ad segments as the core and hydrophilic POEGMAs as the corona. Moreover, a composite system was designed using Ad-terminated POEGMAs and PGCD, which was able to form thermo-responsive pseudo-graft polymer in aqueous solution via host-guest complexation between the β-CD core of the host polymer and the adamantyl moiety of the guest polymer. Such pseudo-graft polymer stabilized not only by single CD/Ad complexes but also by pairs of CD/Ad complexes. The pseudo graft polymer could self-assemble to form nano-sized aggregation above and below the LCST in aqueous solution. As a result of its tunability of thermoresponsive behavior, noncovalent connected micelle forming ability and potential biocompatibility, the pseudo graft polymer can be of potential interest for applications in biomedical science.

## Materials and methods

### Materials

β-Cyclodextrin was purchased from Sigma and used after recrystallization from water and drying at 100 °C under vacuum. Glycidyl methacrylate (GMA) was purchased from TCI, Japan. p-Toluenesulfonyl chloride (p-TsCl), ethanediamine and azoisobutyronitrile (AIBN) were all purchased from Sigma, China. Copper(I) bromide was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried. 2-bromopropiomyl bromide, 1-adamantanamine and 2, 2-bipyridine were all obtained from Aldrich. 2-(2-Methoxyethoxy) ethyl methacrylate ($MEO_2MA$, $M_n$= 188 g/mol), oligo (ethylene glycol) methyl ether methacrylate (OEGMA, $M_n$= 475 g/mol) were all acquired from Aladdin. Tetrahydrofuran (THF) was initially dried over sodium wire and refluxed over potassium for 3 days before use. Dichloromethane ($CH_2Cl_2$), N, N-Dimethylformamide (DMF) was refluxed over $CaH_2$ before use, separately. Ethanol was refluxed over $CaH_2$ before use. All other reagents were used as received without further purification.

**Characterization**

Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet 5100 spectrometer by KBr sample holder method in the fundamental region of 400-4000 $cm^{-1}$. 1H NMR spectra were obtained on a Bruker DMX-400 spectrometer. Deuterated chloroform ($CDCl_3$), deuterated water ($D_2O$), or deuterated dimethyl sulfoxide (DMSO-$d_6$) was used as the solvent. The MALDI-TOF mass spectrum was recorded in the reflector mode on a Bruker autoflex III smartbean mass spectrometer using a nitrogen laser and α-cyano-4-hydroxycinnamic acid (CCA) was used as the matrix. The number-average molecular weight (Mn) and polydispersity index ( Mw/Mn) of each polymer were determined at 35 °C using a Waters 1515 size exclusion chromatograph (SEC) equipped with a Waters 2414 refractive index (RI) detector. DMF or THF was used as the eluant and the columns used were the styragel HR3 and HR4 columns calibrated by narrow PS standards.

The LCSTs of the polymers was determined by UV-vis spectroscopy (U-3010 Spectrophotometer). The transmittance of the polymeric aqueous solution (3 mg mL$^{-1}$) was recorded at temperatures ranging from 20 $^{o}$C to 65 $^{o}$C. The lower critical solution temperature (LCST) value of the aqueous polymer solution at a specific concentration was determined as the temperature corresponding to 10% decrease in the optical transmittance. The hydrodynamic diameters (Dh) of the capsules and their polydispersity indices (PDI) were determined by dynamic light scattering (DLS) on a Malven Zetasizer Nano System (Nano-zs90). The solutions were passed through 0.45 μm filters before DLS measurements. The measurements were conducted in a 3.0 mL quartz cuvette, using a 670 nm diode laser, and the scattering angle used was 90°.

The micro morphology was visualized by transmission electron microscopy (TEM), the samples were prepared by placing polymer aqueous solution on copper grids in a biochemical incubator thermostatted at 25 $^{o}$C (or 50 $^{o}$C), and stained with phosphotungstic acid before TEM observation on a JEM-100CX II microscope operated at 80 kV.

