RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Thermo-responsiveness and biocompatibility of star-shaped poly[2-(dimethylamino)ethyl methacrylate]-*b*-poly(sulfobetaine methacrylate) grafted on β-cyclodextrin core†

Mingming Zhang,*[a, ‡] Wei Shen, [a, ‡] Qingqing Xiong,[b] Hongwei Wang,[a,c] Zhimin Zhou,[a] Wenjuan Chen,[a] Qiqing Zhang *[a,d]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Star-shaped thermo-responsive block copolymers were synthesized by atom transfer radical polymerization (ATRP) of a hydrophilic cationic monomer (2-(dimethylamino) ethyl methacrylate) (DMAEMA) and a zwitterionic monomer (sulfobetaine methacrylate) (SBMA) from cyclodextrin with multi-initiator sites. These star polymers with different arm length and arm density were characterized by ^{1}H NMR and GPC. Thermo-responsive behaviors of the star polymers were investigated not only at different pH values, but also at different NaCl concentrations. The size and morphology of the star polymers and their aggregates were measured by dynamic light scattering and transmission electron microscopy. The star polymers showed only upper critical solution temperature (UCST) behaviors, since zwitterionic PSBMA outer blocks shielded the lower critical solution temperature (LCST) behavior of PDMAEMA midblocks. However, PDMAEMA blocks had significant influences on the thermo-induced associations of the star polymers, and resulted in tunable critical aggregation temperature with varying arm density or pH value of solution. Moreover, enhanced thermo-responsive behavior was also obtained at NaCl concentration up to more than 20 mM, which is much higher than those reported before. Finally, biocompatibility evaluations showed that the star polymers could effectively reduce the adsorption to BSA in PBS solution and had insignificant cytotoxicity to MCF-7 cells. These results demonstrate they are good candidates for potential applications in biomedical relevant fields.

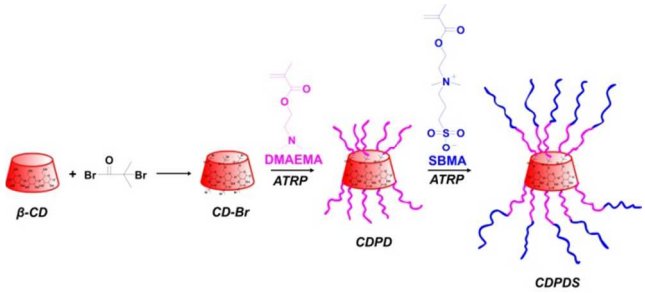
Introduction

Star polymers possess very high molecular weights with three-dimensional branched architectures, but a good solubility and low viscosity comparable to the corresponding linear polymers.[1-5] They can self-assemble into various novel topological architectures with interesting physical and chemical properties,[6-8] which makes them very promising in various fields including drug delivery,[9] gene delivery,[10-15] coatings,[16] and so on.[17] Cyclodextrins (CD) are good junction structures for preparing star polymers, as they offer 18, 21, or 24 hydroxyl groups with high reactive activity for α-, β-, or γ-CD, respectively. Star polymers with CD cores have attracted the increasing attention of researches over the past decades, because they provide not only hydrophobic cores for fabricating supermolecular architectures,[18] but also precursors for designing novel nanomaterials for biomedical and pharmaceutical applications.[19-23] For example, Lu and coworkers[1] synthesized novel macromolecular star polymers with triazole and cyclodextrin (CD) segments as branch points and poly(oligo(ethylene glycol)methacrylate) as dense hydrophilic branches. The thermo-responsive star polymers showed tunable LCST behaviors in aqueous solution, and stable multimolecular micelles can be formed. Moreover, the polymers also exhibited multi-guest encapsulation capacities toward various water-soluble guests. Chen and coworkers[4] prepared carboxybetaine star polymers of different molecular weights from a β-cyclodextrin (β-CD) initiator. The polymer has a long-circulation time in mice, and no appreciable damage or inflammation of major organ tissues, which shows great potential for drug delivery systems.

Thermo-responsive polymers have become very important in polymer science over the last decades. They exhibit phase transition behaviors with changing temperature. During the transition, the polymer chains change the open coil state into the globule state, and subsequently aggregate into larger particles with visible turbidity. Polymers with upper critical solution temperature (UCST) are one kind of important thermo-responsive polymers. They display temperature sensitivity on the basis of hydrogen bonding or electrostatic interactions between polymer chains that are destabilized at higher temperature.[24] Polysulfobetaines are the most popular investigated UCST polymers in aqueous solution, which rely on Coulombic interactions.[25] Great potential applications in the smart materials have been investigated based on polysulfobetaines, such as thermo-responsive gels,[26] multi-layered microshpere,[27] nanoparticles[28] and layer-by-layer films.[29] Besides, due to their

great pH-independent zwitterionic characteristics, they have been widely applied in antifouling surfaces.[30-34]

The aim of this paper is to design and understand hydrophilic star-shaped polymers that will exhibit adjustable UCST behavior by the combination of hyper-branched polycationic and polyzwitterionic blocks. These star polymers were synthesized by atom transfer radical polymerization (ATRP) from β-cyclodextrin with multi-initiator sites (CD-Br). The arm of the star polymer is composed of a cationic block poly[2-(dimethylamino) ethyl methacrylate] (PDMAEMA) and a zwitterionic block poly(sulfobetaine methacrylate) (PSBMA). The representative synthesis route and the typical structure of the star polymer are illustrated in Scheme 1. The influences of arm length, arm density, pH value and salt concentration on thermo-responsive behaviors of the star polymers were investigated in detail by means of dynamic light scattering (DLS) and transmission electron microscopy (TEM). Besides, to investigate the potential application of the star polymers in biological relevant fields, their biocompatibility was evaluated by protein adsorption and cytotoxicity assays as well.



