RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

ARTICLE

Supramolecular nanoparticles constructed by balancing forces between attractive host-guest and repulsive electrostatic interactions in two positively charged polymers

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Yue-Zhu Zhuang,[a] Wen-Xing Gu,[a] Jin-Jun Yang,[b] XiYi Chen,[c] Hui Gao[a,*]

A novel type of supramolecular nanoparticles (SNPs) was self-assembled based on the balance of forces including attractive supramolecular host-guest interactions and repulsive electrostatic interactions between the host and the guest polymers. Positively charged (P+CD), neutral β-cyclodextrin (CD) grafted poly(glycerol methacrylate) (PCD) and negatively charged CD-modified poly(acrylic acid) (PAA-CD) were synthesized respectively as the host polymers, meanwhile 4-tert-butylphenyl group (TBP) grafted positive poly(glycerol methacrylate) (P+TBP) was synthesized as the guest polymer to study the influence of electrostatics in host-guest systems between CD and TBP. The resulting P+CD/P+TBP SNPs possessing well stability will provide a guide for future construction of SNPs and promote the development of SNPs as carriers for biomedical applications such as drug delivery agents.

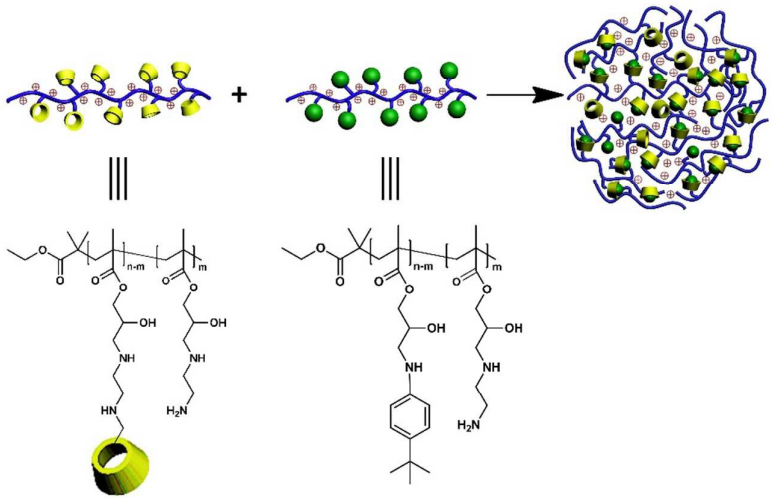
Introduction

Supramolecular nanoparticles (SNPs) are formed by special noncovalent multivalent supramolecular interactions in a multicomponent system. Compared with traditional polymers, supramolecular polymers whose chains are connected by non-covalent forces such as metal coordination, hydrogen bonding, host-guest interactions, electrostatic forces and so on, could be easily obtained in a facile and dynamic way instead of time-consuming and tedious synthesis as a result of the dynamic and reversible nature of noncovalent forces.[1] SNPs could be constructed in several methods, for instance, in our previous study, polyion complexes for siRNA delivery based on electrostatic interactions and hydrogen bonding between a protonated polymer and negatively charged siRNA,[2] and reverse vesicles constructed from supramolecular amphiphilic copolymers via host-guest interactions;[3] a supramolecular system prepared by Tseng[4] in which the SNPs are formed by multivalent host and guest interactions between a positively charged polymer bearing cyclodextrin (CD) and positively charged PAMAM dendrimers containing adamantyl moieties; the dextran-based nanoparticles by Larsen[5] based on host and guest interactions between adamantyl and CD-modified neutral dextran polymers. These systems have been introduced for different biomedical applications, such as photothermal therapy,[6] imaging,[7] gene delivery[8] and drug delivery.[3,9]

Self-assembly of carefully designed CD inclusion complexes into higher order structures is a significant aspect of current CD study.[10] Self-assemblies of CD inclusion complexes into supramolecular polymers are documented in the literature.[11] CDs are nontoxic and biocompatible and therefore CD-based SNPs would have potential applications in biomaterial field. β-CD is cyclic oligosaccharide consisting of seven α-D-glucopyranose units linked by α-1,4-glucosidic bonds.[12] It contains a hydrophilic outer surface and a hydrophobic internal cavity within which can include numerous hydrophobic guests with appropriate size by non-covalent interactions.[13-17] CDs and their derivatives have been suggested to undergo degradation in that the enormous microflora in the colon, breaks them into monosaccharides to be absorbed in the large intestine.[18] As a consequence, we envision biocompatible CDs and their derivatives have a bright future for biomaterial applications.

In the literature published before, the β-CD-based SNPs make use of adamantyl-terminated poly (ethylene glycol) as a monovalent capping agent (stopper), to stop the multivalent supramolecular network, avoiding the continuous growth of the complexes and their aggregation due to the electrostatic forces in the Davis system and the attractive multivalent interactions in the Tseng system.[3,19,20] Despite of the multi-function of SNPs, insufficient study was performed about the interplay of electrostatics, host-guest interactions in the control of particle size and stability. In the Davis system,[19] the electrostatic interactions are attractive; in the Tseng system,[3] the electrostatic interactions are repulsive; and in the Larsen

[a]School of Chemistry and Chemical Engineering, School of Material Science and Engineering, Tianjin Key Laboratory of Organic Solar cells and Photochemical Conversion, Tianjin University of Technology, Tianjin, 300384, China, Email address: ghhigher@hotmail.com (H.Gao), Tel:+86-22-60214251
[b]School of Environmental Science and Safety Engineering, Tianjin University of Technology, Tianjin, 300384, China.
[c]School of Public Health,Dalian Medical University,Dalian, 116000, China.