**Sample Synthesis**

**(a) Synthesis of mono-6-OTs-CD (TCD).** For the synthesis of TCD was synthesized according to previous literature with a few modifications.[33, 34] β-CD (24 g) was suspended in 180 mL of water, NaOH (2.623 g) in 20 mL of water was added dropwise over 30 min, and reacted under vigorous agitation at 0 $^{o}$C for a period of 1 h. then p-toluenesulfonyl chloride (4.032 g) in 20 mL of acetonitrile was added dropwise over 1 h, causing immediate formation of a white precipitate. After 3 h of stirring at 20 $^{o}$C filtered off unreacted toluenesulfonyl chloride, the solution was neutralized and refrigerated, and then the precipitate was recovered by suction filtration and recrystallization in water. The sample obtained was dried at 60 $^{o}$C for 48 h in a vacuum oven, and TCD was obtained as a white solid (5.14 g yield: 21.40%). IR (KBr, cm$^{-1}$): 3388 (s, OH), 2928 (w, $CH_2$), 1597 (s, Ph). $^{1}$H NMR (400 MHz, DMSO-$d_6$): δ

7.42 (2H), 7.74 (2H), 4.75 (7H), 3.49-3.64 (28H), 3.28-3.36 (14H), 2.42 (3H). $^{13}$C NMR (DMSO): δ 144.9 (s), 132.7 (s), 129.9 (s), 127.6 (s), 102.3 (m), 81.5 (m), 71.9-73.1 (m), 69.8, 69.0, 59.5 (t), 21.3 (s) ppm. MALDI-TOF on CCA matrix (TCD + $Na^+$) m/z calcd for $C_{49}H_{76}NaO_{37}S$ (TCD +$Na^+$) 1311.37, measured 1311.13.

**(b) Synthesis of mono (6-amino-6-deoxy)-β-CD (ECD).** 5.0 g of TCD was reacted with excess amount of EDA (30 ml) in 20 ml DMF at 80 °C for 6 h. After the reaction was completed, the mixture was allowed to cool to room temperature, and then precipitated with acetone. The sample obtained was dried at 40 °C for 72h in a vacuum oven, and a white solid of ECD were obtained (3.96 g yield: 79.10%). IR (KBr, $cm^{-1}$): 3388 (s, OH, $NH_2$), 2928 (w, $CH_2$). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 4.85 (7H), 3.54-3.64 (28H), 3.27-3.41 (14H), 2.72-2.92 (4H). $^{13}$C NMR (DMSO): δ 102.0 (s), 81.6 (s), 72.1-73.1 (m), 60.0 (s), 40.1-39.5 (m) ppm. MALDI-TOF on CCA matrix (ECD + $Na^+$) m/z calcd for $C_{44}H_{76}N_2NaO_{34}$ (ECD +$Na^+$) 1199.42, measured 1199.20.

**(c) Synthesis of aminoethyl methacrylate β-cyclodextrin (GCD).** 5.98g ECD was dissolved in 30 ml DMF and a small amount inhibitor was added as. Then 2.49g GMA was added dropwise. After the reaction was completed, the mixture was allowed to cool to room temperature, then precipitated with acetone, and finally dried at room temperature for 2 d under vacuum. (4.90 g yield: 82.0%). IR (KBr, $cm^{-1}$): 3388 (s, OH), 2928 (w, $CH_2$), 1720 (w, C=O). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 6.05 (2H), 4.81 (7H), 4.48 (1H), 3.54-3.62 (28H), 3.29-3.43 (14H), 2.72-2.92 (6H), 1.87 (3H). $^{13}$C NMR: (DMSO) δ 166.6 (s), 135.9 (s), 125.8 (s), 102.0 (s), 81.6 (s), 72.0-73.1 (m), 66.9 (s), 59.9 (s), 56.1 (s), 40.1-39.5 (m), 20.6 (t), ppm. MALDI-TOF on CCA matrix (GCD + $Na^+$) m/z calcd for $C_{51}H_{86}N_2NaO_{37}$ (GCD +$Na^+$) 1341.48, measured 1341.09.