Scheme 1 Synthesis of CDPD and CDPDS star polymer by ATRP.

Experimental

Materials

2-(Dimethylamino) ethyl methacrylate (DMAEMA, Aldrich, 98%) was passed through a basic alumina column and dried with CaH_2 overnight. β-cyclodextrin (β-CD, Sinopharm Chemical Regent Co., Ltd, CR) was recrystallized twice from water and vacuum-dried at 80°C overnight. CuCl (Tianjin, P. R. China, AR) was dissolved in concentrated HCl, precipitated by dilution with water, washed with ethanol and ethyl ether for three times, and then dried under vacuum. $CuCl_2$ (Tianjin, P. R. China, AR) was baked at 120°C to remove the crystal water. [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (SBMA, Aldrich, 97%), 2,2'-bipyridine (Bpy, Sinopharm Chemical Regent Co., Ltd, AR), N,N,N',N",N"-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%), 2-bromoisobutyryl bromide (BiBB, Aldrich, 98%), and anhydrous *N*-methyl-2-pyrrolidone (NMP, Alfa) were used without further purification. Cell Counting Kit-8 was purchased from Dojindo Laboratories. MCF-7 cell line was kindly gifts from School of Pharmacy, Tianjin Medical University. Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were obtained from Thermo Scientific. Bovine serum albumin (BSA) and phosphate buffered saline (PBS) were bought from Solarbio Life Science Co., Ltd. Trypsine-EDTA solution was purchased from Gibco, Life Technology. CD-Br initiator was synthesized by the reaction of β-CD and BiBB.[23]

ATRP of DMAEMA from CD-Br

Star polymer with PDMAEMA arms (CDPD) was synthesized by ATRP of DMAEMA using CD-Br as initiator. CuCl (50.49 mg, 0.51 mmol), $CuCl_2$ (34.29 mg, 0.255 mmol) and Bpy (238.95 mg, 1.53 mmol) were dissolved in 8 mL of acetone/water (95/5, v/v) and degassed with three freeze-pump-thaw cycles. A solution of CD-Br (184.25 mg, 0.05 mmol) and DMAEMA (4.3 mL, 25.5 mmol) in 12 mL of acetone/water (95/5) was added into the catalyst solution under argon atmosphere, and the mixture was then degassed with another two freeze-pump-thaw cycles. The reaction mixture was stirred at 35°C for 20 h. The polymer was further purified by dialysis against deionized water for 2 days (MWCO 7000) and recovered by lyophilization.

ATRP of SBMA from CDPD macroinitiator

CDPD was used as macroinitiator for subsequent ATRP of SBMA. Typically, CuCl (4.75 mg, 0.048 mmol) and PMDETA (20.04 μL, 0.096 mmol) were dissolved in 3 mL of ethanol/water (2/9, v/v) and degassed with three freeze-pump-thaw cycles. A solution of CDPD (0.004 mmol) and SBMA (402.3 mg, 1.44 mmol) in 3 mL of ethanol/water (2/9, v/v) was added into the solution under argon atmosphere, and the mixture was then degassed with another two freeze-pump-thaw cycles. The reaction mixture was stirred at 25°C for 24 h. The obtained star polymer with PDMAEMA-*b*-PSBMA arms (CDPDS) was further purified by dialysis against deionized water for 2 days (MWCO 7000) and recovered by lyophilization.

Characterization

^{1}H NMR was performed on Varian UNITY-plus 400M spectrometer. The apparent molecular weight and polydispersity of the star polymers were determined by gel permeation chromatography (GPC) with a CoMetre 6000 LDI pump and a Schambeck SFD GmbH RI2000 refractive index detector. Na_2SO_4 aqueous solution (0.15 M) was used as mobile phase at a flow rate of 0.5 mL min^{-1}. Polymer solution was injected through Shodex SB-802.5, 803 and 804 HQ columns at 40°C. Polyethylene glycol calibration kit was used as the calibration standard. The sizes of star polymer (D_h) were determined by a Malvern Zetasizer Nano ZS instrument for every 2°C decrement after a 5 min thermal equilibration. The critical aggregation temperature (T_c) is determined by using the D_h data to define two lines, one passing through the data at baseline, above 25°C, and the other passing through the initial rising slope of the aggregation. T_c is defined as the temperature at which the two lines intersect. Transmission electron microscopy (TEM) images were obtained on a Tecnai 2 20 S-TWIN electron microscope. All samples were stained by OsO_4.

Protein adsorption

According to the literature,[35] BSA (2 mg mL^{-1}) was added to equivalent PBS solution or PBS solution containing star polymer (1 mg mL^{-1}), and shaken at 37°C for 40 min. Then the mixture was centrifugated at 12000 rpm for 30 min to remove the large adsorption complexes from the solution. The supernatants were collected for obtaining UV absorbance at 280 nm. The amount of

BSA adsorbed by the star polymer was calculated by the following equation.

BSA absorbed (mg mL^{-1}) $=(C_0V_0-C_sV_s)/V_s$

Where C_0 and V_0 represent the initial BSA concentration and volume; C_s and V_s are BSA concentration and volume after adsorption in supernatant. The experiment was performed in triplicate.

Cytotoxicity evaluation

MCF-7 cells were applied to evaluate the cytotoxicity of star polymers. The cells were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 units mL^{-1} of penicillin, and 100 μg mL^{-1} of streptomycin at 37°C, 5% CO_2. Briefly, cells were seeded into a 96-well plate at a density of 5×10^3 cells per well and incubated for 24 h. The culture media were replaced with fresh media containing polymer with varying concentrations. The media were removed after 48 h, and then a mixture of 10 μL of CCK-8 and 90 μL fresh medium was added to each well. After 1 h, the plate was shaken for 2 min to dissolve formazan crystals. The absorbance was measured using a multifunctional ELISA plate reader at a wavelength of 450 nm.