Scheme 1 Schematic representation of the formation of SNPs formed by P+CD and P+TBP.

system, electrostatics is supposed to play a negligible role due to the absence of formal charges on the building blocks.[4] However, the above systems were independently constructed based on different polymers and different host-guest. Therefore, the property and the stability of the SNP were not comparable. Additionally, the systems published before mainly focus on a single force or the synergy effect between electrostatic and host-guest interactions. In this study, we tried to find a balance between repulsive and attractive forces. In this case, stable and biocompatible complexes were expected to be easily obtained instead of time-consuming synthesis.

Herein, we utilized ring-opening addition of epoxy groups to generate 4-tert-butylphenyl group (TBP) and ethanediamine (EDA) modified positive charged poly(glycerol methacrylate) (P+TBP). TBP could form inclusion complex with CD.[21] Then positively charged or neutral CD grafted poly(glycerol methacrylate) (P+CD/PCD) and negatively charged CD-modified poly(acrylic acid) (PAA-CD) were synthesized and applied to form SNP with P+TBP. Poly(glycerol methacrylate) (PGOHMA) derivatives used in this study contain functional groups such as multi-hydroxyl and amino groups, thus drugs can be easily encapsulated by both electrostatic interaction and hydrogen-bonding interaction,[22] making them good candidates for supramolecular preparation. While PAA is nontoxic, rich with natively charged carboxylate groups, possessing similar backbone structure with PGOHMA.[23] As a result, we demonstrated the possibility of new way to form supermolecules that possess a promising future in biomaterials field.

Experimental

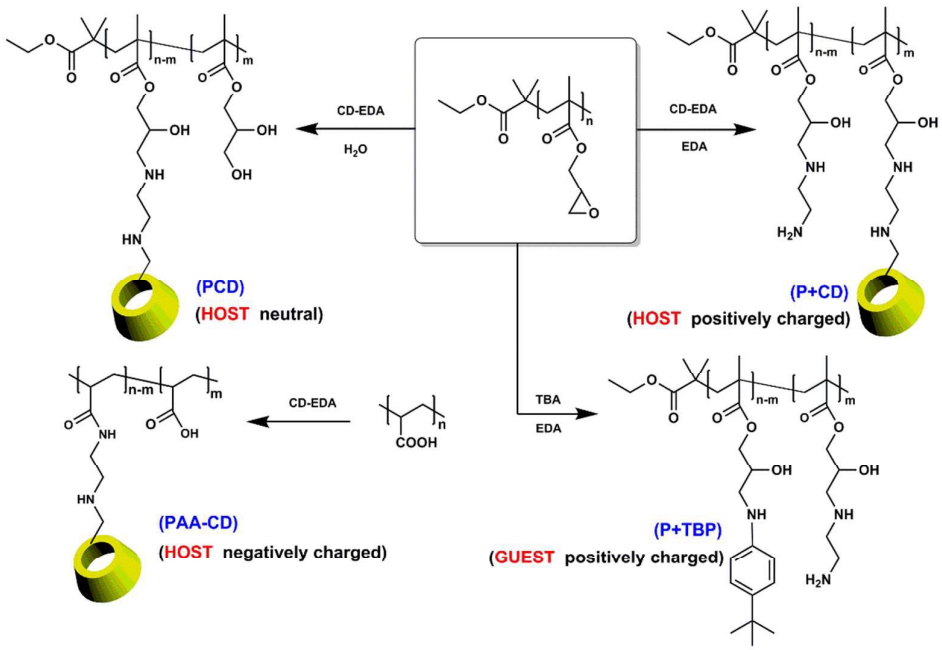
Materials and methods

Materials. Glycidyl methacrylate (GMA), 2-bromoisobutyryl bromide, bipyridyl and CuBr were purchased from Adamas Reagent Co., Ltd. (Shanghai, China). PAA (Mw=250 kDa) was obtained from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). 4-tert-butyl-aniline (TBA) was purchased from Heowns Co., Ltd (Tianjin, China). Prior to use, tetrahydrofuran (THF) was dried over sodium, with benzophenone serving as a dryness indicator. All the other reagents were obtained from Tianjin Chemical Reagent Co. (Tianjin, China). ^{1}H NMR spectra were recorded on a Bruker AV-400 400 MHz NMR spectrometer (400 MHz, Bruker, Freemont, CA). Gel permeation chromatography (GPC) measurements were performed at 40 ^{o}C with THF as mobile phase at a flow rate of 0.5 mL/min.