**(d) Homopolymerization of aminoethyl methacrylate β-cyclodextrin (PGCD).** Radical copolymerizations of GCD were initiated by AIBN. A typical experiment for the polymerization procedure is as follows: in a 50 mL three neck round-bottom flask, GCD (1.5 g) and AIBN (0.1 g) were mixed with 10 mL of DMF. The solution was bubbled under argon for 30 min at room temperature to remove free oxygen. The flask was then immersed in an oil bath thermo stated at 80 °C and the reaction was maintained for 6h under argon. Homopolymer was directly precipitated by acetone and washed at least three times using a large amount of acetone, and then dried at 50 $^{o}$C under vacuum to yield a fine white powder.

**(e) Synthesis of 1-adamantyl-2-bromoisobutyrate (AdBB).** The ATRP initiator, AdBB, was prepared by the esterification reaction of 1-adamantanol with 2-bromoisobutyryl bromide. A 100 mL round-bottom flask was charged with 1-adamantanamine (3.03g), TEA (3.04 g), and dry $CH_2Cl_2$ (30 mL). The mixture was cooled to 0 $^{o}$C in an ice-water bath; 2-bromopropiomyl bromide (6.48 g,) in THF (20 mL) was then added dropwise over 2 h. After the addition was completed, the reaction mixture was stirred at 0 $^{o}$C for another 2 h and then at 30 $^{o}$C for 24 h. The mixture was allowed to cool to room temperature and removed the insoluble salts by suction filtration. The organic solution was washed with aqueous sodium bicarbonate solution (2×100 mL) and distilled water (2×100 mL). Then the organic solution was dried with anhydrous magnesium sulfate and concentrated by rotary evaporator. The concentrated syrup purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3 v/v) as the eluent. After removed the solvents by a rotary evaporator and dried in vacuum oven at 50 $^{o}$C for 2 d. AdBB was obtained as a white solid (1.24 g, yield: 82%). $^1$H NMR ($CDCl_3$): δ (ppm) 5.99 (1H, NHCO), 4.25-4.30 (1H, $BrCHCH_3$), 2.07 (3H,-CH), 1.98 (6H,-$CH_2$), 1.81 (3H, $CH_3$-Br), 1.63 (6H, $CH_2$). $^{13}$C NMR ($CDCl_3$): δ (ppm) 168.1 (C=O), 52.3(C-O), 46.1 (BrCH), 41.1 ($CH_2$), 36.2 ($CH_2$), 29.3 ($CH_3$), 23.1 (CH).

**(f) Synthesis of adamantyl-terminated P($MEO_2MA$-co-OEGMA) (Ad-POEGMAs).** Ad-POEGMAs were synthesized by the ATRP of $MEO_2MA$ and OEGMA monomer using

AdBB as the initiator. A typical procedure is described as follows: the schlenk tube was purged with dry argon for 30 minutes, a degassed mixture of 2-(2-methoxyethoxy) ethyl methacrylate (95 eq.), oligo (ethylene glycol) methyl ether methacrylate (5 eq.), ethanol (monomers/ethanol ~ 1:1.5 v/v), AdBB (1 eq.) initiator and copper bromide (1 eq.) was added to a Schlenk tube, degassed via three freeze-thaw-pump cycles and back-filled with argon. Then 2, 2- bipyridyl (2 eq.) were added. The mixture was heated at 60°C in an oil bath for 6 h. The experiment was stopped by opening the flask and exposing the catalyst to air. The final mixture was diluted in ethanol and passed through a short neutral alumina column (200 mesh) in order to remove copper catalyst. Then, the filtered solution was diluted with ethanol and subsequently purified by dialysis in water (molecular weight cut-off: 3500). Last, freeze-drying in vacuum, Ad-POEGMAs were obtained as viscous solid. The synthetic routes employed for the preparation of β-CD based monomers and PGCD, and adamantane-based initiator and adamantyl-terminated POEGMAs are shown in scheme 1.

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