Results and discussion

Synthesis of CDPD and CDPDS star polymers

Table 1 Characterization of CDPD and CDPDS star polymers

Samples	Arm number [a]	$DP_{n\text{-}DMAEMA}$ (per arm) [b]	$M_{PDMAEMA}$ [b]	$DP_{n\text{-}SBMA}$ (per arm) [c]	M_{SBMA} [c]	M_{star} [d]	$M_{w\text{-}star}$ [e]	PDI (M_w/M_n) [e]
CDPD	12	19	35.8×10^3	0	0	35.8×10^3	28.5×10^3	1.07
CDPDS1	12	19	35.8×10^3	13	43.6×10^3	79.4×10^3	36.3×10^3	1.13
CDPDS2	12	19	35.8×10^3	22	73.7×10^3	109.5×10^3	46.7×10^3	1.13
CDPDS3	12	19	35.8×10^3	27	90.5×10^3	126.3×10^3	53.3×10^3	1.28
CDPDS4	17	18	48.1×10^3	11	52.2×10^3	100.3×10^3	41.1×10^3	1.13

[a] Arm number is considered as initiator site number on one CD molecule, which is calculated from ^{1}H NMR result in our previous work.[23] [b] The practical degree of polymerization of DMAEMA unit per arm ($DP_{n\text{-}DMAEMA}$) and the practical molecular weight of total PDMAEMA ($M_{PDMAEMA}$) are estimated by the weight difference before and after polymerization. [c] The practical degree of polymerization of SBMA unit per arm ($DP_{n\text{-}SBMA}$) and the practical molecular weight of total PSBMA (M_{PSBMA}) are estimated by ^{1}H NMR. [d] $M_{star} = M_{PDMAEMA} + M_{SBMA}$ [e] The apparent molecular weight ($M_{w\text{-}star}$) and molecular weight distribution (M_w/M_n) of the star polymer are measured by GPC.

To obtain CDPDS star polymer, ATRP initiator CD-Br with Br number of 12 was first synthesized by esterification between hydroxyl groups on CD and BiBB as described in our previous work, and confirmed by ^{1}H NMR and ^{13}C NMR.[23] ATRP of DMAEMA was subsequently carried out from CD-Br core using CuCl/$CuCl_2$/Bpy as catalyst and acetone/water as solvent.[23] ^{1}H NMR result of CDPD (Fig. 1A) displays the characteristic signals of protons on PDMAEMA, while the signal related to CD becomes almost invisible, due to the minor contribution of CD to the overall polymer structure.[23, 36] The relatively narrow molecular weight distribution (1.072) measured by GPC suggests the polymerization is successful without occurring intermolecular coupling reactions. The degree of polymerization (DP_n) of DMAEMA unit is estimated to be 19 per chain by the weight difference before and after polymerization.

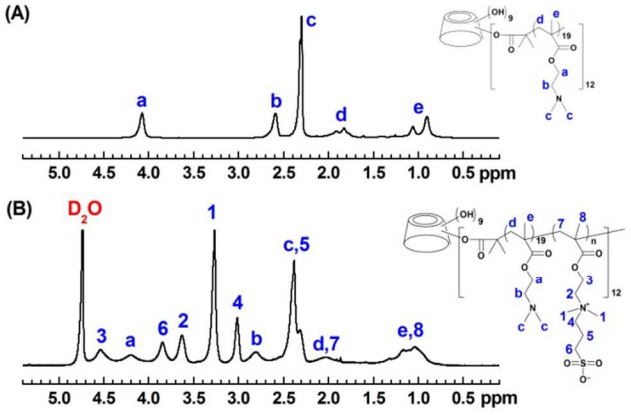


Fig. 1 ^{1}H NMR spectra of CDPD (in $CDCl_3$) and CDPDS (in D_2O) star polymers.

Using CDPD as macroinitiator, a series of polymerization of CDPDS star polymers were then conducted by ATRP of SBMA in ethanol/water at room temperature. DP_n of SBMA is controlled by adjusting the feeding mole ratio of SBMA to CDPD macroinitiator. ^{1}H NMR result in Fig. 1B confirms the structure of CDPDS polymers. The chemical shift at about 3.26 ppm presents the methyl proton adjacent to the quaternary ammonium. Signal at 3.83 ppm is attributed to the methylene protons adjacent to sulfonate group. According to the integration ratio of signal *a* to signal *3*, the DP_n of SBMA is calculated to be 13, 22 and 27 per chain for CDPDS1, CDPDS2 and CDPDS3, respectively. GPC traces in Fig. S1† also demonstrate the successful synthesis of all CDPDS polymers with controlled chain lengths and molecular weight distributions. Besides, to investigate the influence of arm density of CDPDS on thermo-responsive behavior, CDPDS4, which possesses more arm number but almost same arm length with CDPDS1, was also synthesized. Table 1 summarizes the samples investigated in this work along with the molecular characteristics.

Thermo-responsive behaviors of CDPDS star polymers in aqueous solutions

Zwitterionic PSBMA chains exhibit an UCST in water between 16-18°C at a concentration of 0.5 mg mL^{-1} [37] and nonionic PDMAEMA chains exhibit an LCST in the range of 25-78°C in basic solutions.[38] The coexistence of PSBMA and PDMAEMA blocks in a star-form would change the thermo-induced behaviors in aqueous solutions. In this work, the temperature-responsive behaviors of CDPDS star polymers in dilute solutions are investigated in detail at different temperatures and pHs. The concentrations of the star polymers are kept at 1 mg mL^{-1} in all cases. Since the transmittance change of the solution can hardly be detected at such low polymer concentration, DLS is employed to monitor the temperature-dependent hydrodynamic diameters (D_hs) of these polymers.