Synthesis of P+CD. Poly(glycidyl methacrylate) (PGMA) was synthesized via atom transfer radical polymerization (ATRP) reaction according to a method reported previously.[23] The reaction between PGMA and mono(6-(ethanediamine)-6-deoxy)-β-cyclodextrin (CD) was prepared by the ring-opening reaction.[24] Typically, PGMA (0.1 g) and CD (1.8 g) were dissolved in DMF (30 ml). The mixture was heated at 70 ^{o}C for 24 h. Then, EDA (10 ml) was added to the flask and reacted for another 12 h. The crude mixture solution was purified against deionized water by dialysis (cut-off molecular weight 7 kDa) for 48 h at room temperature, then freeze-dried to obtain purified P+CD.

Synthesis of P+TBP. P+TBP was prepared similarly to P+CD: To a solution of PGMA (0.3 g) in acetonitrile (15 ml), was added a solution of 4-tert-butyaniline (TBA) in acetonitrile (15ml). The mixture was reacted overnight at 90 ^{o}C. To the crude production, EDA was added and the unreacted epoxy rings were opened. Afterwards, the crude mixture was purified by dialysis (cut-off molecular weight 7 kDa) for 48 h. Phase separation occurred during dialysis. The aqueous phase was purified by extraction with chloroform. Pure P+TBP was obtained by freeze-drying.

Synthesis of PCD. PGMA and CD were dissolved in DMF, and the mixture was heated at 70 ^{o}C for 24 h.[24] After reaction, the crude mixture was dialyzed against deionized water for 48 h to remove DMF and unreacted CD. After freeze-drying, the product (0.1 g) was dissolved in N-methyl-2-pyrrolidinone (NMP) as less as possible at room temperature. The temperature was then raised to 60 ^{o}C, at which point 0.62 ml water was added dropwise. The mixture was left to react at



Scheme 2 Synthesis of P+TBP, P+CD, PCD and PAA-CD.

120 °C for 24 h. The polymer was dialyzed (cut-off molecular weight 7 kDa) against water for 48 h and then freeze dried.[25]

Synthesis of PAA-CD. 0.90 g (12.5 mmol of -COOH groups) of PAA (250 kDa) was dissolved in 30 mL of NMP at 60 °C for 24 h. Then, 0.43 g (0.37 mmol) of CD (dissolved in 3.0 g of NMP) and 0.10 g (0.48 mmol) of dicyclohexylcarbodiimide (DCC) (dissolved in 2.0 g of NMP) were introduced into the PAA solution under vigorous stirring. After a reaction for 48 h at 60 °C, the system was cooled to room temperature, followed by addition of 35 mL of 40 wt % NaOH aqueous solution to precipitate the polymer. The precipitate was washed twice with 15 mL of hot NMP (60 °C) and then with 20 mL of methanol at room temperature. After filtration under vacuum, the solid product was dissolved in 12.5 mL of DI water and precipitated in 100 mL of methanol (twice). Finally, the product was dissolved into 20 mL of DI water and dialyzed (cut-off molecular weight 3.5 kDa) against DI water for 3 days. The final dry product was obtained by freeze-drying after concentrating the solution to 10 wt % by evaporation.[26,27]

Preparation of the supramolecular nanoparticles. To ensure the sufficient protonation of polymers, we chose acetic acid buffer as solvent. Moreover, different concentration KCl was prepared to adjust the electrostatic repulsive forces between host and guest polymers.

In acetic acid buffer: The SNPs were prepared maintaining the host/guest ratio at 1:4 at 117 mM CD by mixing stock solutions of P+CD (or PCD, PAA-CD) with P+TBP using a vortex and afterwards they were measured by DLS at different times.

In KCl solution: 50 µL of the P+CD (or PAA-CD) were mixed with the P+TBP solutions at 1:4 host: guest ratio at 117 mM CD and a final concentration of KCl of 100 mM, 200 mM and 400 mM. The samples were vortexed and afterwards measured by DLS at different times.

Results and Discussion

The host polymer P+CD and the guest polymer P+TBP were synthesized by ring-opening of PGMA (MW 10 kDa, PDI=1.2). P+TBP derivatives not only has good solubility under proper pH and biocompatibility, but also serve as polymeric supramolecular hosts to form inclusion complexes with suitable guest compounds.

^{1}H NMR spectra of P+TBP (Fig. 1(a)) in DMSO-d_6 shows typical signals of benzene and tertiary butyl, apart from those assigned to PGMA backbone. In ^{1}H NMR spectra of P+CD (Fig. 1(b)), PCD (Fig. 1(c)) and PAA-CD (Fig. 1(d)) in D_2O, the signals of CD are clearly showed, evidencing the successfully introduction of CD groups in PAA and PGMA. And the molecular weights of P+CD, PCD and PAA-CD and P+TBP are 22.1 kDa, 14.3 kDa, 31.3 kDa and 13.4 kDa, with polydispersity index of 1.45, 1.33, 1.48, and 1.36 respectively.

The complexation between host and guest polymers could be confirmed by ^{1}H NMR (Fig. 2). The peak at 5.07 ppm corresponding to methylene groups in CD of P+CD showed significantly upfield shift, and peak at 7.3 ppm corresponding to benzene in the TBP of P+TBP showed downfield shift after mixing the P+TBP and P+CD, indicating the formation of host-guest polymer complexes.