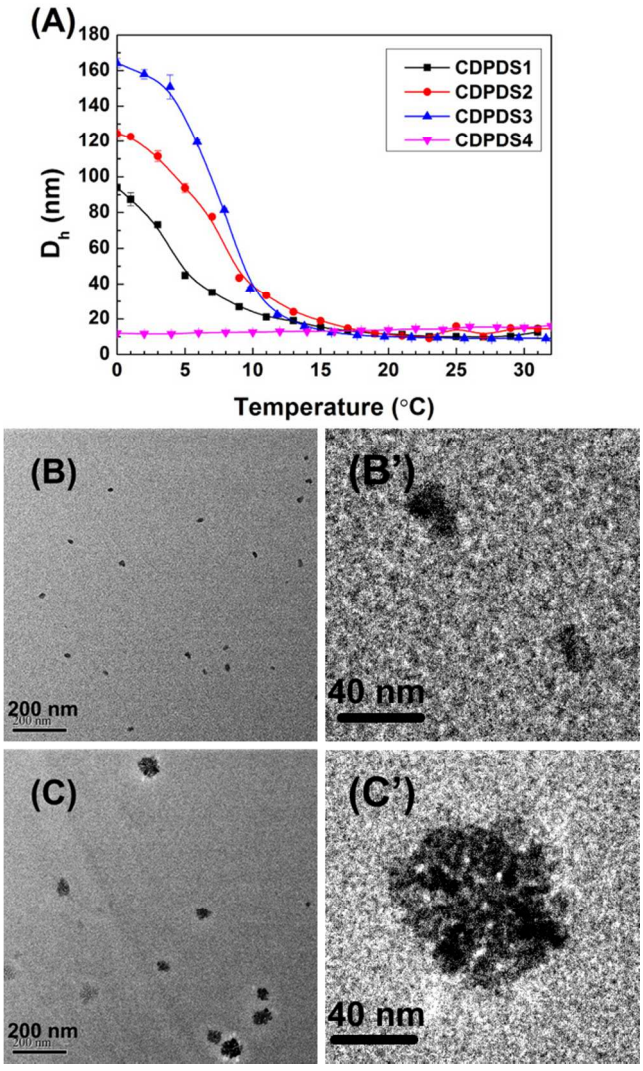


Fig. 2 Influence of temperature on D_hs of CDPDS star polymers in pure water (pH 6.4) (A); TEM images of CDPDS3 in pure water (pH 6.4) at 55°C (B, B') and 5°C (C, C'). The scale bars are 200 nm for (B) and (C), 40 nm for (B') and (C').

As shown in Fig. 2A, in pure water D_h for CDPDS1, CDPDS2 and CDPDS3 increases when temperature goes down to about 15°C, demonstrating these star polymers show significant thermo-responsive behaviors. This result coincides with the UCST of PSBMA block reported by Erel-Goktepe's group[29] and Armes' group.[39] Below 15°C, PSBMA blocks loss the solubility in aqueous solution and become hydrophobic due to the Coulombic attraction between ammonium and sulfonate groups. According to the aggregation models investigated by Lodge,[40] when a hydrophobic block is used as the corona block with a hydrophilic midblock, the hydrophobic block might collapse around the hydrophilic shell to form a thin layer or sticky patches; at higher concentrations, they can associate to form larger aggregated structures. In our work, as illustrated in Scheme 2, the PSBMA blocks are speculated to collapse around the hydrophilic star molecule core to form sticky patches rather than thin layer, because this would be entropically favored for the hydrophilic PDMAEMA midblocks. On decreasing the temperature below 15°C, significant increase in the D_h is observed as a result of the association of the star molecules, and the association degree shows an enhanced trend with increasing the DP_n of SBMA units. TEM is used to directly observe the morphologies of the star polymers. The results obtained from TEM images are in good agreement with that measured by DLS. As shown in Fig. 2B and 2B', typical single star molecule shows elliptical shape with the size about 20 nm at the temperature above UCST (55°C). While decreasing the temperature below UCST (5°C), larger irregular spheres with the size about 50-80 nm are observed (Fig. 2C and 2C'). Interestingly, unlike these samples, CDPDS4 exhibits negligible association behavior under the same condition. This can be attributed to its higher arm density. At pH 6.4, high positively charged PDMAEMA density increases both the intra- and intermolecular electrostatic repulsion, which contributes the stability of the star polymer.

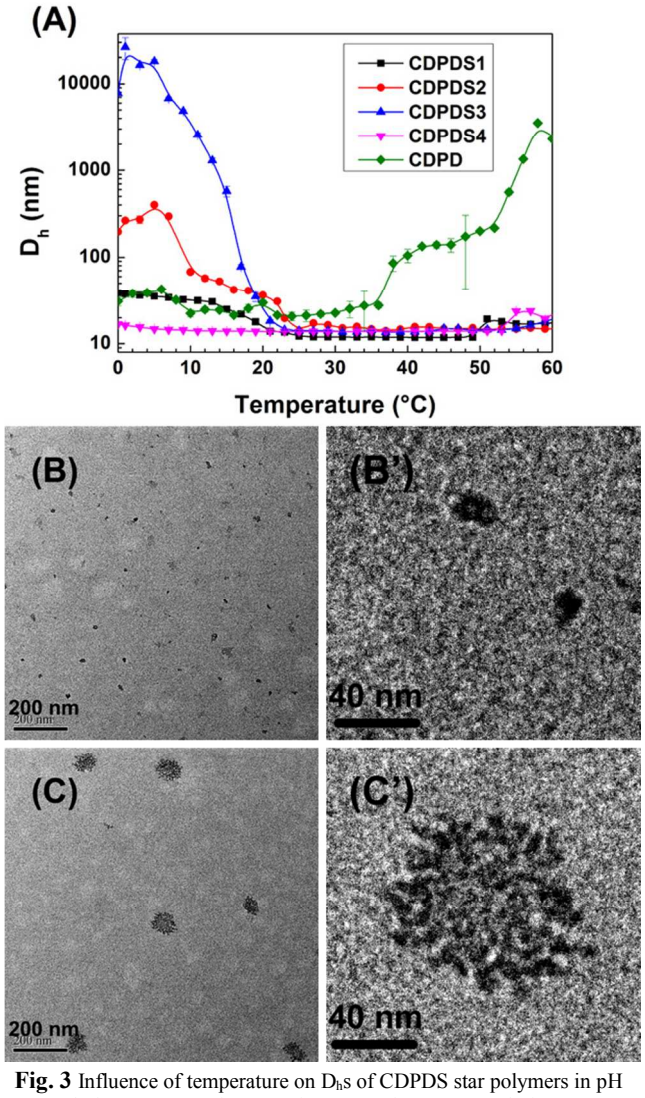
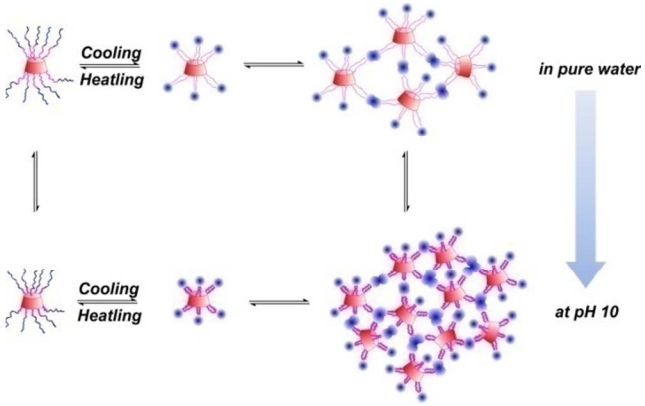


Fig. 3 Influence of temperature on D_hs of CDPDS star polymers in pH 10.0 solution (A); TEM images of CDPDS3 in pH 10.0 solution at 55°C (B, B') and 5°C (C, C'). The scale bars are 200 nm for (B) and (C), 40 nm for (B') and (C').

Since hydrophilic PDMAEMA chains will turn to be hydrophobic and show temperature responsiveness above its pK_a (~7.1),[41, 42] the thermo-responsive behaviors of CDPDS star

polymers are also investigated at pH 10. As shown in Fig. 3A, comparing to the cases in pure water, the critical association temperature of CDPDS1, CDPDS2 and CDPDS3 increases to about 21-23°C. This is caused by the deprotonation of amino groups on PDMAEMA chains at pH 10, which reduces the hydrophilicity of the arm and facilitates the association of the star molecules. The association degree also shows an increase tendency with the increase of DP_n of SBMA units, and large aggregates are formed for CDPDS2 and CDPDS3 samples. TEM images in Fig. 3B and 3C show the typical morphologies of CDPDS star polymer in pH 10 solutions. As expected, when temperature decreases from 55 to 5°C, the spheroid-like single star molecules gradually turn to associate to form spherical assemblies with the size of 50-95 nm, in order to increase the area of the hydrophilic shell to ensure stability of the assembled structures. It is worth noting that, though the CDPD star polymer has significant lower critical solution temperature (LCST) at about 36°C and gets aggregation at the temperature above LCST, no remarkable LCST behaviors are observed in CDPDS star polymers in all cases. This is attributed to the high hydrophilicity of the PSBMA outer corona that stabilizes the star polymer. Unexpectedly, CDPDS4 still does not show visible association or fusion in pH 10 solution. Although PDMAEMA blocks are hydrophobic in pH 10 solution, they remain soluble at relatively low temperature.[43] Thus, we speculate that high chain density provides the star polymer relatively high solubility and therefore prevents the molecules from associating with each other.



Scheme 2 Schematic representative model of thermo-responsive behaviors of CDPDS star polymer in pure water (pH 6.4) and in pH 10 solution.

Thermo-responsive behaviors of CDPDS star polymers in NaCl solution

As well known, zwitterionic (co)polymers display an anti-polyelectrolyte effect. It is, the addition of electrolytes (eg. NaCl) can screen the electrostatic interactions between polyelectrolytes and thus result in the increase of solubility. [20, 44, 45] The influence of salt concentration on the thermo-responsiveness of CDPDS star polymer is therefore investigated. The aggregation curves are shown in Fig. 4A, and the trend of critical aggregation temperature (T_c) vs. NaCl concentration is displayed in Fig. 4B. Increasing NaCl concentration from 10 to 90 mM, expected decrease in T_c is obtained, which is caused by the salt-induced screening of the Coulombic attraction between the negative sulfonate and positive quaternized ammonium sites. The Coulombic attraction is found to be completely disrupted at the highest NaCl concentration (90 mM), as no significant association of the star polymer is detected by DLS measurement. Interestingly, comparing to that in pure water, the T_c of CDPDS star polymer increases to 25 and 19°C in 10 mM and 20 mM NaCl solution. Such a phenomenon has already been found by Bendejacq[46] and Roth.[45] The possible reason is that, in the solution with low NaCl concentration, only a few of charges on PSBMA chains are screened by salt. At this stage, the hydration is very weak, and the polymer chains are still very close to each other. Overall, the charges coming from both of the zwitterionic polymers and salt contribute the intermolecular Coulombic attraction. On the contrary, when in pure water, charges are only coming from zwitterionic polymer. As a result, compared to that in pure water, the association of the star polymer increases, and higher T_c is observed. Moreover, we first find that, the threshold NaCl concentration (higher than 20 mM) that increases T_c is much higher than the cases reported before (between 0.7 and 2 mM). This is probably attributed to the unique star-shaped architecture. That is, the sulfonate and ammonium groups located in the internal layer of the star polymer are more difficult to be screened compared to the linear polymer, which results in high tolerance to salt concentration. Therefore, these star-shaped polyzwitterionic assemblies can extend their applications into more realms of science rather than typically necessitating working in ultrapure water.