The content of CD was determined by Phenol-sulfuric acid method which based on the theory that polysaccharide hydrolysis into monosaccharides under the effect of sulfuric acid, and the ultro violet of monosaccharides could be detected at 490 nm using UV

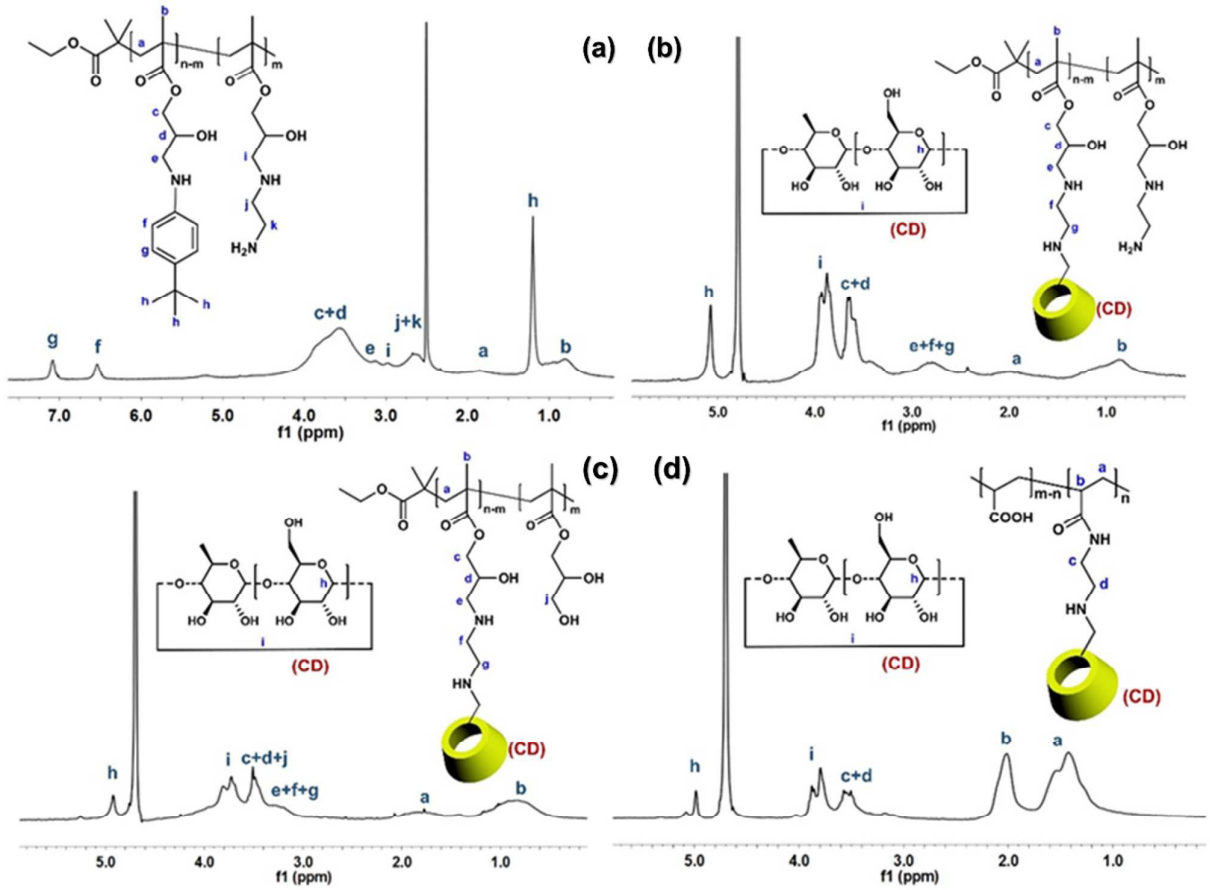


Fig. 1 ^{1}H NMR spectra of (a) P+TBP, (b) P+CD, (c) PCD and (d) PAA-CD.

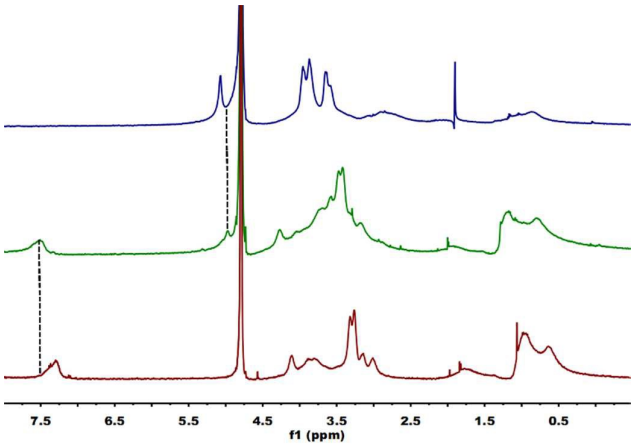


Fig. 2 ^{1}H NMR of (a) P+CD, (b) complex of P+CD and P+TBP, (c) P+TBP in D_2O.

spectrometer.[28] According to Phenol-sulfuric acid method, the P+CD contained eleven CD per polymer backbone. Moreover, there are twenty-one TBP moieties per polymer backbone tested by standard curve meathod.

The particles were readily prepared by mixing the components maintaining a 1:4 ratio between host (CD) and guest (TBP) moieties of the polymers bearing 117 μM of CD. Scheme 1 shows a schematic representation of the assembly of the SNPs from the host and the guest polymers. Both DLS measurements and SEM images confirmed the formation of particles (Fig. 3(a)-(f)). Neither P+TBP nor P+CD could form SNPs according to the DLS and SEM measurement, indicating that the single polymer solution failed to form nanoparticles. Instead, once P+TBP and P+CD were mixed with a proper ratio, DLS measurements of the particles obtained in acetic acid buffer solution (pH=4.5) showed nanoparticles of comparable hydrodynamic diameters with the size of approximately 200 nm (Fig. 4(c)). In addition, SEM images of the host or guest polymers individually revealed no particle formation. However, the SEM of mixture of host and guest polymers (Fig. 4(a)) showed multi-cores of the micelle. This kind of micelles was named as a large compound micelle consisting of numerous small micelles. This confirmed specific multivalent supramolecular interactions occurred between the two positively charged polymers.

The stability of the nanoparticles in acetic acid buffer solution over time at 20 ^{o}C was studied by DLS. The results show that these SNPs are very stable, and the particles size maintained

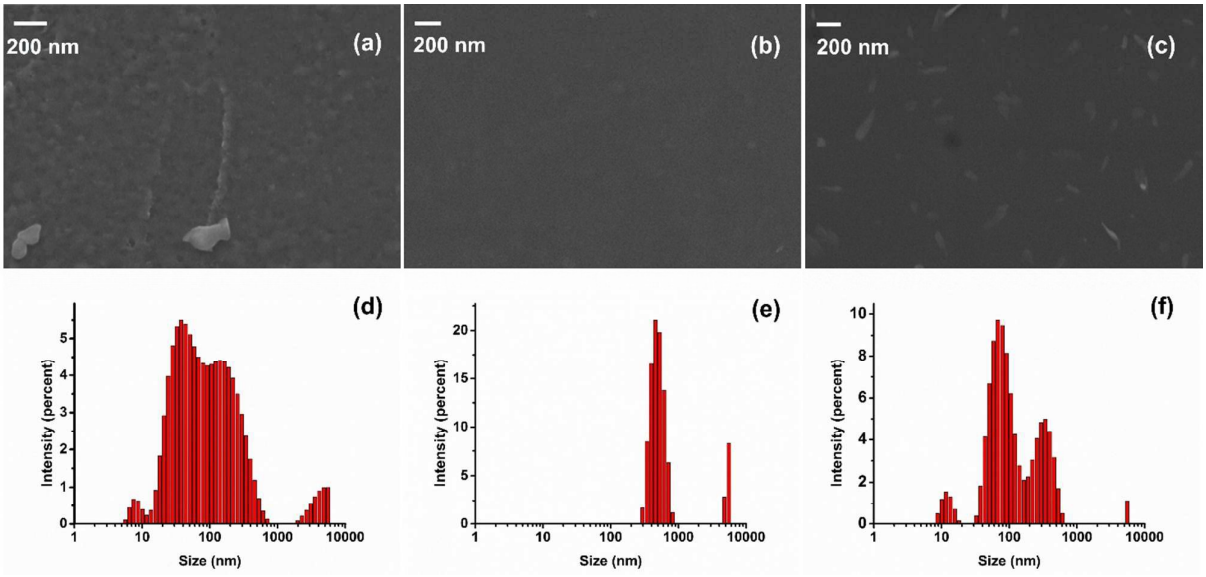


Fig. 3 SEM images of (a) P+TBP, (b) P+CD, (c) PAA-CD and DLS data of (d) P+TBP, (e) P+CD, (f) PAA-CD.