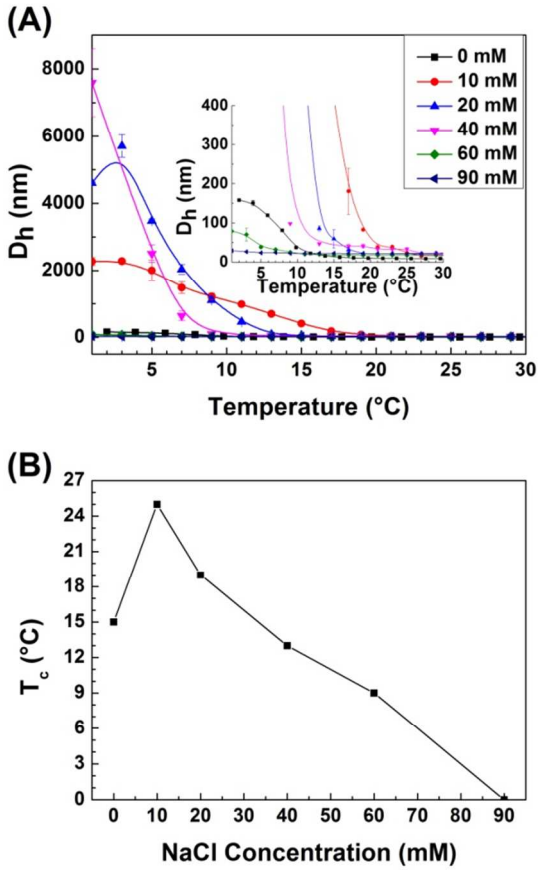


Fig. 4 Effect of NaCl concentration on the aggregation behavior of CDPDS3 star polymer (1 mg mL^{-1}). (A) Aggregation curves for increasing NaCl concentration (the inset is the magnified curves); (B) The

trend of critical aggregation temperature (T_c) vs. NaCl concentration, where 90 mM is the concentration for eliminating the aggregation.

Biocompatibility assays

Zwitterionic polymers have been shown to exhibit ultra-high resistance to non-specific protein adsorption and offer promising alternatives to polyethylene glycol-modified materials. To apply CDPDS star polymers in biological relevant fields, the biocompatibility of the star polymers is also investigated by BSA adsorption and cytotoxicity assessments.

To mimic the cases in physiological environment, protein adsorption assay is conducted in PBS solution (pH 7.4). Fig. 5 shows the amount of adsorbed and remained BSA protein in solutions after incubation with CDPDS or CDPD star polymers at 37°C for 40 min. Unexpectedly, all star polymers including the positively charged CDPD polymer exhibit little BSA adsorption. The result for CDPD polymer is probably attributed to the shielding effects of the anti-charged ions in the solution, that blocks the adsorption between CDPD and BSA. While for CDPDS star polymer, both shielding effects of the ions and the hydrophilic zwitterionic characteristics of PSBMA blocks contribute the reduction of BSA adsorption. These results indicate that the CDPDS star polymers can effectively reduce non-specific serum protein adsorption and escape from being eliminated in blood, which make them suitable for long-time circulation and application *in vivo*.

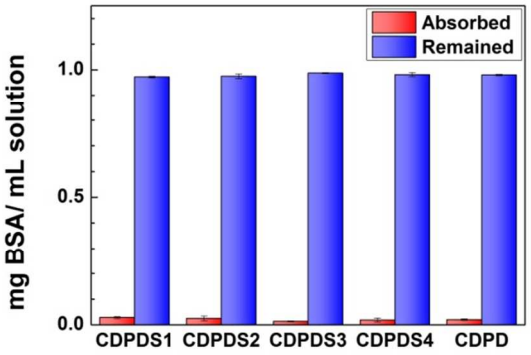


Fig. 5 BSA adsorption for CDPDS and CDPD star polymers in PBS solution.

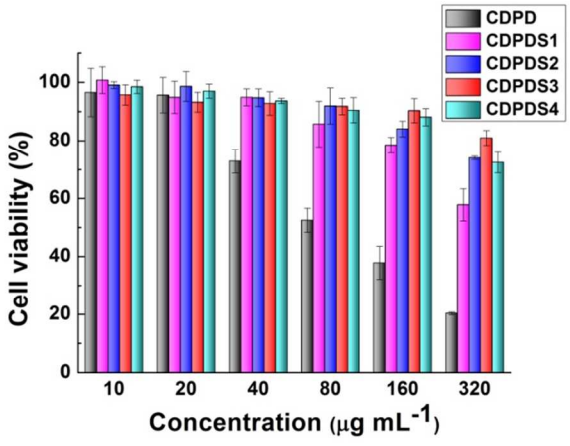


Fig. 6 Cell viability profiles for CDPDS and CDPD star polymers in MCF-7 cells. Data is represented as the mean ± SD (n = 3).