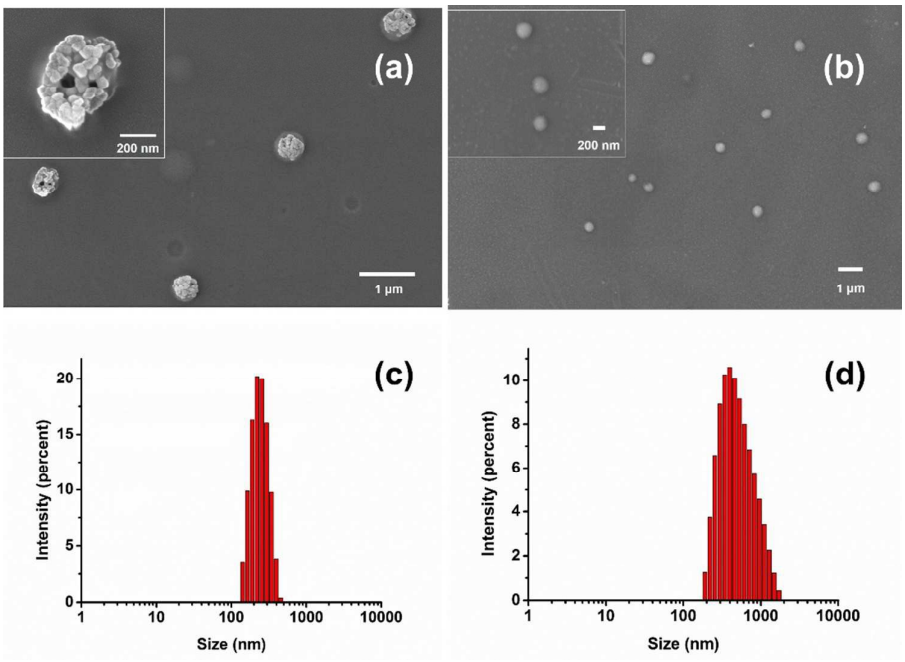


Fig. 4 SEM images (a) and DLS graph (c) of the particles prepared by P+TBP and P+CD, SEM images (b) and DLS graph (d) of the particles prepared by P+TBP and PAA-CD.

similar for almost 2 days, indicating that these SNPs are suitable for biomedical applications. The remarkable stability of the particles owe to the existence of a balance of forces in the SNPs, that is repulsive electrostatic forces and attractive host-guest interaction between the both positively charged host and guest polymers, which obviously led to a stable supramolecular nanoparticle system.

When the NaOH solution was added in the nanoparticles, the amino groups of the polymers are partly deprotonated, leading to a decrease of the overall charge of the host and guest polymers, and consequently aggregation and precipitation.

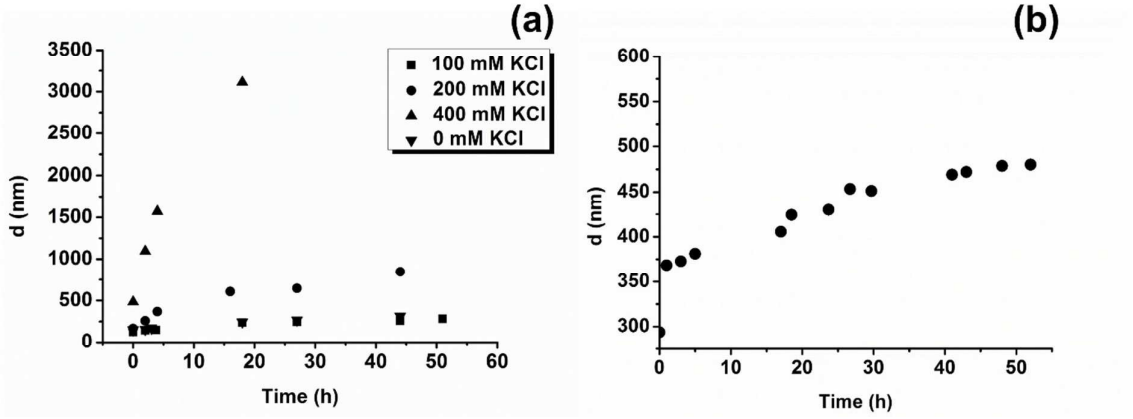


Fig. 5 (a) Influence of ionic strength on particle stability prepared by P+CD and P+TBP, (b) particle stability prepared by P+TBP and PAA-CD.

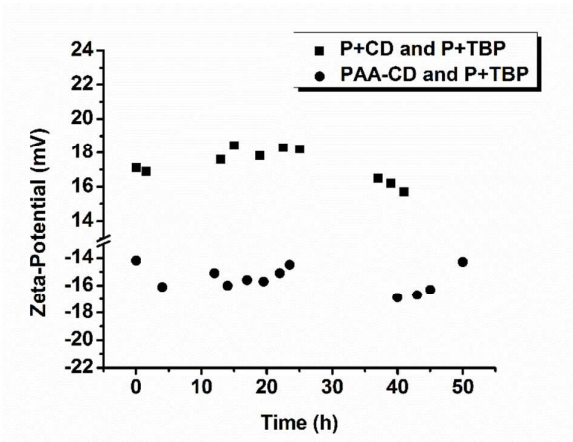


Fig. 6 Zeta-Potential changes of nanoparticles prepared by P+CD and P+TBP, PAA-CD and P+TBP at various time respectively.

Analogously, decrease of the electrostatic repulsive forces between host and guest polymers was achieved by preparing the SNPs at increasing ionic strengths varying from 100 to 400 mM KCl at a 4:1 host/guest ratio (Fig. 5(a)).[29,30] DLS results certainly demonstrated the destabilization of the particles with increasing ionic strength. While in the absence of KCl, the particles size was maintained for almost 2 d. In 200 mM KCl solution, the SNPs began to enlarged at the first 2 hours, and reached almost triple of their original size after 40 hours. In 400 mM KCl, the original size of the particles was nearly 500 nm, and the particle size was continuously increasing and reached six times of their original size after 20 hours. It is noteworthy that the SNPs prepared at 100 mM KCl was more stable than the SNPs prepared without KCl. We speculate that KCl of high concentration was able to decrease the electrostatic repulsions between host and guest polymers. The forces between the attractive host-guest interaction and the repulsive electrostatic force between the positively charged host and guest polymers reached a new balance, leading to an enhanced stability of the complex. These results demonstrate that the balance between the attractive host-guest interaction and the repulsive electrostatic forces between the both positively charged host and the guest polymers can be disturbed by higher pH and/or increasing the ionic strength to some extent, giving rise to the continuous growth of the supramolecular cross-linked network.

Besides using KCl of different concentration to adjust the balance forces, the ratio between host and guest polymers is another important factor to obtain a stable balance between the host-guest interaction and the electrostatic force. The mixture which is prepared by improper ratio was unable to form SNPs. The imbalance between the attractive host-guest interaction and the repulsive electrostatic force lead to unstable SNPs. For example, with the host and guest polymers ratio of 1:2, precipitation can be observed within 2 hours.

To confirm the conclusion that the SNPs are formed by the balance between the attractive host-guest and the repulsive electrostatic forces between the host and the guest polymers, we prepared PCD polymer which only bears small amount of positive charge caused by the EDA modified CD just like P+CD. However, the charge on CD has almost negligible effect on the systems we study. As expected, immediate aggregation and precipitation occurred along with the addition of P+TBP induced by the single force of attractive host-guest interactions and insufficient repulsive force. These results clearly prove that once electrostatic repulsions between host and guest polymers is insufficient, the attractive host-guest interactions become the main force, which results in aggregation of the host and guest polymers.

We then prepared another pair of host and guest polymers with opposite charges, P+TBP and PAA-CD. The complexes are formed by host-guest inclusion complexation and electrostatic attraction. And the SNPs are prepared by the same 1:4 ratio between host (CD) and guest (TBP) moieties of the polymers.

The SEM image and DLS graph was showed in Fig. 4(b) and Fig. 4(c). It is noteworthy that the polydispersity index was apparently higher than SNPs formed by P+CD and P+TBP, indicating a better distribution of the latter. The difference forces with electric charge offered between the two pairs of polymers can be confirmed by ζ-potentials. The ζ-potential of complexes formed by P+TBP and P+CD was 17 mV. While the ζ-potential of complexes formed by P+TBP and PAA-CD was -15.0 mV as a result of redundant negative charge of PAA-CD. Zeta-Potential changes of the particles prepared by P+CD and P+TBP, PAA-CD and P+TBP upon time variation were measured (Fig. 6), and the surface charge kept consistently over time. The stability of particles prepared by P+TBP and PAA-CD is observed at 20 ^{o}C as well (Fig. 5(b)). Compared with the SNPs formed by P+TBP and P+CD, the complexes formed by P+TBP and PAA-CD were more stable. However, the size of ~450 nm was rather big, making the complexes difficult to be applied as biomaterials. The influence of iron strength on the particle stability was also conducted at increasing concentrates of KCl varying from 100 mM to 400 mM. It is noteworthy that immediate aggregation and precipitation occurred along with the addition of P+TBP in the presence of different concentration of KCl, indicating that the complexes formed by PAA-CD and P+TBP are sensitive to iron strength.

Conclusions

In summary, we have developed a multicomponent SNP system using the positively charged polymer P+TBP and P+CD. The SNPs are prepared by multivalent supramolecular interactions between the guest TBP and the host CD, and their stability and size are controlled by the balance of forces between attractive host-guest interactions and repulsive electrostatic interactions. The novel SNPs we formed possessing well stability will promote the development of SNPs as carriers for biomedical applications, such as drug delivery agents. Moreover, PGOHMA can be easily functionalized with different groups such as target ligands, providing us an opportunity to integrate functional groups into the complex, which hold potential for different applications.

Acknowledgements

The authors thank National Natural Science Foundation of China (21374079 and 21244004), Program for New Century Excellent Talents in University (NCET-11-1063), and Program for Prominent Young College Teachers of Tianjin Educational Committee for financial support.