Next, the cytotoxicities of all materials are evaluated in MCF-7 cell line by CCK-8 assay and the results are shown in Fig. 6. Although the positively charged CDPD star polymer shows little adsorption to BSA as discussed above, they exhibit significant dose-dependent cytotoxicity. The viability of the cells decreases with the increase of the polymer concentration, which is caused by the great quantity of positive amino groups on PDMAEMA blocks.[23, 47] However, after polymerization of SBMA on CDPD, no significant cytotoxicity is observed up to the polymers concentration of 160 μg mL^{-1}. Moreover, increasing DP_n of SBMA unit or the arm density of the star polymer can further enhance the cell viability. As shown in Fig. 6, for CDPDS3 sample, the cell viability is still greater than 80% even after incubation at highest polymer concentration (320 μg mL^{-1}). These results demonstrate that the cytotoxicity caused by positively charged CDPD polymers can be effectively reduced by the introduction of zwitterionic PSBMA blocks in the outer corona. In other words, CDPDS star polymers are low cytotoxicity and can be potential candidates for biological applications, such as drug delivery, gene delivery and biomolecules encapsulation.

Conclusions

Hydrophilic CDPDS star polymers have been successfully synthesized by sequential ATRP of cationic DMAEMA and zwitterionic SBMA monomers from CD core. The thermo-responsive behaviors of these star polymers in dilute solution are investigated by DLS and TEM. CDPDS star polymers exhibit only UCST behaviors owing to PSBMA blocks, because zwitterionic PSBMA outer blocks shield the LCST behavior of PDMAEMA midblocks. However, PDMAEMA midblocks show strong influences on the thermo-responsiveness and aggregation behaviors of the CDPDS polymer. In pure water, the critical aggregation temperature is measured as 15°C, while it increases to about 21-23°C in pH 10 solution, due to the deprotonation of amino groups on PDMAEMA blocks. Moreover, higher PDMAEMA density enhances the solubility of star polymer, and results in no significant thermo-induced aggregation whether in pure water or pH 10 solution. Besides, enhanced thermo-responsiveness of the star polymer is firstly obtained at relatively high NaCl concentration (above 20 mM), which probably benefits from the unique star-shaped architecture. Further biocompatibility evaluations show that CDPDS star polymers can effectively reduce the adsorption of BSA and exhibit insignificant cytotoxicity to MCF-7 cells. These results provide that the CDPDS star polymers have adjustable thermo-responsiveness and good biocompatibility, which can be good candidates for potential applications in biomedical fields, such as drug delivery, gene delivery, antifouling coatings and so on.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China under Contract No. 51203190 and 31271023. Major Research Plan of National Natural Science Foundation of China under Contract No. 91323104, and Science

and Technology Committee of Tianjin under contract No. 11JCYBJC02300 and 15JCQNJC14400.

Notes and references

[a] *Tianjin Key Laboratory of Biomedical Materials, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300192, P. R. China. Fax: +86 22 87890868; Tel: +86 22 87890868; E-mail: zmm_zmm_zmm@hotmail.com; zhangqiq@126.com*
[b] *Department of Hepatobiliary Surgery, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060, P. R. China*
[c] *Institute of Chinese Materia Medica, Chengde Medical University, Hebei Key Laboratory of Research and Development for Traditional Chinese Medicine, Chengde, Hebei 067000, P. R. China*
[d] *Institute of Biomedical and Pharmaceutical Technology, Fuzhou University, Fuzhou 350002, P. R. China.*
† Electronic Supplementary Information (ESI) available: GPC traces of CDPDS and CDPD star polymers. See DOI: 10.1039/b000000x/
‡ These authors contributed equally.