Notes and references

1 K. Jie, Y. Zhou and X. Ji, *Polym. Chem*., 2015, **6**, 218-222.
2 H. Gao, H. Takemoto, Q. Chen, M. Naito, H. Uchida, X. Liu, K. Miyata and K. Kataoka. *Chem. Commun.*, 2015, **51**, 3158-3161.
3 W. Gu, Q. Li, H. Lu, L. F, Q, Chen, Y. Yang and H. Gao. *Chem. Commun.*, 2015, **51**, 4715-4718.
4 J. H. Lee, K. J. Chen, S. H. Noh, M. A. Garcia, H. Wang, W. Y. Lin, H. Jeong, B. J. Kong, D. B. Stout, J. Cheon and H.-R. Tseng, *Angew. Chem. Int. Ed*., 2013, **125**, 4480-4484.
5 C. Amiel, V. Wintgens, T. T. Nielsen and K. L. Larsen, *Macromol. Symp*., 2012, **75**,317-318.
6 S. Wang, K.-J. Chen, T.-H. Wu, H. Wang, W.-Y. Lin, M. Ohashi, P.-Y. Chiou and H.-R. Tseng, *Angew. Chem. Int. Ed*., 2010, **49**, 3777-3781.
7 H. Wang, S. T. Wang, H. Su, K. J. Chen, A. L. Armijo, W. Y. Lin, Y. J. Wang, J. Sun, K. Kamei, J. Czernin, C. G. Radu and H. R. Tseng, *Angew. Chem. Int. Ed*., 2009, **48**, 4344-4348.
8 Y. Liu, H. Wang, K.-I. Kamei, M. Yan, K.-J. Chen, Q. Yuan, L. Shi, Y. Lu and H.-R. Tseng, *Angew. Chem. Int. Ed*., 2011, **50**, 3058-3062.
9 M. E. Davis, *Adv. Drug Delivery Rev*., 2009, **61**, 1189-1192.
10 N. Nayak and K. R. Gopidas. *J. Mater. Chem. B*, 2015, **3**, 3425-3428.
11 M. Miyauchi and A. Harada, *J. Am. Chem. Soc.*, 2004, **126**, 11418-11419; Y. Hasegawa, M. Miyauchi, Y. Takashima, H. Yamaguchi and A. Harada, *Macromolecules*, 2005, **38**, 3724-3730; A. Harada, Y. Takashima and H. Yamaguchi, *Chem. Soc. Rev.*, 2009, **38**, 875-882; X. Ma and H. Tian, *Acc. Chem. Res*., 2014, **47**, 1971-1981; H. X. Zhao, D. S. Guo, L. H. Wang, H. Qian and Y. Liu, *Chem. Commun.*, 2012, **48**, 11319-11321; A. Harada, Y. Takashima and M. Nakahata, *Acc. Chem. Res*., 2014, **47**, 2128-2140; X. Yan, F. Wang, B. Zheng and F. Huang, *Chem. Soc. Rev*., 2012, **41**, 6042-6065; A. Tamura and N. Yui, *Chem. Commun*., 2014, **50**, 13433-13446.
12 L. Wang, Y. Yang, M. Zhu, G. Qiu, G. Wu and H. Gao. *RSC Adv*., 2014, **4**, 6478-6485.
13 S. Shang, W. Zeng and X. M. Tao, *RSC Adv*., 2012, **2**, 4675-4682.
14 Y. Liu, Y. W. Yang, L. Li and Y. Chen, *Org. Biomol. Chem*., 2004, **2**, 1542-1548.
15 J. L. Manasco, C. D. Saquing, C. Tang and S. A. Khan, *RSC Adv.*, 2012, **2**, 3778-3784.
16 J. Szejtli, *Chem. Rev.,* 1998, **98**, 1743-1753.
17 M. L. Denadai, D. Ianzer, A. F. C. Alcantara, M. M. Santoro, C. F. F. Santos, I. S. Lula, A. C. M. Camargo, A. FaljoniAlario, R. A. S. Santos and R. D. Sinisterra, *Int. J. Pharm*., 2007, **336**, 90-98.
18 Z. Z. Shao, Y. P. Li, T. Chermak and A. K. Mitra, *Pharm. Res*., 1994, **11**, 1174-1179.
19 L. G. Suarez, W. Verboom and J. Huskens. *Chem. Commun*., 2014, **50**, 7280-7282.
20 M. E. Davis, Mol. Pharmaceutics, *Mol. Pharmaceutics*., 2009, **6**, 659-668.
21 M. R. de Jong, J. Huskens and D. N. Reinhoudt, *Chem. Eur. J.*, 2001, **7,** 4164-4170.
22 W. Gu, Y. Ma, C. Zhu, B. Chen, J. Ma and H. Gao. *Eur. J. Pharm. Sci.,* 2012, **47**, 556–563.
23 H. Gao, M. C. Jones, P. Tewari, M. Ranger and J. C. Leroux; *J Polym. Sci. Pol. Chem*., 2007, **45**, 2425-2435.
24 W. Gu, Y. Yang, J. Wen, H. Lu and H. Gao, *Polym. Chem.,* 2014, **5**, 6344-6349.
25 Y. Chen, Z. Shen, E. Barriau, H. Kautz and H. Frey, *Biomacromolecules.*, 2006, **7**, 919–926.
26 J. Wang, D. T. Pham, X. Guo, L. Li, S. F. Lincoln, Z. Luo, H. Ke, Li Zheng and R. K. Prud'homme, *Ind. Eng. Chem. Res.*, 2010, **49**, 609-612.
27 L. Li, X. Guo, J. Wang, P. Lin, R. K. Prud'homme, B. L. May and S. F. Lincoln, *Macromolecules.,* 2008, **41**, 8677-8681.
28 Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. *Analytical Chemistry*, 1956, **28**, 350–356.
29 J. Hierrezuelo, A. Vaccaro and M. Borkovec, *J. Colloid Interf. Sci.,* 2010, **347**, 202-208.

30 I. P. D. Picola, K. A. N. Busson, A. H. Casé, F. D. Nasário, V. A. O. Tiera, S. R. Taboga, J. R. Neto and M. J. Tiera, *J. Exp. Nanosci.*, 2013, **8**, 539-552.