1. Y. Li, H. Guo, J. Zheng, J. Gan, Y. Zhang, X. Guan, K. Wu and M. Lu, *RSC Advances*, 2014, **4**, 54268-54281.
2. D. Konkolewicz, M. J. Monteiro and S. b. Perrier, *Macromolecules*, 2011, **44**, 7067-7087.
3. J. Wang, X. Wang, F. Yang, H. Shen, Y. You and D. Wu, *Langmuir*, 2014, **30**, 13014-13020.
4. W. Lin, G. Ma, F. Ji, J. Zhang, L. Wang, H. Sun and S. Chen, *Journal of Materials Chemistry B*, 2015, **3**, 440-448.
5. R. Plummer, D. J. T. Hill and A. K. Whittaker, *Macromolecules*, 2006, **39**, 8379-8388.
6. Y. Liu, C. Yu, H. Jin, B. Jiang, X. Zhu, Y. Zhou, Z. Lu and D. Yan, *Journal of the American Chemical Society*, 2013, **135**, 4765-4770.
7. E. Doganci, M. Gorur, C. Uyanik and F. Yilmaz, *Journal of Polymer Science Part A: Polymer Chemistry*, 2014, **52**, 3406-3420.
8. Z. Tian, C. Chen and H. R. Allcock, *Macromolecules*, 2014, **47**, 1065-1072.
9. M. Mooguee, Y. Omidi and S. Davaran, *Journal of Pharmaceutical Sciences*, 2010, **99**, 3389-3397.
10. M. Zhang, Q. Xiong, J. Chen, Y. Wang and Q. Zhang, *Polymer Chemistry*, 2013, **4**, 5086-5095.
11. Q. Xiong, M. Zhang, Z. Zhang, W. Shen, L. Liu and Q. Zhang, *International Journal of Pharmaceutics*, 2014, **474**, 232-240.
12. Y. Wen, Z. Zhang and J. Li, *Advanced Functional Materials*, 2014, **24**, 3874-3884.
13. Y. Y. Durmaz, Y.-L. Lin and M. E. H. ElSayed, *Advanced Functional Materials*, 2013, **23**, 3885-3895.
14. S. Matsumoto, R. J. Christie, N. Nishiyama, K. Miyata, A. Ishii, M. Oba, H. Koyama, Y. Yamasaki and K. Kataoka, *Biomacromolecules*, 2009, **10**, 119-127.
15. F. J. Xu, Z. X. Zhang, Y. Ping, J. Li, E. T. Kang and K. G. Neoh, *Biomacromolecules*, 2009, **10**, 285-293.
16. B.-S. Kim, H. Gao, A. A. Argun, K. Matyjaszewski and P. T. Hammond, *Macromolecules*, 2009, **42**, 368-375.
17. J. Iocozzia, H. Xu, X. Pang, H. Xia, T. Bunning, T. White and Z. Lin, *RSC Advances*, 2014, **4**, 50212-50219.
18. Y. Bai, X.-d. Fan, W. Tian, H. Yao, L.-h. Zhuo, H.-t. Zhang, W.-w. Fan, Z. Yang and W.-b. Zhang, *Polymer*, 2013, **54**, 3566-3573.
19. M. Adeli, Z. Zarnegar and R. Kabiri, *European Polymer Journal*, 2008, **44**, 1921-1930.
20. P.-F. Gou, W.-P. Zhu and Z.-Q. Shen, *Biomacromolecules*, 2010, **11**, 934-943.
21. J. Li, Z. Guo, J. Xin, G. Zhao and H. Xiao, *Carbohydrate Polymers*, 2010, **79**, 277-283.
22. J. Li and X. J. Loh, *Advanced Drug Delivery Reviews*, 2008, **60**, 1000-1017.
23. M. Zhang, Q. Xiong, Y. Wang, Z. Zhang, W. Shen, L. Liu, Q. Wang and Q. Zhang, *Polymer Chemistry*, 2014, **5**, 4670-4678.
24. J. Seuring and S. Agarwal, *Macromolecular Rapid Communications*, 2012, **33**, 1898-1920.
25. J. Seuring and S. Agarwal, *ACS Macro Letters*, 2013, **2**, 597-600.
26. J. Ning, G. Li and K. Haraguchi, *Macromolecules*, 2013, **46**, 5317-5328.
27. N. Morimoto, K. Muramatsu, T. Wazawa, Y. Inoue and M. Suzuki, *Macromolecular Rapid Communications*, 2014, **35**, 103-108.
28. M. Tian, J. Wang, E. Zhang, J. Li, C. Duan and F. Yao, *Langmuir*, 2013, **29**, 8076-8085.
29. P. Yusan, I. Tuncel, V. Butun, A. L. Demirel and I. Erel-Goktepe, *Polymer Chemistry*, 2014, **5**, 3777-3787.
30. Z. Zhang, J. A. Finlay, L. Wang, Y. Gao, J. A. Callow, M. E. Callow and S. Jiang, *Langmuir*, 2009, **25**, 13516-13521.
31. Y. Chang, S. Chen, Z. Zhang and S. Jiang, *Langmuir*, 2006, **22**, 2222-2226.
32. S. Chen and S. Jiang, *Advanced Materials*, 2008, **20**, 335-338.
33. Z. Zhang, T. Chao, S. Chen and S. Jiang, *Langmuir*, 2006, **22**, 10072-10077.
34. P.-S. Liu, Q. Chen, X. Liu, B. Yuan, S.-S. Wu, J. Shen and S.-C. Lin, *Biomacromolecules*, 2009, **10**, 2809-2816.
35. J. Sun, F. Zeng, H. Jian and S. Wu, *Biomacromolecules*, 2013, **14**, 728-736.
36. Y. Wu, P. Ni, M. Zhang and X. Zhu, *Soft Matter*, 2010, **6**, 3751-3758.
37. Y.-J. Shih, Y. Chang, A. Deratani and D. Quemener, *Biomacromolecules*, 2012, **13**, 2849-2858.
38. F. A. Plamper, M. Ruppel, A. Schmalz, O. Borisov, M. Ballauff and A. H. E. Müller, *Macromolecules*, 2007, **40**, 8361-8366.
39. J. V. M. Weaver, S. P. Armes and V. Butun, *Chemical Communications*, 2002, 2122-2123.
40. C. Zhou, M. A. Hillmyer and T. P. Lodge, *Macromolecules*, 2011, **44**, 1635-1641.
41. M. Zhang, L. Liu, C. Wu, G. Fu, H. Zhao and B. He, *Polymer*, 2007, **48**, 1989-1997.
42. M. Zhang, L. Liu, H. Zhao, Y. Yang, G. Fu and B. He, *Journal of colloid and interface science*, 2006, **301**, 85-91.
43. Y. Zhao, K. Guo, C. Wang and L. Wang, *Langmuir*, 2010, **26**, 8966-8970.
44. M. Tian, J. Wang, E. Zhang, J. Li, C. Duan and F. Yao, *Langmuir*, 2013, **29**, 8076-8085.
45. P. A. Woodfield, Y. Zhu, Y. Pei and P. J. Roth, *Macromolecules*, 2014, **47**, 750-762.
46. P. Mary, D. D. Bendejacq, M.-P. Labeau and P. Dupuis, *The Journal of Physical Chemistry B*, 2007, **111**, 7767-7777.
47. P. van de Wetering, E. E. Moret, N. M. E. Schuurmans-Nieuwenbroek, M. J. van Steenbergen and W. E. Hennink, *Bioconjugate Chemistry*, 1999, **10**, 589-597.